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Hydrogel forms of hydroxypropylmethylcellulose and bentonite clay based on plant extract *Tamarix Hispida*

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NORMATIVE REFERENCES

The following standards are referenced in this dissertation:

STSD 6709-72 Distilled water. Technical conditions
STSD 31460-2012 Medicinal products. Ointments
STSD 23932-90 Laboratory glassware and equipment. General technical conditions
STSD 1770-74 Measuring laboratory glassware. Cylinders, beakers, flasks, test tubes. General technical conditions
STSD 9147-80 Porcelain tableware and laboratory equipment. Technical conditions
STSD 10749.1-80 Technical ethyl alcohol. The method of determining the appearance.
STSD 24104-2001 Laboratory scales.
STSD 28115-89 (ST SEV 6347-88) Drying apparatuses and installations.
STSD R 56327-2014 Medical electrical products. Ultrasonic devices of expert class.

DESIGNATIONS AND ABBREVIATIONS

The following terms and definitions are used in this dissertation:
BAC – Biological Active Compounds
BAS – Biologically Active Substances
BC – Bentonite Clay
CH – Chitosan
CuNP – Copper Nanoparticles
DNA – Deoxyribonucleic Acid
HPLC – High-performance Liquid Chromatography
HPMC – Hydroxypropyl Methylcellulose
HPC – Hydroxypropyl Cellulose
HEC – Hydroxyethyl Cellulose
EC – Ethyl Cellulose
MC – Methylcellulose
Na-CMC – Sodium Carboxymethyl Cellulose
NMR spectroscopy – Nuclear Magnetic Resonance spectroscopy
TH – *Tamarix Hispida*
TH-10 – water-alcohol extract of Tamarix Hispida (10%)
THU-10 – water-alcohol extract of Tamarix Hispida (10%) obtained by ultrasound extraction
PEG – Polyethylene Glycol
PG – Polypropylene glycol
PVA – Polyvinyl Alcohol
PSMs – Plant Secondary Metabolites
PVP – Polyvinylpyrrolidone
WHO – World Health Organization
RNA – Ribonucleic Acid
SEM – Scanning Electron Microscope
UV - Ultraviolet
LC-MS – Liquid Chromatography-Mass Spectrometry
DCM - Dichloromethane
Asp – Aspartic acid
Arg – Arginine
His – Histidine
Met – Methionine
Lys – Lysine
Phe – Phenylalanine
Tyr – Tyrosine
Val – Valine
T – temperature, °C
°C – Celsius degree by temperature
t – time, min
W – quantity of yield from gel (%, mol/g)
α - extent of swelling (g/g)

m_0 – mass of dry gel, g
m – mass of swelling gel, g

INTRODUCTION

General description of the work. The dissertation is devoted to the development of hydrogel forms based on HPMC and its compositions with extracts from the plant *Tamarix Hispida*, obtained through ultrasonic treatment of the raw material; study of the anti-inflammatory and antioxidant activity of extracts, assessment of the physicochemical characteristics of polymer forms.

The work was carried out at the Department of Chemistry and Technology of Organic Substances, Natural Compounds and Polymers and the Research Institute of New Chemical Technologies and Materials of the Al-Farabi Kazakh National University.

The relevance of the work.

Among polysaccharide-based gel matrices, cellulose ether derivatives are favored due to the ability to obtain modified forms that allow variation in solubility, duration of action, and drug release. They are also widely available and accessible due to their derivation from various raw material sources. In the production of pharmaceutical materials, hydroxypropylmethylcellulose (HPMC), also known as hypromellose, is one of the most in-demand cellulose ethers used as a carrier, filler, and coating – especially in combination with plant-derived biologically active compounds (BACs) for their antibacterial, wound-healing, antimicrobial, mucoadhesive, anti-inflammatory, and antioxidant properties. Plants of the *Tamarix* genus from the *Tamaricaceae* family are a valuable source of various biologically active compounds, extracts and isolated compounds have demonstrated antioxidant, anti-amnesic, antimicrobial, antifungal, and cytotoxic activity. One of the important compatible and structure-enhancing components for polymer compositions is the inorganic polymer bentonite clay. The presence of large deposits and reserves of bentonite clay in Kazakhstan is an additional strong incentive for the development of organo-mineral hydrogels. Using extracts of *Tamarix Hispida* as an aqueous drug component in HPMC and its clay-based compositions allows for the development of hydrogels with the required physicochemical properties, making them suitable for widespread use in medicine and pharmacology.

The aim of the dissertation is to obtain new hydrogel forms based on hydroxypropylmethylcellulose (HPMC), bentonite clay and extract from aerial part of the *Tamarix Hispida Willd*.

To achieve the stated goal, the following **tasks** were formulated:

1. Obtaining extracts based on *Tamarix Hispida* using ultrasonic treatment. Determination of the optimal extraction conditions, as well as the quantitative and qualitative composition of the obtained extracts.

2. Determination of the antioxidant and anti-inflammatory activities of the extracts from the aerial part of *Tamarix Hispida.*

3. Preparation of hydrogels based on hydroxypropylmethylcellulose, bentonite clay and their composites with THU-10 extracts.

4. Determination of physicochemical properties and study of rheological characteristics of the obtained hydrogels based on hydroxypropylmethylcellulose, bentonite clay and their composites with THU-10 extracts.

The object of the study: Medicinal plant material - the aerial part of the plant of the species *Tamarix Hispida* of the genus *Tamaricaceae*, its extract (THU-10) and hydrogels of HPMC, bentonite clay with THU-10

The subject of the study: Selection of optimal conditions for obtaining an extract from the aerial part of the plant species *Tamarix Hispida* of the genus *Tamaricaceae* by the method of ultrasonic extraction. Obtaining hydrogel forms based on HPMC, bentonite clay and their composites with THU-10.

Research methods: titrimetric analysis, thin-layer chromatography (TLC), HPLC, liquid chromatography–mass spectrometry (LC-MS), UV-visible spectroscopy (UV-Vis), scanning electron microscopy (SEM). The antioxidant activity of the extracts was determined using the DPPH radical scavenging assay, providing insight into free radical neutralizing capacity. In vivo anti-inflammatory activity was assessed using the carrageenan-induced paw edema model in rats, with statistical comparison to standard anti-inflammatory drugs.

The scientific novelty of the thesis

For the first time, extracts from the above-ground part of the plant species *Tamarix Hispida* of the genus *Tamaricaceae* were obtained using the ultrasonic extraction method. The method of obtaining the extract by ultrasonic extraction was optimized. The anti-inflammatory and antioxidant activity of the hydroalcoholic extract from the above-ground part of the plant species *Tamarix Hispida* was established. They exhibit pronounced anti-inflammatory and antioxidant activity. The THU-10 extract demonstrates a similar level of DPPH radical inhibition with gallic acid standards and quercetin at concentrations of 400 μg/mL and higher (% DPPH inhibition 96,08±0,48). Hydrogels based on HPMC, Bentonite clay with an extract of water-ethanol extract from the above-ground part of the plant species *Tamarix Hispida* were obtained for the first time. Composite hydrogels with HPMC and Bentonite clay with THU-10 extract were also obtained.

The main provisions for the defence:

1. The optimal conditions for obtaining a water-alcohol extract from the aerial part of the *Tamarix Hispida* by ultrasonic extraction are time of extraction 25 minutes, a raw material-to-solvent ratio of 1:6, a temperature of 20-40°C, and an ultrasound frequency of 35 kHz, which ensure the highest yield of biological active compounds.
2. Aqueous-ethanol extract from the aerial part of the *Tamarix Hispida* (THU-10) demonstrates pronounced anti-inflammatory and antioxidant activity. The THU-10 extract demonstrates a similar level of DPPH radical inhibition with gallic acid and quercetin standards at concentrations of 400 μg/mL and higher (% DPPH inhibition 96,08±0,48)
3. Based on the analysis of rheological properties, the optimal hydrogel compositions are: a hydrogel based on bentonite clay 25%; a hydrogel based on HPMC 2-4%; a composite hydrogel based on HPMC:BC 4% (7:3) with the addition of 1% THU-10 extract.

Theoretical significance of the work:

This study expands the scientific basis for the use of hydrogels based on cellulose ethers, in particular hydroxypropylmethylcellulose (HPMC), in the development of modern drug delivery systems. The work deepens the theoretical understanding of the action of plant bioactive compounds by exploiting their potential for integration with polymer matrices for therapeutic applications. The obtained results can serve as a basis for the creation of "smart" hydrogels capable of controlled and prolonged release of medicinal substances, which contributes to the development of biomedical materials science.

Practical significance of the study:

The study offers practical solutions for the development of advanced transdermal drug delivery systems using HPMC-based hydrogels with Tamarix Hispida extracts and bentonite clay. These hydrogels eliminate the key limitations of traditional biologically active compounds by increasing their stability, prolonging the therapeutic effect, biocompatibility, and economic availability, which is especially relevant for Kazakhstan due to its rich reserves of medical plants and bentonite clay. The resulting hydrogel dressings have potential for use in wound healing, local anesthesia, and infection control, indicating high promise for their integration into pharmaceutical practice.

Relation of the thesis with research and government programs:

The dissertation work was carried out within the framework of the grant funding for young scientists under the "Zhas Galym" project for 2024–2026 by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan, project No. AP22684531, titled "Development of hydrogel forms of plant extracts based on HPMC," under the priority area "The science of life and health".

The main results of the study:

1. Alcoholic extracts based on *Tamarix Hispida* were obtained using ultrasonic treatment. The optimal conditions for the extraction method using ultrasonic extraction were determined. By using the ultrasound method, the yield of components from plant raw materials increases by 3-4 times and the extraction time is significantly reduced (from 48 hours to 30 minutes) with an emitted ultrasound power of 20 to 35 kHz. The quantitative and qualitative compositions of the obtained extracts were determined.

2. The biological activities of THU-10 were determined. Anti-inflammatory, antioxidant activities and cytotoxicity of the extracts were investigated. The THU-10 extract demonstrated a similar level of DPPH radical inhibition with gallic acid standards and quercetin at concentrations of 400 μg/mL and higher (% DPPH inhibition 96,08±0,48).

3. Hydrogels based on hydroxypropylmethylcellulose (HPMC), bentonite clay, and its composites with THU-10 extract at various concentrations and compositions were obtained.

4. The physicochemical and rheological characteristics of hydrogels based on HPMC, bentonite clay and its composites with THU-10 extract were determined, and the optimal compositions of hydrogels were determined: based on bentonite clay

25% with the addition of 1% THU-10 extract; based on HPMC 2-4% with 1% THU-10; composites based on HPMC:BC 4% (in a ratio of 7:3) with 1% THU-10.

The personal contribution of the author was to independently planning and carrying out the experimental part of the study, interpret and process the obtained data, and work with scientific consultants to resolve issues and discuss the results of the study.

1. Ultrasonic extraction of biologically active substances *Tamarix Hispida Willd* // Chemistry of plant materials (Direct participation in the collection of experimental data, processing and interpretation of results, preparation of the article);
2. Hydrogel Delivery Systems for Biological Active Substances: Properties and the Role of HPMC as a Carrier // the review article was published in the journal Molecules (analysis of literature data, collection of empirical data, publication of the article (first author));
3. **Materials of reports at scientific conferences and symposia (collection and description of experimental data, discussion of analysis results, preparation of materials**);
4. Method for obtaining extract from plant raw materials // Utility model patent, published by the RSE "National Institute of Intellectual Property" (Direct participation in the collection of experimental data, processing, and interpretation of the results).

Approbation of the work.

The results and key findings of the scientific work were presented at the following conferences:

5th International Conference on Chemical Investigation and Utilization of Natural Resources, Ulaanbaatar, Mongolia, October 14–15, 2021: "New polymer forms of Tamarixidin and their properties". XXXI Russian Youth Scientific Conference on Problems of Theoretical and Experimental Chemistry, Yekaterinburg, April 20–23, 2021: "Synthesis and study of the properties of polymer forms of Tamarixidin". Galissultanova A., Amanzholkyzy A., Abilov Zh. A. “Preparation and rheological properties of HPMC gels” // International Chemistry and Chemical Technology Congress “XI International Congress of Birimzhanov” – Almaty, 2021. – P.142.

Publications.

Article in the journal “Chemistry of Plant Raw Material”: “Ultrasonic Extraction of Biologically Active Substances from *Tamarix Hispida* Willd,” 2021, No. 3, pp. 283–289. percentile – 23 (in the Scopus database), https://doi.org/10.14258/jcprm.2021038695.

Utility model patent, published by RSE "National Institute of Intellectual Property", 2022, No. 28: “Method for obtaining extract from plant raw material”.

Article in the journal ***Molecules*** (Switzerland), which in 2024 was ranked in Q1 in the field of "Chemistry (miscellaneous)" with an SJR of 0.865 and a percentile of 80 (according to the Scopus database): “Hydrogel Delivery Systems for

Biologically Active Substances: Properties and the Role of HPMC as a Carrier" *Molecules* **2025**, *30*(6), 1354, **https://doi.org/10.3390/molecules30061354**.

Volume and the structure of the thesis. The dissertation is presented in 121 pages of text and consists of an introduction, 3 chapters, a conclusion, 21 tables, 68 figures, and a list of references comprising 193 sources.

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1 LITERATURE REVIEW

1.1 Hydrogels and their types

In the field of development of medical drugs and products, among all popular forms of drug delivery, hydrogel delivery systems have a number of advantages. Hydrogel-based drug delivery systems are easy to use, painless, and offer enhanced efficiency due to the prolonged release of the active ingredient. An important distinction of hydrogel systems is their convenience in use. This difference allows them to be considered an alternative to traditional forms. By selecting and combining an active ingredient with a hydrogel base, transdermal systems that have antiviral, antibacterial, and local anesthetic effects are developed [1–4]. Hydrogels also have the ability to respond to changes in external conditions, such as pH, temperature, electric field, light, ionic strength, magnetic oscillations, etc., which allows them to be classified as intelligent materials with adjustable properties. Additional properties of hydrogels include biodegradability, biocompatibility, and sensitivity to various factors. Biodegradability is currently [1, 5, 6] an important property for the development of environmentally friendly materials and technologies.

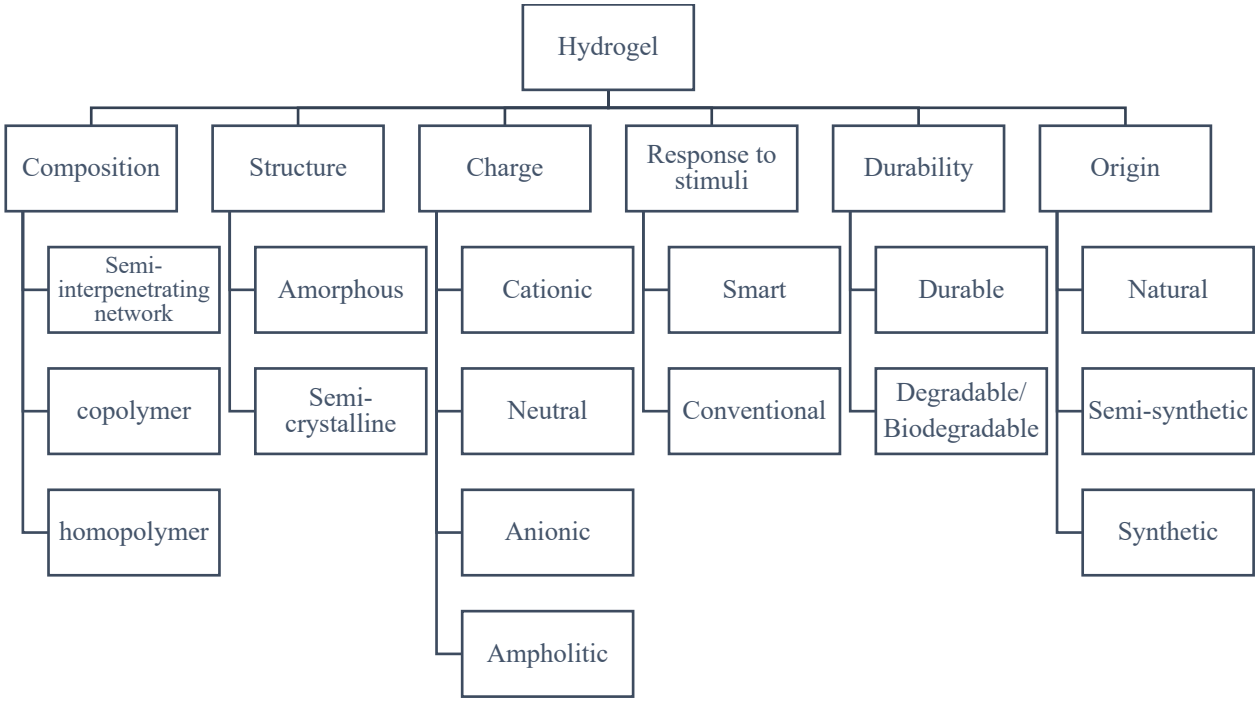


Figure 1 – Classification of hydrogels based on their properties.

Hydrogels, which are three-dimensional polymer networks crosslinked through physical and chemical interactions, are synthesized from polymers of synthetic origin, semi-synthetic, and natural origin [7–9]. Polymers of different origins have both advantages and disadvantages. Synthetic and semi-synthetic polymers, while possessing the ability to polymerize and excellent mechanical properties, cannot surpass natural ones in terms of biocompatibility with the human body, and are also

not capable of creating an accessible environment for other cells. Among the wide range of natural polymers, including those of protein, nucleic acid, and polysaccharide origins, this review will focus on cellulose ether derivatives, in particular, HPMC and hydrogels based on them [10, 11]. As recent literature reviews show, cellulose derivatives are among the leading materials for pharmaceutical hydrogel production due to their widespread availability in nature, non-toxicity, and low cost.

Hydrogel systems are capable of swelling in water and aqueous solutions, which makes them compatible with the human body. Hydrogels also function as matrices in which biologically active substances can be immobilized, including synthetic and natural compounds, and plant extracts, for biomedical applications and enhancing biocompatibility [7]. Especially promising "green" sources of biologically active substances are plants rich in phenolic compounds, flavonoids, tannins, and others. In addition to biocompatibility and safety, another important property of hydrogels is their ability to prolong the action of a drug. This is particularly significant when developing medications for long-term use and high-toxicity treatments, in cancer therapy, and in the treatment of slow-healing wounds using pain-relieving agents. For instance, studies [12–14] have reported the development of drug formulations containing lidocaine and prilocaine microemulsions, which are local anesthetics with prolonged action, targeted delivery, and enhanced drug efficacy, demonstrating the promise of research in this direction. The potential of using cellulose derivatives as drug carriers, mathematical models of drug release, and the feasibility of creating ointment and gel drug formulations with prolonged action have also been demonstrated in [15–20]. Previously, the authors of [21–23] obtained hydrogels of the anesthetic substances Rihlokaine and AK-29. New hydrogel dressings were developed using the radical crosslinking method based on poly-N-vinylpyrrolidone. These dressings contain the plant-derived medicinal substance "Alchidine", extracted from the plant camelthorn (Alhagi kirghisorum Shrenk.), which grows in Kazakhstan [24]. The authors of [25] also obtained extracts from the plant Tamarix of the Tamaricaceae family, which were subsequently immobilized into polymer matrices in the form of films [26]. Previously, polyphenolic, terpenoid and other biologically active compounds from plants of this genus were found and characterized. Extracts and individual components have shown antioxidant, anti-amnesic, antimicrobial, antifungal, and cytotoxic activity. Using extracts of Tamarix Hispida as the aqueous drug component of HPMC hydrogel, we can obtain hydrogels with the abovementioned properties and use these widely in medicine and pharmacology [27].

The proposed review is a logical continuation of the studies of prolonged systems with biologically active substances based on polymer hydrogels and their compositions of synthetic and natural origin and will be used to study the possibility of creating hydrogel carriers of plant extracts based on HPMC and its compositions.

Among natural polymers for medical purposes, cellulose is advantageously distinguished by its diversity due to its modified forms. The introduction of additional groups into the structure of cellulose allows for varying their solubility

and provides the opportunity for immobilization of medicinal substances and regulation of release. As mentioned above, hydrogels are classified into natural, semi-synthetic, and synthetic based on their origin (Figure 2, [7]). The classification of hydrogels is based on their physical and chemical properties, as well as their structural features.

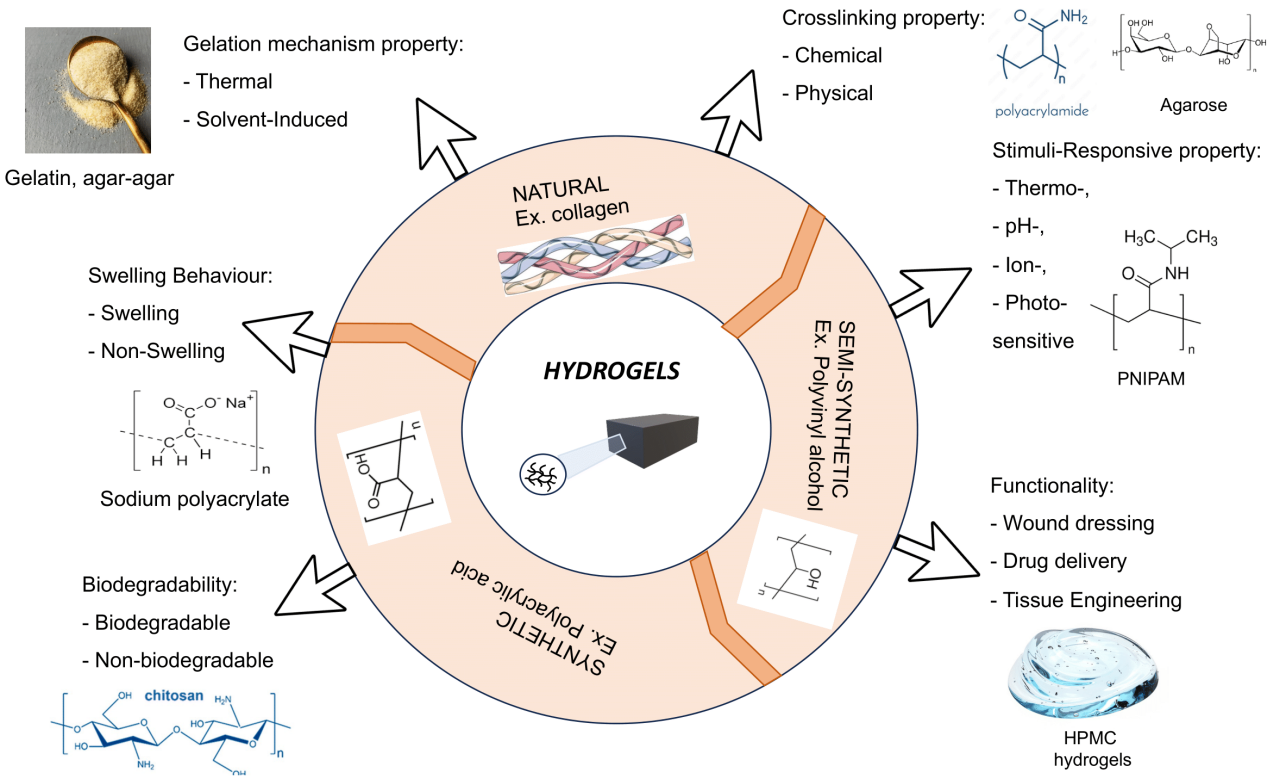
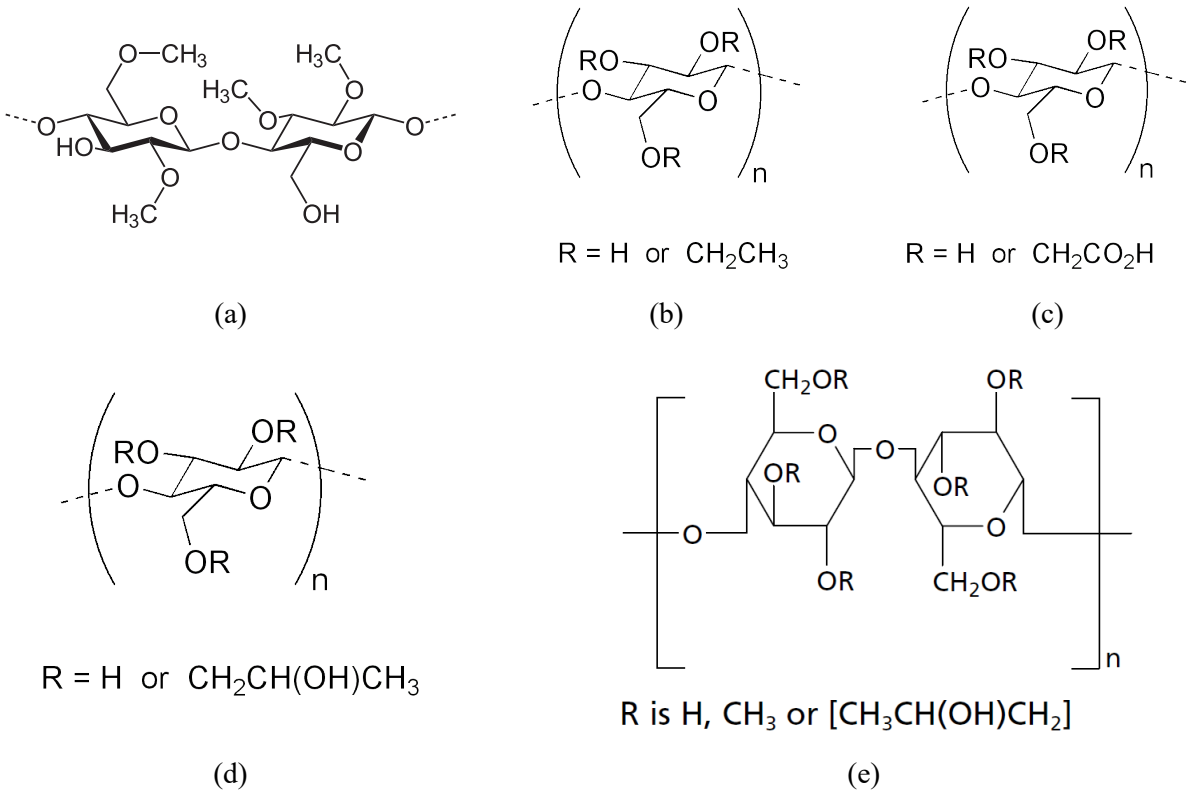


Figure 2 – Classification of hydrogels based on their properties [7].

It should be noted that it is hydrogels based on natural polymers that have been put to use in such an important field as medicine. Hydrogels are used in the production of contact lenses, matrices for cell proliferation, bases for targeted drug delivery, stabilizers for nanoparticles, composite materials, and more [28, 29].

1.1.1 Cellulose ether derivatives as hydrogel bases

Cellulose and its derivatives, particularly cellulose ethers [30–32], are rightfully considered the favorites among natural raw materials for hydrogels. This is evidenced by the studies of various authors [18, 19, 29, 33–36]. A significant advantage of cellulose derivatives is their abundance, which allows for a wide range of raw materials to be used for their production, such as various grains and cotton [37]. Cellulose derivatives, as complex esters, find widespread application in the pharmaceutical, food, and cosmetic industries. Among cellulose derivatives, the most widely used are methylcellulose (MC), hydroxypropyl cellulose (HPC), hydroxyethyl cellulose (HEC), and hydroxypropyl methylcellulose (HPMC) [38, 39] (Figure 3).



(a) (b) (c) (d) (e)

Figure 3 – Cellulose ether derivatives methylcellulose (MC) (a), ethyl cellulose (EC) (b), carboxymethylcellulose (CMC) (c), hydroxypropyl cellulose (HPC) (d), hydroxypropyl methylcellulose (HPMC) (e) [39].

Among the cellulose derivatives mentioned above, the most popular in the production of medicines is HPMC, which, in many literary sources, is called “hypromellose”. Hypromellose is a key component of drug coatings and fillers. This cellulose ether has advantages over other derivatives such as solubility in aqueous media, good swelling capacity, polymer viscosity, biocompatibility and biodegradability [40]. Hypromellose, like chitosan, has the advantage of having a complex-forming ability [41, 42]. Polymer complexes and the modification of HPMC are also widely used to obtain hydrogel carriers of medicinal substances and materials with the necessary rheological and swelling characteristics. Thus, a number of studies [43–47] are dedicated to the development and study of the properties of hydrogels of cellulose derivatives crosslinked with acrylic acid and the delivery of such medicinal substances as galantamine hydrobromide and benzophenone-4. The structural and rheological properties of the hydrogel matrix play an important role in the development of drug delivery systems. These properties can be regulated by selecting a crosslinking agent, copolymerization, and altering external conditions such as temperature, ionic strength, and pH. To improve the physical, mechanical, rheological and bioadhesive properties, three-dimensional hydrogels with polyvinylpyrrolidone (PVP) were synthesized, which have swelling and water-retaining capacity for transporting medicinal substances.

In general, the processes for producing hydrogels are quite simple. In pharmaceutical production, hydrogels can be used both with and without the inclusion of a medicinal substance. For example, the medications Hitopran (Prontosan), Purilon, Matopat, Granugel are used in medical practice as wound-healing dressings in the form of hydrogel bandages and do not contain an active ingredient. Medical gels based on cellulose additionally include the following components: antimicrobial preservatives (methylparaben, propylparaben, chlorhexidine gluconate), stabilizers (disodium edetate), dispersing agents (alcohol, glycerin, propylene glycol, sorbitol), and homogenizers. The gelling ability depends on the type and molecular weight of the cellulose derivatives, but the minimum gelling concentration ranges from 4% to 6%. The convenience of using cellulose-derivative-based gels lies in their simple preparation, the absence of additional crosslinking agents, and the use of purified water as the hydrophilic phase. The methods for obtaining HPMC hydrogels are based on both traditional and environmentally friendly alternative methods, which increases their applicability.

1.1.2 Methods for obtaining hydrogels based on HPMC

The successful development of hydrogel-based drug delivery systems is determined by the initial stage of creating the hydrogel matrix. This involves using both traditional methods and, increasingly in recent years, innovative techniques employing more environmentally friendly approaches. The search for new approaches to hydrogel synthesis is driven by the fact that hydrogels produced using traditional methods often exhibit low mechanical stability and slow responsiveness to external conditions [48].

The order of mixing components plays an important role in the preparation of gel formulations. Mixing the components above with the gelling agent should be carried out considering their influence on the gelling process. If these ingredients affect the rate and extent of swelling of the gelling agent, they are mixed after gelling. If such an intervention does not occur, the drug and other additives are mixed before the swelling process; in this case, it is also important to consider the impact of swelling time, mixing temperature, and other processing conditions on the physicochemical stability of the formulation and additives. Typically, the following order of mixing is recommended:

1. The active ingredients are dissolved or suspended in the hydrophilic phase necessary for preparing the gel.
2. Other additives are dissolved in the obtained solution or in a small amount of the hydrophilic phase, accordingly.
3. If necessary, the dispersion of the active ingredient is mixed with the solution of additives.
4. The gelling agent powder is added to the resulting dispersion solution with gentle stirring and left to swell.

When preparing cellulose-derivative-based hydrogels, temperature and pH of the dispersion are critical parameters, as the gelation mechanism of these polymers

depends on temperature, and their optimal stability is pH-dependent. Thus, it is recommended to heat the macromolecule dispersion either before or after the polymer swelling.

Overall, the method for producing most hydrogels based on cellulose derivatives involves dispersing the polymer powder in cold water using mechanical stirring to form a homogeneous, single-phase dispersion. This is followed by heating the dispersion to approximately 60–80 °C and then gradually cooling it to room temperature to form the gel, a process known as the “hot/cold” method (Figure 4) [49, 50]. Additionally, there are differences in the pH values of the dispersion medium favorable for gel formation: NaCMC, MC, and HPMC gels form over a wide pH range (4–10), while HPC and HEC form gels at a pH of 6–8 and under alkaline pH conditions, respectively. Another important parameter in the preparation of hydrogels from cellulose derivatives is the swelling duration of the polymer. Typically, a swelling time of about 24–48 h is required to obtain homogeneous gels.

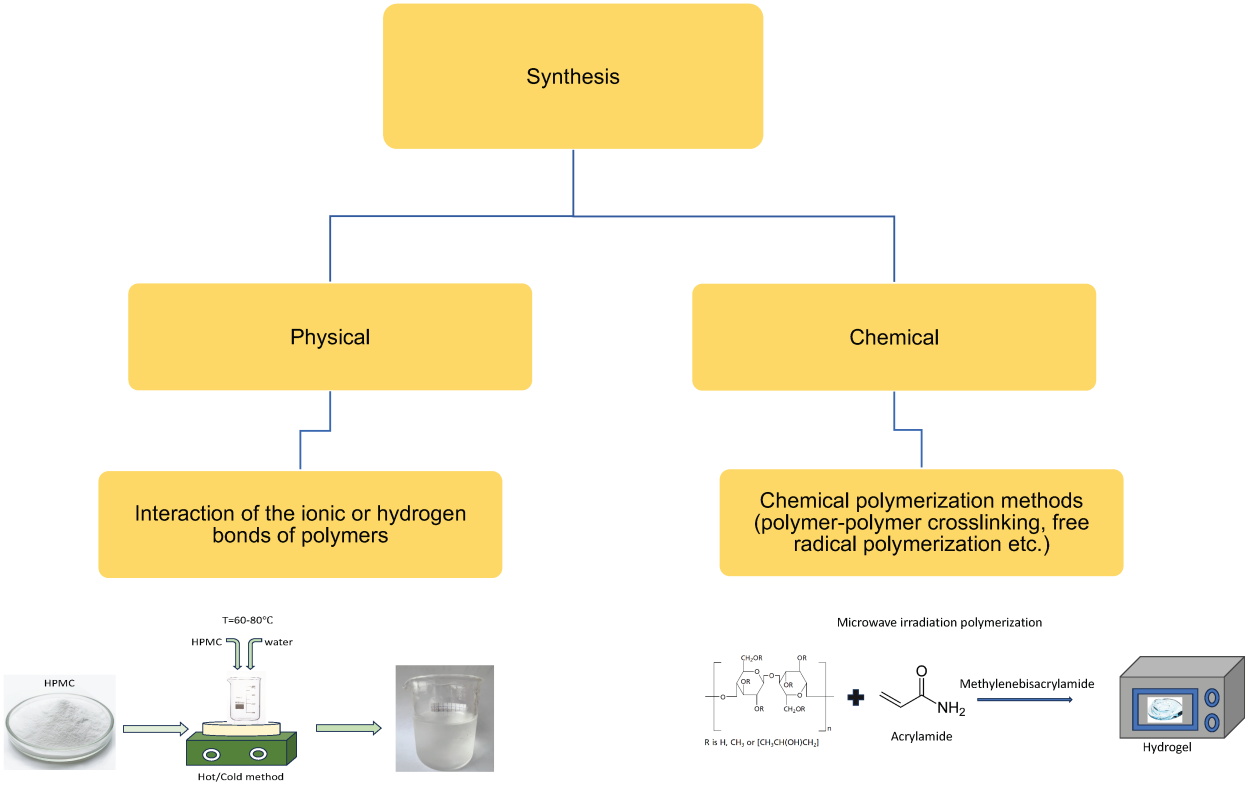


Figure 4 – Method for preparing hydrogels based on HPMC [49, 50].

Finally, the removal of entrapped air is also an important consideration in the preparation of HPMC hydrogels as the presence of air bubbles in the gel inevitably affects its transparency. The inclusion of air bubbles in the gel can be minimized by positioning the stirrer at the bottom of the mixing container. Typically, air bubbles are further removed using various methods, including prolonged storage, storage at low temperatures, ultrasonic treatment, or the addition of silicone-based defoamers. Additionally, in large-scale production, vacuum deaerators are used to remove entrapped air. In small-scale production, the preparation of hydrogels by manually

or mechanically dispersing the polymer in hot/cold water or a co-solvent is carried out using simple equipment and utensils available in a pharmaceutical laboratory, such as porcelain or glass mortars and pestles, measuring cylinders, magnetic stirrers, and various propellers and mixers. In large-scale production of pharmaceutical cellulose derivatives, various mills, separators, mixers, deaerators, and switches are used. In the pharmaceutical application of HPMC gels, the physical method of production is the most convenient and popular, especially when creating medicinal systems with plant extracts.

1.1.3 Important physicochemical properties of HPMC and its hydrogels

HPMC (hydroxypropyl methylcellulose) is a modified propylene glycol ether of methylcellulose, consisting of methoxy and hydroxypropyl groups along the linear polysaccharide chain (P. Timmins [39]).

Cellulose derivatives, particularly HPMC, are among the widely used natural biodegradable polymers applied in the development of oral drug delivery systems with controlled release [51]. The variation in cellulose derivatives regarding molecular weight, solubility, viscosity, and degree of dissociation allows for their extensive use in various applications. The main property of HPMC, which is used in the creation of immobilized systems and in the mechanism of drug release, is its solubility and swelling of its gels, which allows for a variation in parameters to achieve different degrees of prolongation.

The physicochemical properties of HPMC, such as surface activity, solubility, and gel formation, depend on three factors: the presence of methoxy groups, the presence of hydroxypropyl groups, and molecular weight. HPMC is soluble in cold water due to the targeted alkylation of the hydroxyl group of natural cellulose with methoxy and hydroxypropyl groups [15]. After dissolving in water, it forms a clear viscous gel. Hypromellose also dissolves very well in other solvents, such as ethanol, isopropanol, dichloromethane, as well as in their aqueous solutions at various ratios.

Temperature significantly affects the properties of the gel formed from HPMC. Aqueous solutions of hypromellose exhibit sol-gel thermal transitions at high temperatures, which most often manifest as turbidity of the solution caused by the process of hydrophobic interaction between the methoxyl groups in the hypromellose [52]. For hypromellose, the gelation process begins with the dissolution of the polymer, and with a further increase in temperature, the formation of terminal separated sections and hydrophobic associations of chains occurs. Hydration of hypromellose is highly temperature-dependent; as the temperature increases, HPMC loses hydration water, which leads to a decrease in the relative viscosity of the polymer, and the loss of hydration water contributes to the strengthening of polymer–polymer interactions, such as hydrophobic ones, primarily due to methoxy substituents [53]. Increased hydrophobic interactions lead to increased viscosity, which is the point of gelation. The gelation temperature of hydroxypropyl methylcellulose (HPMC) ranges from 50 °C to 90 °C and depends, like the solidification temperature, on the number of methoxy groups in the molecule

[54]. Substitution with ethoxy and dioxy groups contributes to an increase in the gelation temperature. The results of the study [55] showed that the gelation temperature can be reduced to 36 °C by replacing the hydroxypropoxy groups.

As the temperature increases, the gelation process occurs, leading to the precipitation of polymer molecules [56]. The gelation point and the turbidity point are independent of each other; so, the solution may become turbid before reaching the gelation temperature. If a polyelectrolyte is present in the solution, both the turbidity and the gelation temperature decrease [53].

HPMC is not used in materials that require thermal treatment because its decomposition temperature is 170–180 °C, and its melting point is 190–200 °C. Moreover, the decomposition temperature of HPMC depends on the structure of the side chains [57]. The lack of thermal stability prevents the use of further processing methods for the polymer that require its melting. Thus, in [58], attempts were made to improve the polymer's thermal stability. The development by the chemical company "Dow" (USA) for the extrusion and spray drying process required the use of hot molten polymer. A new product under the brand name "Affinisol HPMC HME" has been proposed, which features a low glass transition temperature, low viscosity, color stability at high temperatures, and extrusion capability. The authors also achieved improved solubility in organic solvents, which allows the polymer to be used in spray drying.

The source of hydroxypropyl methylcellulose (HPMC) is cellulose fibers, which are heated with an acoustic alkaline solution during the etherification process [16]. It is then treated with propylene oxide and methyl chloride. The resulting product has a specific ratio of substituted hydroxypropyl and methyl groups, which, in turn, depends on the ratio of methyl chloride and propylene oxide introduced after alkalization. The ratio of substituted groups affects the viscosity and gelation temperature of aqueous solutions of hypromellose.

The glass transition temperature of the polymer plays an important role in the development of controlled drug delivery systems. It was previously mentioned that hypromellose is widely used as a filler in pharmaceutical formulations and for the development of controlled drug delivery systems. When taking drugs with controlled delivery, some properties affect the release of the active drug. For example, it is known that a decrease in the glass transition temperature of the polymer reduces the mobility of macromolecules within the structure. At the same time, as the temperature increases, the mobility of macromolecules begins to rise, leading to an increased rate of drug transport. In the work of Doelker E. et al. [59], the glass transition temperature of the HPMC polymer was investigated. Various methods, such as thermomechanical analysis, dynamic mechanical analysis, differential scanning calorimetry, and torsion analysis, were used to determine and compare the results. However, different analysis methods show different glass transition temperature results for HPMC. Therefore, it is recommended to conduct research and comparisons using one of the methods. In determining the glass transition temperature, it is important to consider the degree of substitution as well as the molecular weight of the polymer [15]. All the listed properties of HPMC are

important in the creation of medicinal hydrogel forms. The nature and structure of the active pharmaceutical ingredient adjust its properties, especially rheological characteristics, swelling properties, solubility, compatibility, uniformity, and physico-mechanical strength.

Hydrogel systems with phytocompounds based on polysaccharides show improved properties such as biocompatibility, controlled release of active substances, mechanical strength, and hydrophilicity. Cellulose ethers have all of the above properties, and HPMC occupies a worthy place. The works summarize research on the production of polysaccharide hydrogels with phytocompounds in various dosage forms using solution casting technologies, with porogenic agents, gas foaming, lyophilization, cryogelation, extrusion, coacervation, emulsification, spray drying and electroforming [60, 61]. In [62], cellulose and CMC are mentioned among the popular carriers, but it should be noted that, in recent years, there has been increasing interest in obtaining hydrogel systems of synthetic biologically active substances, phytocompounds with hypromellose (HPMC) [43, 63–72]. The main focus in [65] is on properties such as thermosensitivity, swelling capacity, physical and mechanical properties, and the release of biologically active substances. The high swelling tendency, hydrophilicity, and thermal sensitivity of HPMC made it possible to use it to obtain a “smart” and biocompatible film with a uniform surface. The formation of hydrogen bonds between the NH- and OH-groups of chitosan and HPMC increases the strength, and the degree of swelling reaches the minimum value of the CH:HPMC 40:60 system. Due to chitosan, the hydrogel system acquires pH sensitivity and the phase transition temperature of HPMC increases. The introduction of essential oils into the composition of the CH-HPMC hydrogel increases the thixotropic and rheological properties and enhances the penetration of fluconazole [66]. In the binary PVP-HPMC gel, improved adhesive properties and rheological characteristics are observed. Bases made from both pure HPMC and the binary PVP-HPMC mixture [43] contribute to the slow release of the model drug benzophenone-4. Additionally, the release of the active substance is further slowed by changes in viscosity, which significantly increases at concentrations above 8%. Upon contact with the skin and intense rubbing, viscosity rapidly decreases, ensuring gel spreadability. The addition of PVP enhances the prolongation of drug action due to the electrostatic interaction between PVP and benzophenone-4. An HPMC-based hydrogel [67] containing PGA microfibers and ofloxacin, formed through blending, exhibits high wound-healing activity, a swelling degree of 531.8–1700%, and elongation within the range of 70–120%. The synergistic antimicrobial effect of the microfibers and the active ingredient is enhanced by the strong swelling capacity of HPMC, which plays a crucial role in the tissue granulation stage and promotes rapid wound healing.

Studies have demonstrated the possibility of obtaining a porous HPMC gel containing carvedilol, dissolved in Tween-20, lactic acid [68], and polysaccharides from *Agaricus blazei Murill* [69], with controlled release and rheological properties suitable for bone tissue regeneration. The gelation temperature of HPMC was reduced to 37 °C due to the presence of polysaccharides, making it comfortable for

the oral mucosa and dental restoration. Additionally, the porosity of the HPMC gel allowed for the regulation of the release of poorly soluble carvedilol. As is well known, the rheological characteristics, swelling properties of HPMC, and gelation temperature are essential for the development of pharmaceutical dosage forms, particularly prolonged-release tablets with controlled drug release [70–72].

Researchers have described the kinetics of release of the active drug substance from a controlled delivery system based on hypromellose [73, 74]. Various methods of analysis have been used, such us NMR spectroscopy, to characterize the mobility of water in the gel layer of the HPMC polymer [75].

1.1.4 Relevance of pharmaceutical application of HPMC with BAS

According to the WHO, more than 80% of medicines are of plant origin. Most diseases require the use of medicinal herbs along with synthetic drugs. Enhancing the efficiency of plant extracts and the rational use of natural resources are among the pressing global issues included in the Sustainable Development Goals due to the growing number of patients with skin diseases, trophic ulcers, and purulent wounds of various etiologies, necessitating a range of affordable applicative materials and their production [76, 77].

The growing interest in the field of natural compound chemistry research is explained by several factors:

– The diversity of both the chemical structures and biological activities of naturally occurring secondary metabolites;

– The use of new bioactive natural compounds as biochemical probes;

– The development of new and sensitive methods for detecting biologically active natural products;

– Improved isolation methods;

– Purification and structural characterization of these active components, as well as the demand for natural products [76].

The scientific literature includes studies on the development of wound-healing pharmaceutical formulations based on natural polymers and plant extracts [78, 79]. The biological activity of medicinal plants and their extracts is determined by their components, which define their beneficial properties such as antioxidant, wound-healing, anti-inflammatory, antibacterial, anti-tumor, and other effects. Among the natural components of plants, phenolic compounds and secondary metabolites that exhibit the abovementioned activities stand out. An example of the application of natural polymers is the development of a wound-healing dosage form based on alginates. Leading positions in the creation of polymeric dosage forms are held by biopolymers that are biodegradable and typically derived from natural sources, such as plants, algae, animal organisms, and bacteria. Examples of such biopolymers include polysaccharides, lipopolysaccharides, and proteins, which hold a significant share of the pharmaceutical sector [80]. Thus, Klemm D. et al. [81] propose a range of affordable, biodegradable biopolymers with low cost, which can serve as the basis for medicinal products, particularly medical wound-healing dressings. Among

biopolymers used as carriers for plant-based medicinal substances, HPMC stands out, as evidenced by data on HPMC-based materials combined with biologically active substances, extracts, and synthetic compounds, demonstrating enhanced effectiveness in wound treatment. Examples of HPMC applications as a drug carrier can be found in the works of many researchers. Below are some examples of soft dosage forms based on HPMC with medicinal substances and plant components (Table 1).

Table 1 – List of HPMC-based formulations with biologically active substances (BAS).

Carriers	Active Pharmaceutical Ingredient (Natural and Synthetic)	Type of Wound Dressing	Advantages and Application	Ref.
HPMC/ CH	Essential oil/Fluconazole	Hydrogel	Treatment of superficial fungal infections	[66]
HPMC/ PG	Lidocaine and prilocaine	Film	A hydrophilic additive (propylene glycol), when used in a water-insoluble membrane, acts as a channelizer, and increases the rate of release of the drug.	[82]
HPMC	Lawsonia inermis (henna) and Matricaria chamomilla	Gel	Stability, good viscosity, uniformity, extrudability, good extract release, effectiveness in the treatment of burns.	[83]
	Liposomal farnesol	Gel	HPMC with farnesol in the ratios of 1:2 and 2:1 had a good effect in the treatment of burns of the third degree.	[84]
	Bacteriophage	Gel	The gel obtained at a temperature of 37 °C in a percentage of 3% HPMC was found to be stable, effective in the treatment of wounds with an antibacterial effect, especially against Klebsiella pneumoniae bacteria.	[85]
	CuNPs-licorice and phenytoin	Gel	It is effective in acute inflammation by suppressing	[86]

			JAK 3 inflammation and synthesis of type I procollagen.	
	Honey and Aloe vera	Hydrogel	The hydrogel obtained in a percentage of 3% HPMC was found to be quite viscous when applied to the wound after a burn. The hydrogel showed good antibacterial activity on Klebsiella pneumoniae.	[87]
	Cefotaxime sodium	Hydrogel	After 4 h, the hydrogel containing 3 percent HPMC 400 had a strong spread ability and had released all of the medication content.	[88]
	Haruan/fusidic acid	Film	Elongation at break and water vapor permeability were acceptable in films containing 1% and 2% plasticizers, respectively.	[89]
	Epigallocatechin-3-gallate	Film	Tensile strength and water vapor barrier properties were improved.	[90]
	Coper nanoparticles	Film	Antibacterial activity that is appropriate.	[91]
	Ibuprofen	Nanocrystals	To make nanosuspensions of several polymers, microfluidization and sonication were used.	[92]
HPMC/ Chitosan/Sodium alginate	Lidocaine chloride and polymyxin B sulphate	Bio membrane	Mechanical parameters (elasticity, tension, stiffness) and thickness are appropriate; in vivo, the material has a strong antibacterial action that aids tissue regeneration.	[93]
HPMC/ PVA/PVP-I/PEG	Aloe vera (2%, 4%, 6%)	Fibers	Fibers containing 6% aloe vera were thinner and lacked beading, resulting in a larger porosity of the fibers.	[94]
HPMC/ Poly(lactic acid)	Tetracycline hydrochloride	Nanofibers	Antimicrobial activity and high-water sorption rate.	[95]
HPMC K100M/	Fluconazole and ofloxacin	Sponge	The gum Odina-HPMC K100M: gelatin (1:1)	[96]

Gum Odina/ Gelatin			formulation cured chronic wounds with good physicochemical qualities and antibacterial activity.	
Na-CMC/ HPMC Citric Acid	Tetracycline Methylene Blue	Hydrogel films	Inhibitory activity against pathogenic microbes S. aureus and E. coli	[97]
HPMC/ AMPS	Loxoprofen sodium	Hydrogel	pH-sensitive hydrogel with controlled drug release, exhibiting intense release in alkaline environment.	[98]
HPMC/ Gelatin	Lamontrigin	Hydrogel	Hydrogel-based material produced using 3D printing for pediatrics.	[99]
HPMC/ alginate	Albumin	Hydrogel	Controlled release of albumin	[100]
HPMC/ PEG	Fibrion, extract of B. mori silkworm cocoons	Film	It possesses stability, rapid swelling, and bioadhesiveness, suitable for transmucosal delivery.	[101]
Hydrofobic HPMC/ alginate	Diclofenac	Capsules	Controlled release of diclofenac	[102]
HPMC	Tramadol Hydrochloride	Film	Hydrogel film, analgesic agent for peripheral neurotherapy.	[103]
HPMC/ co-PAA-co-MAA	Insulin	Hydrogel	Hydrogel, rectal suppositories for regulating blood glucose levels, hypoglycemic effect.	[104]
HPMC/ collagen /sesame oil	Diclofenac	Hydrogel, Emulgel, Bigele	The best properties for a diclofenac carrier are exhibited by a bigele.	[105]

The analysis of studies and data presented in Table 1 shows that the geographic application of HPMC as a carrier of biologically active substances (BASs) is quite extensive. The advantageous properties of HPMC for the creation of pharmaceutical formulations enable the development of soft transdermal systems incorporating biologically active substances (BASs). Among the developed formulations, gel and film forms with HPMC are the most popular [65–80]. The studies on obtaining

capsules, biomembranes, nanocrystals, and nanofibers are particularly interesting. The soft biomaterials developed are proposed as antibacterial, wound-healing, antimicrobial, and anti-inflammatory agents for the treatment of burns of varying degrees, as membranes, sorbent materials, and drug delivery carriers. As the data presented in the table show, HPMC plays an important role in hydrogel formulations, providing gelation, moisture retention, controlled release and prolongation of the action of medicinal components.

In the process of wound healing, plant extracts show activity and advantages due to the content of biologically active substances such as flavonoids, terpenoids, fatty acids, phenolic compounds, alkaloids, catechins, quinones and carbohydrates [106, 107]. The main disadvantage of plant-based biologically active substances is their short duration of action and instability, which can be solved by the creation of hydrogel dressing materials based on natural polymers. Secondary metabolites of plants exhibit antibacterial, anti-inflammatory, antioxidant and regenerative effects. The stages of the physiological process of wound healing include platelet aggregation, the appearance of a hemostatic effect (hemostasis), the formation of macrophages that cleanse damaged tissue with further tissue growth (inflammatory phase), the stage of re-epithelialization and the formation of granulation tissue with the participation of fibroblasts, keratinocytes and endothelial cells (proliferative phase), and the stage of replacing type III collagen with type I collagen with strengthening of the formed tissue (remodeling). Plant extracts participate in stimulating the process of formation of new blood vessels, the flow of nutrients to damaged tissue, the removal of degradation products, and the formation of granulation tissue and collagen.

Among plant extracts, aloe vera [70, 94], henna [83], curcuma [108], Araucaria heterophylla [109], and others are popular in wound treatment and healing. These medicinal plants are used in the preparation of dosage forms with cellulose ethers, and with HPMC for the treatment of wounds and burns with the properties necessary for treatment. For wound healing, the most commonly used compounds are flavonoids—rutin, quercetin, kaempferol, phenolic compounds and terpenoids—tannins, anthocyanins, ellagic acid, and alkaloids—berberine, embelin, etc.

The anti-inflammatory activity of plant extracts is due to the presence of flavonoids (curcumin, quercetin, quercetin-3,7-di-O-α-L-rhamnopyranoside, Epigallocatechin-3-gallate, 5-Hydroxy-7,8-dimethoxyflavanone, 6,7-Dimethoxycoumarin, liquiritigenin, isoflavone, etc.), terpenes (8-o-acetylharpagide, schizanol, stigmasterol, 7-B-hydroxysitosterol, marinoid D, maslinic acid, oleanolic acid, glycyrrhetinic acid etc.), essential oils (eugenol, linalool, camphor, borneol, thymol, citral, menthol, γ-terpinene, thymoquinone, carvone, α-terpineol, etc.), alkaloids (colchicine, sinomenine, capsaicin, berberine), and phenolic compounds (10-Shogaol, 4-hydroxybenzoic acid, cis-mellitoside, trans-mellitoside, salicin, ferulic acid, dihydromellitoside, etc.) [110].

Plant extracts can be used individually or in combination as antibacterial components of medicines with well-known antibacterial drugs, providing a synergistic effect [111]. The antimicrobial properties are due to the presence of

natural compounds such as allicin, piperine, curcumin, eugenol, chlorogenic acid, quercetin, carvacrol, thymol, etc.

The mechanisms of action of plant secondary metabolites (PSMs) on microbial cells include disrupting the structure and function of the bacterial cytoplasmic membrane, preventing complex formation with membrane proteins, inhibiting enzyme synthesis, blocking the synthesis and functions of DNA and RNA, and increasing the coagulation of cytoplasmic components [112]. Antimicrobial action based on the destruction of the membrane of microbial cells is exhibited by flavonoids, terpenes and terpenoids, essential oils. For alkaloids, the literature describes effects on key stages of the pathogenesis process, such as inhibiting the production of staphylococcal α-hemolysin (e.g., capsaicin from *Capsium L.*).

It is important to note that the extraction of plant biologically active substances is carried out through the preparation of decoctions, infusions and alcohol tinctures. In the study [111] of the antibacterial activity of plant extracts using the dual reporter system Dualrep2, it was shown that alcoholic extracts exhibit greater activity compared to aqueous ones, and that alcoholic extracts are better obtained from dried raw materials than from fresh ones.

Medicinal systems based on HPMC and its compositions are obtained both with individual synthetic biologically active substances and on the basis of plant extracts individually and in combination with known drugs. The Aloe vera plant (Aloe barbadensis) is rich in polyphenolic compounds with antioxidant activity and is abundant in vitamins. The anthraquinones and saponins in aloe gel exhibit antimicrobial activity against Gram-positive bacteria such as *Shigella flexneri* and *Streptococcus pyogenes* [113]. The presence of various classes of natural compounds in Aloe vera has enabled its use in an HPMC-based gel as a burn treatment for skin wound healing [87]. Aloe vera gel is an excellent and accessible alternative to antimicrobial agents among plant-based sources with a rich composition of phytometabolites. In the wound-dressing material with Aloe vera gel, HPMC is included into the composite base as the main film-forming and moisture-retaining component [94]. The similarity of the nature of HPMC and the active compounds of Aloe vera, such as polysaccharides (glucomannans), glycoproteins, amino acids, flavonoids, provides the fibrous material with strength and heat resistance due to the formation of bonds between these components. HPMC also facilitates the dispersion of Aloe vera in the polymer mixture, thus ensuring its uniform distribution.

For the treatment of burn infections caused by Gram-positive bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, a composition based on cellulose ether derivatives, extracts of henna (*Lawsonia inermis L.*) and chamomile (*Matricaria chamomilla L.*) was proposed [83]. The antibacterial effect of the composition is due to terpenoids, phenolic compounds of the alcohol extract of chamomile, and the presence of such active components of henna as Lawson (2-hydroxy-1,4-naphthoquinone), tannins. Also, the presence of a powerful antioxidant gallic acid, flavonoids, mucilagin, and mannitol contributes to an important stage,

skin healing, in the treatment of burn wounds. The base of HPMC and CMC provides controlled release of active components by a diffusion mechanism [114].

1.2 *Tamarix Hispida* plant extracts and its biological activities

Plants of the genus Tamarix (comb-shaped) of the family Tamaricaceae (comb-shaped) are a valuable source of various biologically active substances. Polyphenolic, terpenoid and other biologically active compounds were previously isolated and characterized from plants of this genus. Extracts and individual components showed antioxidant, antiamnesic, antimicrobial, antifungal and cytotoxic activity [115-117] (Figure 5).



Figure 5 - *Tamarix Hispida* plant

It is known that extraction of biologically active substances from plant raw materials is one of the longest stages of its processing. Extraction is usually carried out by labor-intensive and time-consuming methods such as percolation, maceration and Soxhlet extraction. One of the most innovative methods of intensifying the extraction of plant components, along with vibration-cavitation, two-phase, supercritical fluid CO2 extraction, is ultrasound [118, 119]. The use of ultrasound allows not only to significantly speed up the production process, but also to increase the extraction of the main product compared to other methods. There is evidence that ultrasonic extraction processes can be used to improve processing in the food

industry, including the production of biocomponents from plant and animal materials [120, 121]. Recently, there has been an increasing number of studies using ultrasonic extraction from plants. Thus, ultrasound is used to extract flavonoids from *Herba Epimedii* [122], hawthorn fruits [123], green mass of buckwheat [124]; terpenoids from *Loquat* leaves (Japanese medlar) [125]; polyphenols of walnuts [126], meadow mint, sage, savory, hyssop [127]; anthocyanins of mulberry [128]. The authors Sharma and Gupta [129] used this method to isolate oils from almond, apricot and *Jatropha curcas L*. In this regard, the use of ultrasonic extraction method for extracting components from plant materials is very relevant.

In turn, medicinal plants containing a wide range of biologically active substances, such as flavonoids, triterpenoids, tannins, help to reduce blood glucose levels [130], which contributes to the search for and development of new effective, low-toxic means for the treatment of diabetes among plant objects.

Currently, considerable interest is directed to obtaining new and more advanced means for healing and soothing burns of wounds on a polymer basis in the form of a soft dosage form with the *Tamarix Hispida* plant. This is mainly due to the unique antimicrobial and antioxidant properties of the medicinal plant [131] and the great interest in polymer hydrogels today [51].

Currently, many discoveries have been made in the field of medicine. Polymer hydrogels with active medicinal substances have become an evolutionary approach to the treatment of wounds. Thanks to the healing active substance in the composition, they were able to accelerate the healing of deep wounds after operations or bruises. Various alternative substances, including medicinal plants, can be used in such a polymer hydrogel. One of the most affordable and effective wound healing and analgesic plants is *Tamarix*.

The *Tamarix* plant has unique properties that help heal wounds and ease the pain when receiving fresh wounds. The Tamarix plant exhibits antibacterial and antimicrobial properties that stimulate the rapid healing of open wounds. At this time, the antioxidant property is one of the very popular properties in the preparation of medicines. The Tamarix plant is known in the circles of botanists under the name "salt cedar". This halophytic plant belongs to the Tamaricaceae family. At the moment there are more than 60 species of this type of plant growing in many parts of the world. This plant has leaves in the form of needles covered with salt since the needles have the properties to secrete salt with salt glands [27]. Tamarix plants grow in many places, regardless of the climate. Although this plant can withstand very high air temperatures, so they are very often found in large quantities in areas of arid climates [132]. The species of this plant are specially cultivated in hot arid places in order to reduce the number of sand dunes [78]. At the same time, it is undesirable to grow this type of plant in places of high humidity, as it can have a bad effect on the growth of other plants [133].

In a number of different studies in the field of phytochemistry, important conclusions have been made those plants of the Tamaricaceae family contain very important biochemical substances, such as polyphenolic compounds, tannins, phenolic acids and others. In many countries of the Middle East, this plant is grown

naturally, and the plant in these countries is used for medicinal purposes in everyday life. These countries include Pakistan, Algeria, India and others [27].

Based on well-known works in the study of photochemical properties, it was proved that polyphenolic substances have an anti-inflammatory, antitumor, antibacterial character. Polyphenols are divided into such categories as phenolic acids, flavonoids, terpenes and others. At the same time, as for flavonoids, they are divided into subgroups (Figure 6). Polyphenols are widely used in human food [78].

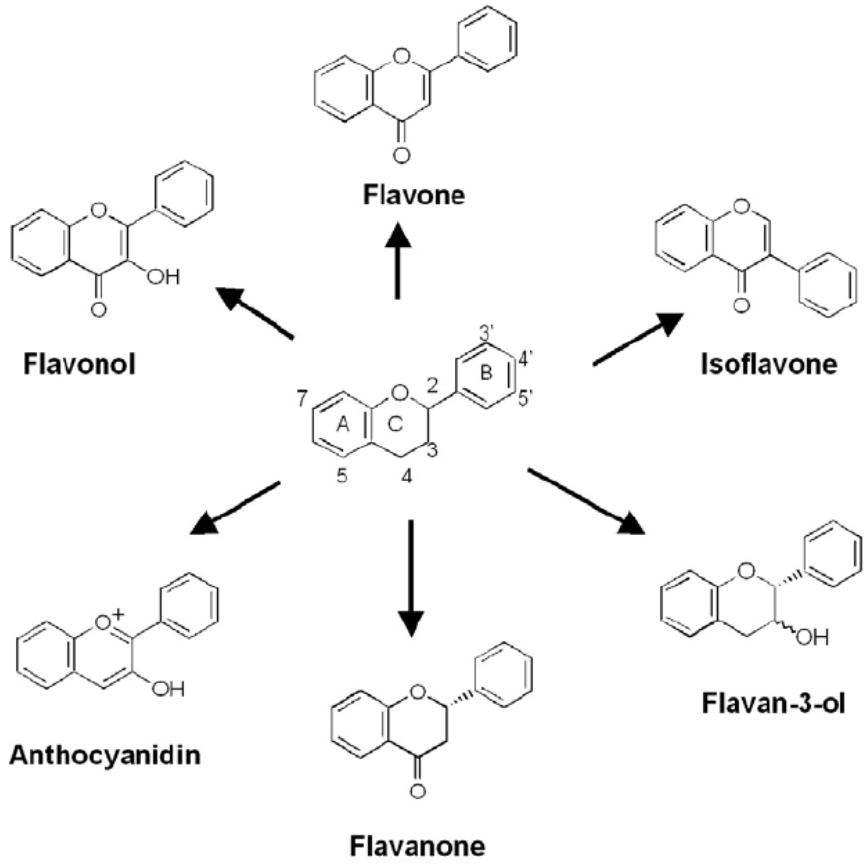


Figure 6 - Subgroups of polyphenols

Tannins are a subclass of phenolic chemicals that exhibit astringent wound-healing qualities. Tannins have active properties to bind to proteins, as they can form strong bonds between functional groups. The basic types of substances included in the tannins include gallic acid and its derivatives, as well as ellagic acid and its derivatives. In addition, there are also proanthocyanides and condensed tannins [132, 134].

Terpenes act as secondary metabolites of plants, including one or more isoprene subunits. Low molecular weight terpenes, which include monoterpenes, diterpenes and monoterpenes, are present mainly in essential oil plants and are responsible for their aroma. High-molecular triterpenes, since they are usually non-volatile and are isolated primarily with the use of nonpolar solvents [135]. Phenolic acids are organic acids containing phenolic functional groups (Figure 7) [115].

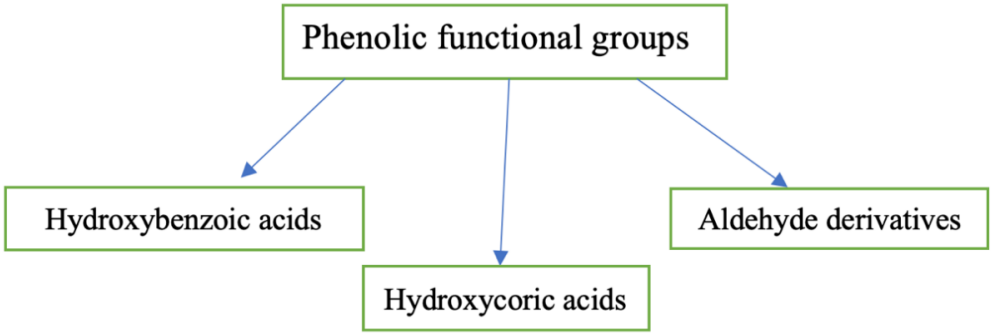


Figure 7 - Classes of phenolic functional groups

Some phenolic acids have been found in plants of the Tamaricaceae family. Phenolic acids are found in all parts of this plant, but a large number of phenolic acids falls on the lower parts of the plant in the stem area. The medicinal properties of the plant are based on the fact that phenolic acids have very good healing properties. Phenolic acids include acids: ellagic, ferulic, gallic and others.

The other phytochemicals that occur in the composition of the Tamarix plant are alcohols, aliphatic hydrocarbons, ketones, and aldehydes. All these substances are the basis of essential oils [136].

This herb has historically been used to cure a variety of ailments and wounds [134]. The extract of leaves and stems (biologically active compound) contains anti-inflammatory effects that are essential in the healing of burn wounds. Algerian scientists have researched to confirm the antioxidant and antibacterial properties of extracts of Tamarix plant leaves and flowers, as well as their phenolic composition. As a consequence, the flowers were shown to have more antioxidant effects than the Tamarix leaves. Simultaneously moment, the therapeutic plant's stems contain high concentrations of phenol.

1.3 Composite polymer materials with the addition of clay minerals and cellulose ethers

To improve the properties of natural polymers, particularly cellulose derivatives, composite materials are often developed in which a mineral component serves as a filler. Bentonite clay has proven to be an effective property enhancer in composite formulations, as it is compatible, also swells in water, and possesses gel-forming ability.

Clays are formed in nature under the influence of atmospheric factors that act on silicates, transforming them into aluminosilicate compounds with a characteristic layered structure. Therefore, clays have a plate-like structural organization. Depending on the arrangement of tetrahedral and octahedral layers, clays are classified into three main groups and are designated as: 1:1, 2:1, and 2:1:1 [137] (Table 2).

Among these groups of clays, smectites exhibit a high degree of swelling. This is due to the fact that their three -layer structure has a negative charge that occurs as

a result of isomorphic replacement in a crystal lattice. Such a charge is formed due to the substitution of trivalent elements (Al^{3+}, Fe^{3+}) with divalent ones (Al^{2+}, Fe^{2+}) in the octahedral layer, as well as the substitution of tetravalent silicon (Si^{4+}) with trivalent aluminum (Al^{3+}) in the tetrahedral layer.

Table 2 – Main groups of clays

Title	Examples	Structural features
1:1	Kaolinite Halloysite	One layer of tetrahedrons alternates with one layer of octahedrons
2:1	Smectites (montmorillonite, laponite, hectorite, etc.), vermiculites, talc-pyrophyllites, mica	One layer of octahedra is sandwiched between two layers of tetrahedrons
2:1:1	Cloisitis	Two tetrahedral layers and one octahedral layer have an additional octahedral layer.

This exchange process can occur simultaneously in both layers, leading to the formation of an interlayer negative charge. To compensate for this charge, the interlayer space contains mono-, divalent and trivalent cations, which, binding with water molecules, form hydrate shells. The presence of these hydration layers determines the swelling capacity of smectites. Alkali metals, such as sodium, exhibit the highest hydration ability, which is particularly important in the development of nanocomposite materials, where a controlled degree of swelling plays a key role in forming a porous structure and enabling controlled drug release. Montmorillonite, one of the representatives of smectite clays, has a high swelling capacity due to the presence of sodium as the primary exchangeable cation. Clay containing at least 70% montmorillonite mineral is referred to as bentonite [138, 139]. Another advantage of bentonite is its antibacterial activity against various types of bacteria [140]. In study [141], the process of obtaining nanocomposite films based on hydroxyethyl cellulose (HEC) and bentonite was examined, with a focus on their structural and functional properties. The composite materials were formed by dispersing bentonite in a polymer solution, which ensured uniform distribution of the mineral filler within the matrix. According to the results of X-ray diffraction analysis, the introduction of bentonite led to an increase in the interlayer spacing within its structure, indicating the formation of an intercalated nanocomposite. The addition of bentonite significantly improved the mechanical properties of the films, including tensile strength and elasticity, and also enhanced their resistance to swelling. Moreover, it was found that the resulting composites exhibit fungistatic activity against *Candida*

albicans, which broadens their potential areas of application. The obtained results confirm that the incorporation of bentonite into the composition of polymer matrices that do not have their own antimicrobial activity can significantly improve the physicochemical properties of materials and provide them with additional functions. Nanocomposite films based on chitosan and bentonite were also developed for use in wound therapy. The films were synthesized by solution casting, which ensured uniform distribution of the components in the polymer matrix. The interaction of negatively charged bentonite and positively charged chitosan was confirmed by IR Fourier spectroscopy, and their morphology was studied using scanning electron microscopy. Physicochemical studies have shown that the addition of bentonite significantly increases mechanical strength and moisture absorption capacity, which is an important property for wound dressings. The presence of bentonite also improved the flexibility of the material, increasing its resistance to repeated bending. Furthermore, studies on antibacterial activity demonstrated a pronounced inhibitory effect of the nanocomposite films against both Gram-positive and Gram-negative bacteria, confirming their potential effectiveness in infection prevention [142].

Large natural deposits of sodium bentonites in Kazakhstan are represented by the Manrak deposit in the east, the Kyzyl-Zhar deposit in the west, and the Kyngrak deposit in the south of the country [140]. The widespread distribution of bentonite clays across Kazakhstan creates favorable conditions for the development of research in the field of composite materials based on them. Domestic researchers have studied composite hydrogels based on sodium carboxymethyl cellulose (NaCMC) and bentonite, aiming to improve their mechanical and sorption properties. For instance, in study [143], hydrogels were obtained at various weight ratios of NaCMC to bentonite (75:25, 50:50, 25:75). Swelling analysis showed that the swelling degree of clay in NaCMC solutions reaches its maximum at a low polysaccharide concentration (0.1%). However, with a further increase in concentration (0.3%), the swelling decreases by nearly half, which is associated with particle aggregation. Electrophoresis studies confirmed the stability of the complexes due to hydrogen bonding, while scanning electron microscopy (SEM) revealed the formation of homogeneous structures. Additionally, the sorption and release of the local anesthetic Rihlokaine were studied. It was found that sorption reaches 75–95% within 3–4 hours, while the desorption process continues for up to 24 hours, indicating gradual and prolonged release of the local anesthetic. Thus, the results of this study confirm the potential of developing composite materials based on NaCMC and bentonite with enhanced mechanical and sorption properties, making them promising candidates for use as wound dressings and sorbent materials for biomedical applications.

It is important to note that cellulose derivatives and clay minerals are environmentally friendly, natural, and readily available components of multifunctional materials. In recent years, clay-based composite carriers [144–150] have been developed for nanoparticles, enzymes, proteins, and pharmaceutical substances for use in various fields of medicine. The limited number of existing studies on the development of composite materials based on cellulose ethers and

bentonite clay offers researchers a wide field for further investigation. The properties of these materials themselves demonstrate promising potential for the creation of wound dressings and carriers for biologically active substances.

Conclusion of the literature review

The main types of hydrogels of cellulose ethers and their methods of synthesizing, classification, uses and chemical properties of hydrogel forms with BAS are reviewed in this article. Hydrogels of cellulose ethers have become a popular material for thorough research and practical use in a variety of fields due to their capacity to absorb and hold large amounts of liquid and due to its economical availability. Cellulose-based products are the most eco-friendly and biodegradable; thus, as hydrogels, when they reach the aquatic environment, they have the unique ability to maintain a three-dimensional structure. Hydroxypropyl methylcellulose is a good material for drug transfer as tablets, ointments, gels, films with prolonged effect.

In the therapeutic treatment of wounds of various etiologies, antibacterial, anti-inflammatory and antioxidant effects are important for the active principle, which can be characteristic of both individual synthetic drugs and the BAC of plant extracts. In recent years, there has been a trend to search for natural sources of biologically active substances that meet the requirements for wound treatment and can be a worthy alternative to well-known drugs, especially antimicrobial agents, to which resistance has already developed. The advantage of using plant extracts is that phytocomponents can act at several stages in the wound-healing process, from hemostasis to remodeling, which is possible due to phytometabolites such as flavonoids, terpenes, tannins, polysaccharides, terpenoids, phenolic compounds, alkaloids, essential oils, saponins. The similarity in nature and structure between phytometabolites and cellulose ether derivatives makes them promising for the development of biocompatible, biodegradable, effective, and controlled wound dressings, gels, films, fibers, and membranes.

To sum up, hydrogels of hydroxypropyl methylcellulose with plant extracts with biological active substances are of great interest to researchers as regards biomedical applications in the form of ointments, gels, and films for local antibacterial, antioxidant activities. A review of the literature showed that there is currently insufficient research on the biodegradation of drug delivery systems based on cellulose derivatives with biologically active substances, which is a broad field for further research.

2 EXPERIMENTAL PART

2.1 Characteristics of the Starting Materials

Tamarix Hispida– the main plant raw material used in this research was the aerial part of *Tamarix Hispida*. Most of the material was collected between August and September in the years 2020 to 2022. Prior to use in the study, the plant material was dried and ground into powder. The coordinates of the collection and growth site are located in the Almaty region (N=43.588006°, E=77.884779°).

Ethanol was obtained from the company "Talgar-Spirt" and used without further purification.

Hydroxypropylmethylcellulose (HPMC) with a viscosity of 2,600–5,600 cP, 2% in H_2O at 20°C (CAS No. 9004-65-3), molecular weight: approximately 86 kDa, HPMC has a methoxy content of 28-30% and a hydroxypropoxyl content of 7-12% (Figure 8).

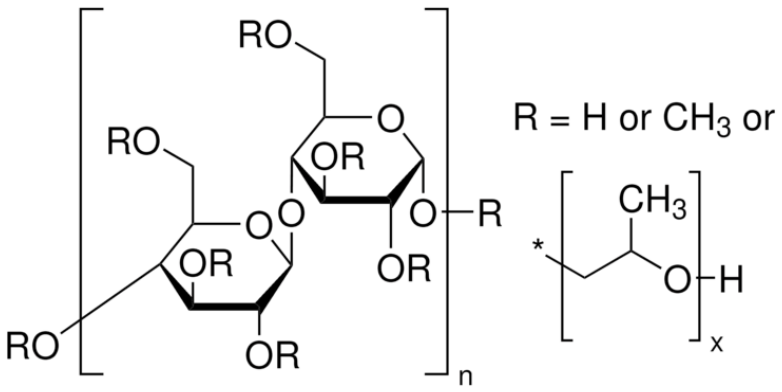


Figure 8 – Hydroxypropylmethylcellulose (HPMC) with a viscosity of 2,600–5,600 cP.

Potassium sorbate ≥99% (CAS No. 24634-61-5), and Tween-80, d = 1.064 g/cm³ (POE (20) sorbitan monooleate, polyethylene glycol sorbitan monooleate, polysorbate 80) (CAS No. 9005-65-6) were purchased from Sigma-Aldrich and used without further purification.

Water was purified using a purification system.

Methanol for HPLC-Gold Ultra gradient grade, dichloromethane, n-hexane, and chloroform for HPLC-Isocratic grade >95% were purchased from Carlo Erba Reagents.

All the reagents used in the study were of analytical grade and were obtained from various sources. 2,2-diphenyl-1-picrylhydrazil (DPPH, Sigma) (99%), gallic acid (99%, Sigma), indomethacin, DMSO.

Bentonite clay was purified by repeated sedimentation in distilled water [151]. The montmorillonite content of the clay exceeds 70%. The chemical composition of the raw and purified clay is presented in Figure 9.

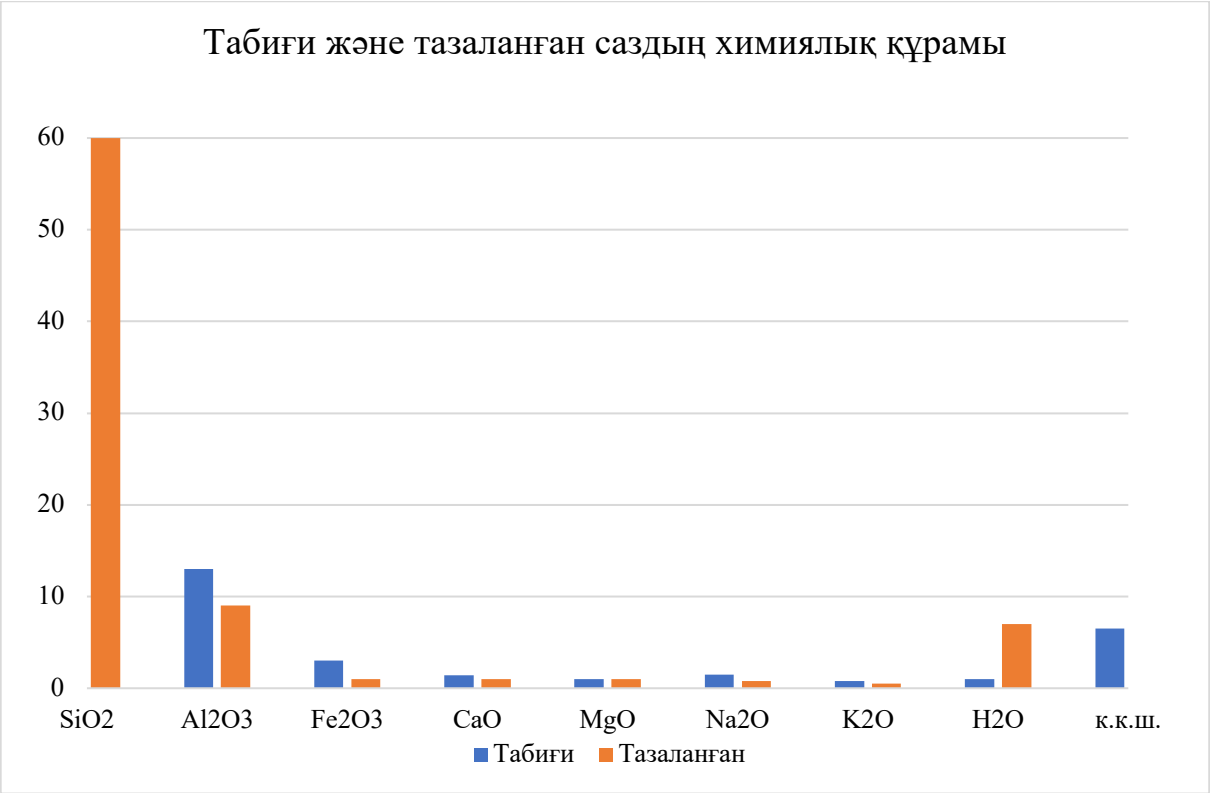


Figure 9 – The chemical composition of the raw and purified clay

Optical microscopy analysis was conducted at the National Nanotechnology Laboratory (NNL), located at the Faculty of Physics and Technology of Al-Farabi Kazakh National University, using a Leica DM 6000 M microscope.

SEM analysis was carried out at the National Nanotechnology Laboratory (NNL), located at the Faculty of Physics and Technology of Al-Farabi Kazakh National University. This analysis allows the determination of the size, shape, and structure of nanoparticles. The work was performed using a Quanta 200i 3D microscope (FEI Company, USA).

2.2 Isolation of 10%, 50%, and 70% aqueous-alcoholic extracts from *Tamarix Hispida* plant

The plant material, specifically the aerial parts (leaves and stems) of *Tamarix Hispida Willd.*, was collected during the flowering phase in the Almaty region of the Republic of Kazakhstan. Herbarium specimens are stored at the Plant Resources Laboratory of the Institute of Botany and Phytointroduction, Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan (Almaty).

The raw material was dried to an air-dry state at room temperature without exposure to sunlight and stored in a cool, dry place. The dried plant material was then ground to a particle size of 1 mm.

Extraction procedure: 200 g of dried and ground plant material was treated with an extracting agent under varying extraction parameters. The obtained extracts were concentrated to dryness using a rotary evaporator, and the yield of extractive

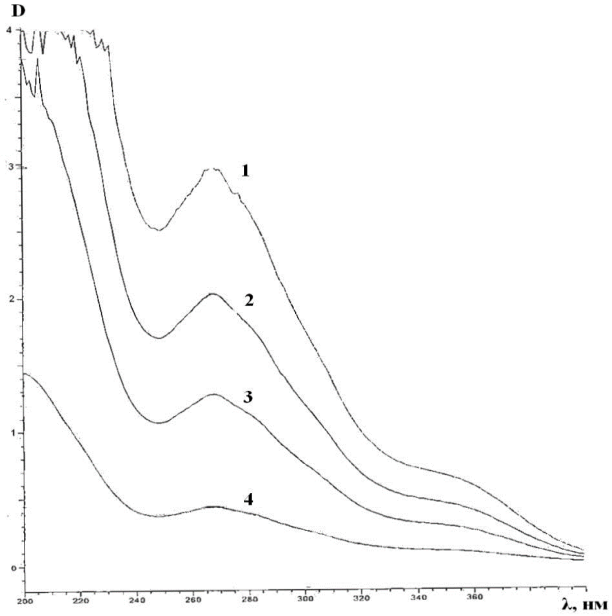
substances was determined. Extraction was performed using two different ultrasonic baths: “Ultrasonik” (20 kHz) and “PSB-Galb” (35 kHz) [25].

2.3 Determination of the qualitative and quantitative composition of extracts from the aerial parts of the *Tamarix Hispida* plant

2.3.1 Qualitative composition of the extracts from the aerial parts of the *Tamarix Hispida* (THU-10) plant

UV spectra of the dry substance in the concentration range from 0.1% to 0.7% were recorded using a Shimadzu UV-1700 PharmaSpec spectrophotometer (Shimadzu, Japan) in the wavelength range of 200 to 500 nm.

The UV spectroscopy method was used to obtain quantitative data on *Tamarixidin*. UV spectra were recorded for aqueous solutions of *Tamarix Hispida* extract at various concentrations. The wavelengths corresponding to functional groups in the solution and the optical density for each concentration were determined (Figure 10-11) [25].



(1) [THU-10] – 0,7%; (2) [THU-10] – 0,5%; (3) [THU-10] - 0.3%; (4) [THU-10] – 0,1%

Figure 10 – UV spectra of aqueous solutions of the extract of *Tamarix Hispida* plant

For quantitative determination, it was necessary to construct a calibration graph of the dependence of optical density on the concentration of the aqueous solution of the extract (THU-10). UV spectra were recorded on an Agilent Cary 60 spectrophotometer (USA) at a wavelength of 268 nm. This wavelength is typical for gallic acid and its derivatives. To construct a calibration graph, it was necessary to

prepare aqueous solutions of the following concentrations in advance: 0.1%; 0.3%; 0.5% and 0.7%.

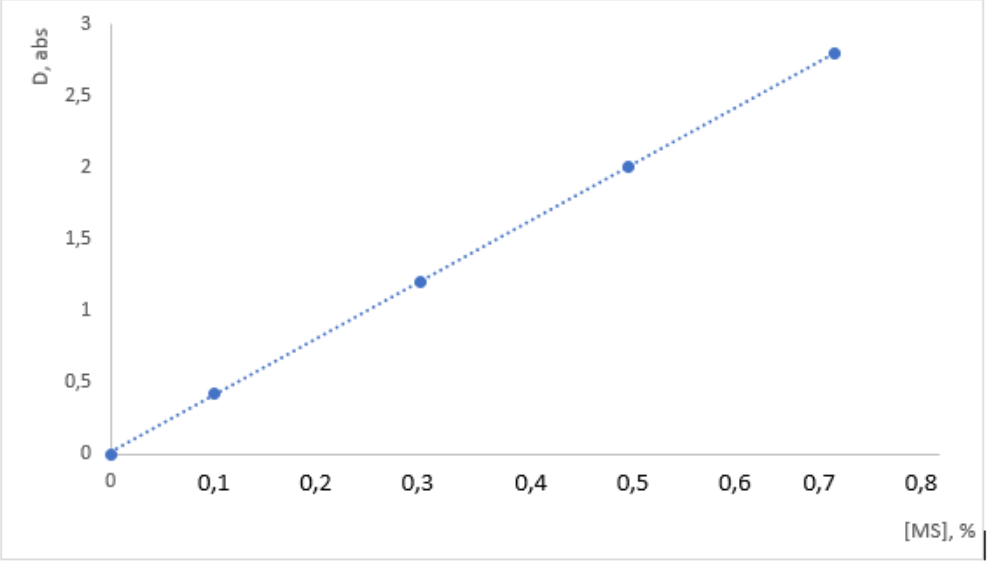


Figure 11 - Calibration curve for the determination of the extract of *Tamarix Hispida* plant

2.3.1.1 Determination of the amount of extractive substances

Extractivity of plant raw material refers to the quantitatively determined dry residue remaining after the extraction of organic and inorganic substances contained in the plant raw material using appropriate solvents [152].

For the analysis, 5 g of ground plant material (particle size up to 1 mm) is placed in a conical flask, 25 mL of solvent is added, the flask is sealed with a stopper, weighed (with an accuracy of ±0.01 g), and left to infuse for 1 hour. Extraction is then carried out under a reflux condenser by slow boiling for 2 hours. After cooling, the flask is reweighed, the loss is replenished with physiological saline, shaken, and filtered into a volumetric flask (150–200 mL). From the filtrate, 15 mL is taken, evaporated in a porcelain dish on a water bath, cooled in a desiccator, and the dry residue is weighed.

The content of extractive substances is calculated using the formula [153]:

$$X=\frac{m\cdot V}{m_0\cdot \vartheta} \quad (1)$$

where:
X – content of extractive substances, %;
m – dry residue mass, g;
V – total volume of filtrate, ml;
ϑ – volume of collected filtrate, ml;
m_0 – mass of raw material sample, g.

2.3.1.2 Determination of raw material moisture content

Humidity is the content of colloidal liquid remaining in the tissues of plant raw materials after drying to a constant mass. It serves as a quantitative indicator of quality and should not exceed 12-15%, since excess moisture reduces the shelf life of the raw materials.

For analysis, 3–5 g of crushed raw material is placed in a weighing bottle and dried at a temperature of 100 – 105°C to a constant mass, periodically cooling in a desiccator and weighing. Constant mass is considered at a difference between weightings of ≤0.1 g.

Moisture content (W, %) is calculated using the following formula:

$$X = \frac{(A - B) \times 100}{A} \quad (2)$$

where:

A – mass of raw material before drying, g;

B – mass of raw material after drying, g [153].

2.3.1.3 Determination of ash content of raw materials

Ash content of plant materials is an indicator of the content of inorganic substances remaining after combustion of the organic part.

To determine 5 g of crushed raw material is placed in a crucible, charred on a hot plate, then pierced in a muffle furnace at 550 - 560 ° C until constant weight. After cooling in a desiccator, the crucible is weighed.

Ash content (X, %) is calculated using the following formula:

$$X = \frac{M_2 \times 100 \times 100}{M_1 \times (100 - W)} \quad (3)$$

where:

M_2 – Mass of ash, g;

M_1 – Mass of raw material, g;

W – Mass lost during drying of the raw material, % [154].

2.3.1.4 Determination of tannins in raw material by the permanganate method

For analysis, 2 g of powdered plant material is placed in a 125 ml conical flask. 65 ml of boiling distilled water is added to the flask and a reflux condenser is attached. The mixture is boiled in a water bath with periodic stirring for 30 minutes.

The resulting extract is cooled to room temperature and filtered through a cotton filter. Then 62 ml of the filtrate is pipetted into a 250 ml conical flask, 125 ml of water and 6.25 ml of indigo carmine solution are added. The resulting mixture is

titrated with a 0.02 mol/l potassium permanganate solution until a yellow-green color appears.

Two control determinations are carried out in parallel. According to the method, 1 ml of potassium permanganate solution corresponds to 0.004157 g of tannins in terms of tannin [152].

The mass fraction of tannins (T, %) in raw materials is calculated using the following formula:

$$X = \frac{V \times 0.004157 \times 100}{m} \quad (4)$$

where:

V – volume of potassium permanganate solution used for titration (0.02 mol/l), ml;

m – mass of plant material sample, g;

0,004157 – the number of tannins in g corresponding to 1 ml of potassium permanganate solution.

2.3.1.5 Quantitative determination of flavonoids in dry extract

The mass of 1 g of crushed raw material (up to 1 mm) is poured with 30 ml of 1% HCl in 90% ethanol, heated for 30 min in a water bath under a reflux condenser. After cooling, it is filtered into a measuring flask (100 ml), the extraction is repeated 3 times. The filtrate is brought to the mark with 90% ethanol - this is solution A.

From solution A, 2 mL is taken, 1 mL of 1% $AlCl_3$ (in 95% ethanol) is added, and the volume is adjusted to 25 mL with the same solvent. After 20 minutes, the optical density is measured at 430 nm. The control is solution A without $AlCl_3$.

The flavonoid content (X, %) calculated as quercetin equivalent is determined using the following formula:

$$X = \frac{D \cdot a \cdot V \cdot 100}{D_0 \cdot m \cdot V_1} \quad (5)$$

Where:

D – optical density of the test solution;

D_0 – optical density of standard quercetin solution;

a – quercetin content in standard solution, mg/ml;

V – total volume of solution A, ml;

V_1 – total volume of solution A taken for analysis, ml;

m – mass of absolutely dry raw material sample, g.

2.3.2 Determination of the chemical composition of the plant extract *Tamarix Hispida* (TH-10) by the liquid chromatography mass spectrometric method (LCMS)

The chemical composition of the plant extract Tamarix Hispida (THU-10) is determined by liquid chromatograph mass spectrometry (LC-MS).

2.3.2.1 Determination of the phenolic composition of the extracts

Ethanol-water extracts of the Tamarix Hispida extracts (8 mg) were diluted in the methanol. After filtration through 0.45 µm PTFE filters, 20 µl of the sample was injected into the HPLC-DAD. The phenolic components of the extracts were analyzed using HPLC-DAD according to the method of Tokul-Olmez et al. (2020). The phenolic composition of the ethanol-water extracts of the plant determined at 254 nm at Inertsil ODS-3 column (4 µm, 4 mm x 150 mm). Mobile phase was 0.1% acetic acid-methanol (gradient elution). The flow rate was 1mL/min. The injection volume was 20 µL. A diode array detector (DAD) was used to monitor phenolic compounds at a wavelength of 254 nm [154].

2.3.3 VLC fractionation of THU-10 extract

The plant extract of Tamarix Hispida (125 g) was subjected to vacuum liquid chromatography (VLC) using silica gel 60 PF 254 (187 g) as the stationary phase and eluted using a gradient elution system with increasing polarity including n-hexane, DCM (100%) DCM/methanol (85:15, 50:50 by volume), methanol (100%).

2.4 Determination of biological activity of *Tamarix Hispida* extract (THU-10)

The biological activities were investigated in the laboratories of the H.E.J. (Husein Ebrahim Jamal) Research institute of chemistry of the International Center for Chemical and Biological Sciences (ICCBS) of the University of Karachi, Karachi, Pakistan.

2.4.1 Anti-inflammatory activity

2.4.1.1 Preparation of blood samples for membrane stabilization assays

In vitro anti-inflammatory activity of *Tamarix Hispida* extract (THU-10) was evaluated by human red blood cell (HRBC) membrane stabilization method [157].

The human red blood cell (HRBC) membrane stabilization method has been used as a method to study the in vitro anti-inflammatory activity. The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.5 % citric acid and 0.42 % NaCl). All the blood samples were stored at 4 °C for 24 h before use. It was centrifuged at 2500 rpm for 5 min and the supernatant was removed. The cell suspension was washed with sterile saline solution (0.9 % w/v NaCl) and centrifuged at 2500 rpm for 5 min. This was repeated three times till the supernatant was clear and colorless and the packed cell

volume was measured. The cellular component was reconstituted to a 40 % suspension (v/v) with phosphate buffered saline (10 mM, pH 7.4) and was used in the assays.

2.4.1.2 Hypotonicity-induced haemolysis

CCE and CHF of *C. cujete* leavesand bark were prepared (100 and 1000 µg/ml), respectively using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of HRBC suspension were added. It was incubated at 37 °C for 30 min and centrifuged at 3000 rpm for 20 min. The hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Aspirin (100 µg/ml) was used as reference standard and a control was prepared by omitting the extracts. The percentage inhibition of haemolysis or membrane stabilization was calculated according to modified method described by Shinde et al. [158].

$$\% \text{ Inhibition of haemolysis} = 100 \times (OD1 - OD2/OD1)$$

where:
OD1 = Optical density of hypotonic-buffered saline solution alone.
OD2 = Optical density of test sample in hypotonic solution.

2.4.2 Antioxidant activity studies (DPPH assay)

DPPH quenching ability of ethanolic extract (THU-10) was evaluated in order to scavenge the 2,2-diphenyl-1-picrylhydrazil free radical. 1 mL of the extract at known concentration was added to 0,25 ml of methanolic solution DPPH. The mixture was placed at 37°C for 30 min in the dark, the ability of the extracts to decrease the stable radical DPPH to the yellow-colored was assessed before evaluating the absorbance at high-quality monochromator-based UV-VIS spectrophotometer (Multiskan GO) at 517 nm, and the ability of extracts to decrease the stable radical DPPH to the yellow-colored DPPH was also measured. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect } \% = \frac{(A_0 - A_1)}{A_0} \times 100 \qquad (6)$$

where:
A_0 – optical density of control solution DPPH (without extract).
A_1 – optical density of solution with extract.

The analysis of all the samples were done in triplicate. A lower IC_{50} value showed the higher antioxidant activity of the plant extract. The antioxidant activity of the studied sample THU-10 was compared with reference antioxidants – quercetin and gallic acid.

2.4.3 Cytotoxicity studies

For cytotoxicity brine shrimp: Artemia Salina (shrimp's eggs), Sea Salt (38 g/L of D/W, pH 7.4), Hatching tray with perforated partition, Lamp to attract brine-shrimp larvae, Micro pipette (5, 50, 500 µl), Vials tray, 9 vials samples Organic solvents (Methanol/Ethanol/Acetone/water), Dist.water.

Cytotoxixicity of the *Tamarix Hispida* ethanol-water extracts (10, 50, 70%) was evaluated by brine shrimp lethality assay.

Bioactive compounds are often toxic to *Artemia salina* (Leach) shrimp larvae. The eggs of the brine shrimp *Artemia salina* are readily available as fish food in pet shops. When placed in artificial seawater, the eggs hatch within 48 hours, providing large numbers of larvae. This is a rapid, inexpensive, general bioassay, which has been developed for screening, fractionation and monitoring of physiologically active natural products.

B-**Hatching Techniques**

Half-filled the hatching tray (a rectangular dish (22x32 cm)) with filtered brine solution then sprinkle (50 mg) eggs of brine shrimp. Incubate at 37º C.

C- **Sample Preparation**

Dissolve test sample (20mg) in 2ml of respective solvent and from this solution transfer 5, 50 and 500 µl to vials (3vials/ concentration). The concentration will be 10, 100 and 1000 µg/ml respectively. Allow the solvent to evaporate overnight. After 2-days of hatching and maturation as nauplii, place 10 larvae/ vials, using a Pasteur pipette. Make the volume to 5 ml with seawater. Incubate at 25-27º C for 24 hrs. under illumination. Supplement other vials with solvent, and reference cytotoxic drug serving as negative and positive controls, respectively. Analyze data with Finney computer program to determine LD_{50} values with 95% confidence intervals. Shrimp can be used 48-72 hours after the initiation of hatching. After 72 hours they should be discarded [159-163].

2.4.4 Animal studies

Conditions for conducting experiments on laboratory animals.

The study was conducted on mature male rats weighing 180–220 g, raised in the vivarium of the Center for Molecular Biology and Diagnostics (PCMD, ICCBS). The animals were kept in a room with controlled environmental parameters: the air temperature was maintained at 23±1°C, with a change in light and dark cycles every 12 hours.

The animals were not fed for 24 hours before the experiment but had free access to water. During the experiment, all animals were provided with a standard diet. All manipulations with animals were carried out in accordance with current ethical standards for the treatment of laboratory animals.

(A) Carrageenan-induced paw edema model in rats

Edema was induced by injecting 100 µl of a 1% suspension of lambda-carrageenan in saline into the plantar region of the right hind paw of each rat.

Tamarix Hispida plant extract (THU-10) at doses of 200 and 400 mg/kg, as well as indomethacin (5 mg/kg, as a standard drug) were administered intraperitoneally 30 minutes before carrageenan injection.

Hind paw volume was measured using a plethysmometer before carrageenan administration and at 1, 2, 3, 4 and 5 hours after it.

The degree of edema was assessed by the difference:

$$\Delta V = a - b \quad (7)$$

where,

a – paw volume after carrageenan administration;

b – initial paw volume before carrageenan administration.

2.5 Synthesis of hydrogels based on hydroxypropylmethylcellulose

During the work, hydrogel ointments based on hypromellose were obtained by dissolving HPMC in water using the "hot/cold" method. The polymer was dispersed in two-thirds of the required amount of hot water [114]. The temperature of the hot water was 80 °C. Then, the remaining amount of cold water was added under magnetic stirring until a clear, viscous solution was formed (Figure 12).

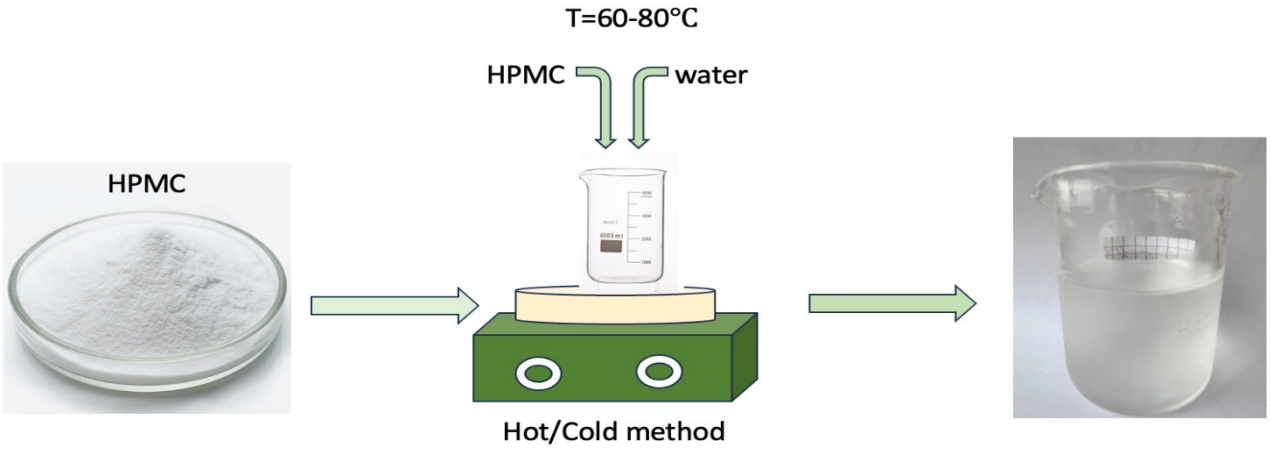


Figure 12 – Method for obtaining hydrogels based on HPMC with different concentrations

Before further use, the solution was left at a temperature of 5°C for at least 24 hours. The “HPMC solution” had a concentration of 2, 3, 4, 5, 7%.

2.5.1 Determination of the degree of swelling of the gel

The degree of swelling of the gels was determined by the gravimetric method of equilibrium swelling and calculated based on the ratio of the mass of the swollen sample to the mass of the dry sample using the formula:

$$\alpha = \frac{m - m_0}{m_0}, \quad (7)$$

where,

m - mass of swollen sample, g;
m_0 - dry sample weight, g;
α - swelling degree, g/g.

2.5.2 Release kinetics of THU-10 extract from hydrogels

To study the release kinetics of the THU-10 extract, samples of the obtained polymer gels containing the aqueous-alcoholic extract in certain concentrations were pre-dried. A 0.01 g portion of the dried sample was placed in 50 ml of distilled water or physiological solution.

At specified time intervals, 5 ml samples were taken from the outer glass vessel and the optical density of the active substance under study was determined using an Agilent Cary 60 UV-Vis spectrophotometer.

Based on the obtained optical density values and a previously constructed calibration graph showing the dependence of optical density on the concentration of THU-10, the amount of drug released from the polymer gel into an aqueous or physiological environment was determined.

The amount of substance released over time τ was calculated using the following formula:

$$W = \frac{n_{\text{in solution}}}{n_{\text{in gel}}} \quad (8)$$

where,
W – the amount of THU-10 released from the gel over time, %;
$n_{\text{in solution}}$ – concentration of TH-10 ions in solution over time τ, mg/ml;
$n_{\text{in gel}}$ – ionic concentration of THU-10 in gel.

2.5.3 Determination of rheological properties

The rheological properties of the obtained gels and ointments with different THU-10 contents were determined using a modular compact rheometer Anton Paar 102 (Austria). Measuring system PP25 (d=24,981). Measuring cell – P-ETP AIR 200.

The calculation of dynamic viscosity was carried out using the formula:

$$\eta = \tau/\Upsilon \cdot 100, \quad (9)$$

where,

n – dynamic viscosity, Pa*s;
τ – shear stress, Pa;
ϒ – shear rate, s^{-1}.

The degree of clay swelling was determined by the volumetric method based on the ratio of the volume of the swollen sample to the volume of the dry sample.

The morphology and structure of bentonite clay and compositions with THU-10 were studied using cryo-scanning electron microscopy images on an S-4800 Hitachi electron microscope (Japan).

3 RESULTS AND DISCUSSION

3.1 Tamarix Hispida Plant and Its Indicators, Authenticity

Pharmaceutical analysis is a section of pharmaceutical chemistry, the main task of which is to assess the quality of drugs at all stages of their life cycle - from development to use and disposal of low-quality drugs. An integral part of pharmaceutical analysis is pharmacopeial control, which includes a set of methods for studying medicinal substances and forms approved in the State Pharmacopoeia and regulatory documentation used to assess their quality. In accordance with the methods of the State Pharmacopoeia, studies were conducted on the qualitative characteristics of plant materials, including the determination of moisture, ash content and content of extractive substances.

3.1.1 Determination of the quantitative content of tannins, flavonoids and amino acids in the dry extract THU-10 obtained from the plant Tamarix Hispida

Tannins are used as an anti-inflammatory agent for inflammatory diseases of the respiratory system, such as tonsillitis, gingivitis and stomatitis. And also as a hemostatic agent for diseases accompanied by bleeding (pulmonary, uterine, gastric, hemorrhoidal). Due to their astringent and bactericidal properties, tannins are used externally to treat minor wounds and abrasions. In addition, tannins have an activity similar to vitamin P. In dentistry, they are used for chronic inflammatory processes in the oral cavity.

Flavonoids are the most common group of phenolic compounds among all compounds contained in plants of the genus Tamarix. In the composition of the plant, flavonoids are predominantly present in the oxidized form - in the form of flavones, flavanols and their glycosided forms.

Due to their wide distribution in plants and structural diversity, medicinal plants containing flavonoids are currently considered one of the promising sources of medicines. Flavonoids are the most common class of natural phenolic compounds, characterized by high biological activity and low toxicity. The wide range of biological activity of flavonoids is associated with their diverse chemical structure and, as a result, various physicochemical properties. Interest in medicinal preparations based on these compounds is due to the fact that flavonoids have antioxidant, angioprotective, hepatoprotective, diuretic, neurotropic and other important pharmacological properties.

The qualitative characteristics of the dry extract of the Tamarix Hispida plant, obtained by the maceration method of traditional extraction, are given in Table 3.

Table 3 - Quality indicators of the Tamarix Hispida plant extract obtained by maceration method (The data are presented as the Mean values ± standard deviation (n=3)).

Quality indicators	TH-10% M extract, %	TH-50% M extract, %	TH-70% M extract, %
Humidity	1,58±0,07	2,58±0,13	4,79±0,27
Ash content	9,70±0,19	9,40±0,21	7,46±0,05
Extractive substances	8,67±0,03	41,33±0,08	33,87±0,10
Tannins	8,00±0,21	12,15±035	13,71±0,23
Flavonoids	1,00±0,16	2,91±0,12	3,72±0,13

In addition, the amino acid composition of plant material *Tamarix Hispida* was determined (Figure 13).

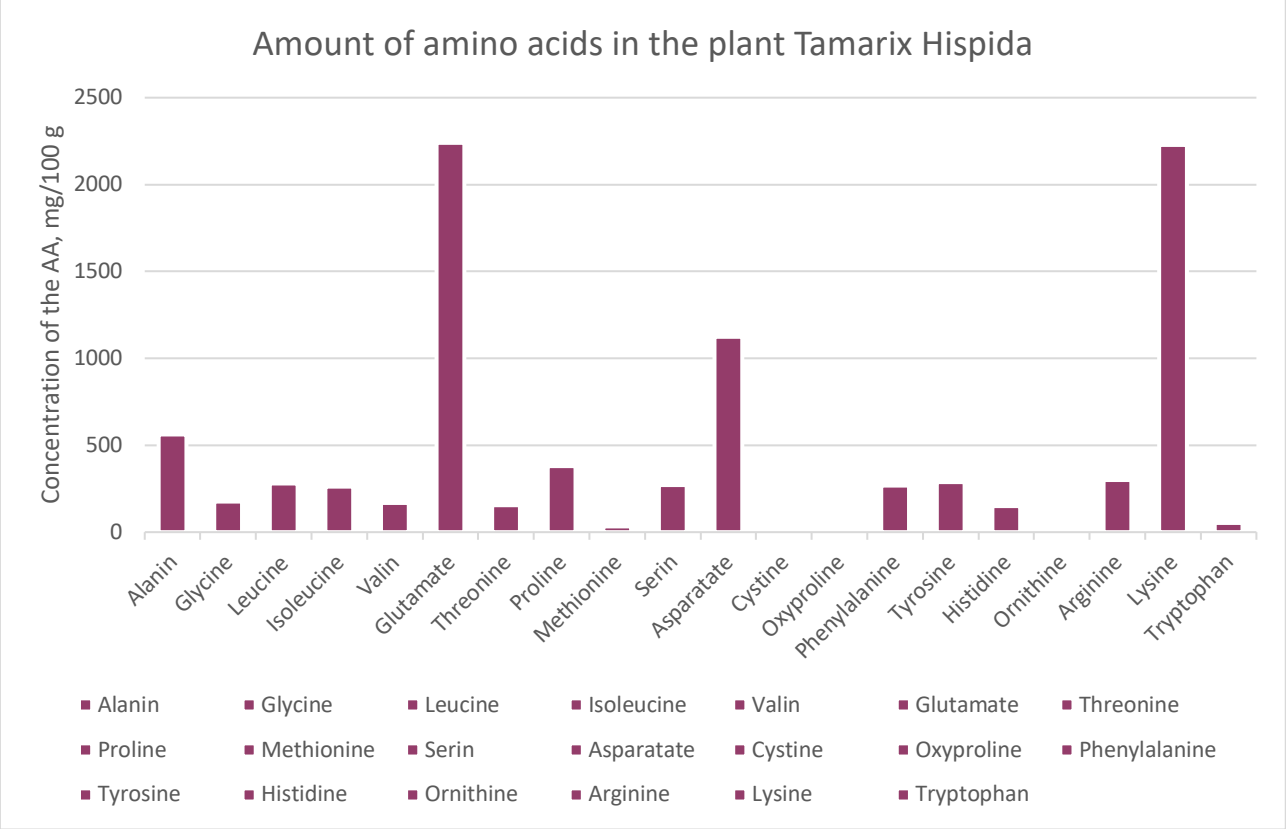


Figure 13 – Amino acid composition of plant material *Tamarix Hispida*

3.2 Optimization of the process of extraction of biologically active substances from the aerial part of plants of the species *Tamarix Hispida Willd.*

A comparative study was conducted on the extraction of extractive substances from the plant raw material *Tamarix Hispida Willd*. using maceration and ultrasonic-assisted extraction methods, considering various extraction factors that influence the efficiency of the process. Considering that the studied plant sample is rich in various polyphenolic compounds (including hydrolysable tannins, glycosylated and sulfated forms of flavonoids, phenolic acids, carbohydrates, and others), an aqueous-ethanolic solvent was chosen for the extraction.

Ultrasonic method was used to extract the hydroalcoholic extract of *Tamarix Hispida* plant because it extracts faster than the standard extraction method. First, the dried and powdered *Tamarix Hispida* plant is weighed on an analytical scale in the amount of 25 grams. Then the powder is placed in a round-bottomed flask and 100 ml of the prepared 10% water-alcohol solution is poured in a ratio of 1:6. The extract is then extracted for 25 minutes in a «Sapphire 1.3» ultrasonic bath at a temperature of 20-40°C. The resulting mixture is then filtered using filter paper.

To determine the optimal volume of solvent at constant mass, time and single extraction, the volume of solvent was changed from 4 to 8 times its excess. From the obtained experimental data, it follows that the optimal ratio of raw materials and solvent with the maximum yield of the substance is 1:6.

The extraction time was worked out with all other parameters (weight, volume, number of extractions) remaining constant, which varied from 6 to 96 hours. Based on the data obtained, a 48-hour extraction time was used. The optimum temperature is 20-25°C, since with increasing temperature there is a tendency for hydrolysis of active substances, which is an undesirable process during their extraction. Maceration was carried out twice, since it is precisely the 2-fold process, as the experiment showed, that contributes to the maximum extraction of the complex of biologically active substances. Figures 14–16 show data on the optimal method for obtaining the substance from *Tamarix Hispida Willd*., considering the above-described extraction parameters by the maceration method. Thus, when extracting from raw materials with 10% water-ethyl alcohol in a ratio of 1:6 (raw materials-extractant), extraction time of 48 hours at room temperature, the yield of extractive substances is 8.67%.

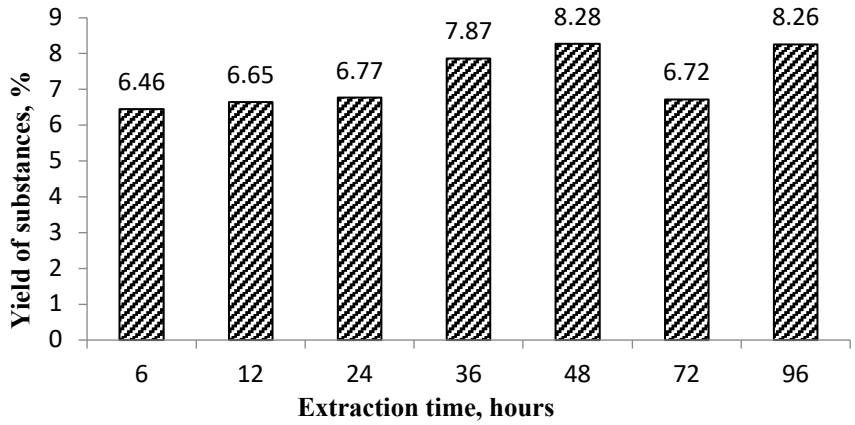


Figure 14 – Yield of extractive substances with a raw material-reagent ratio of 1:4 (maceration method)

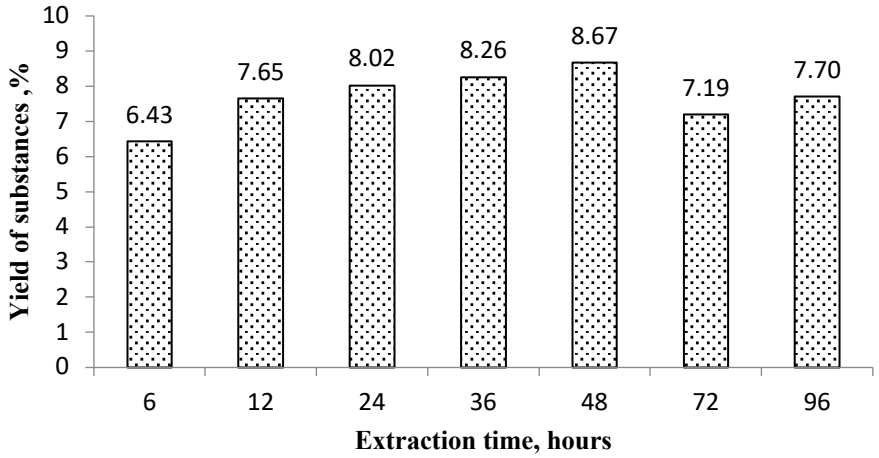


Figure 15 – Yield of extractive substances with a raw material-reagent ratio of 1:6 (maceration method)

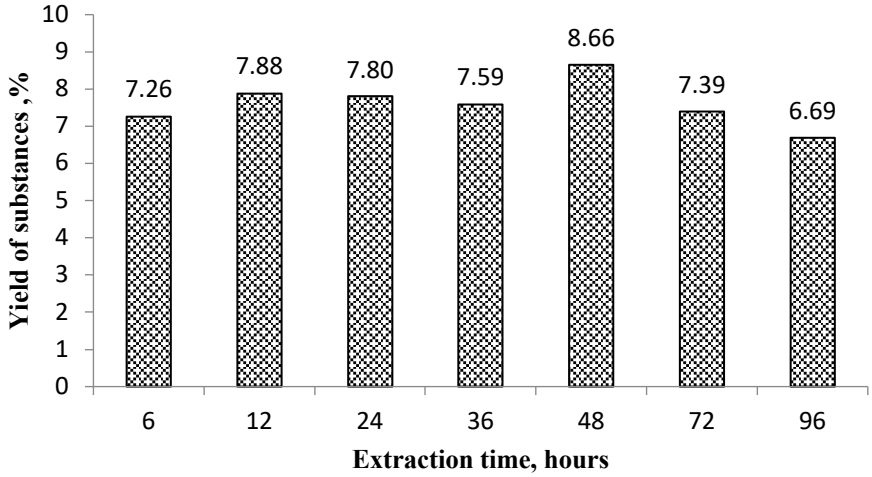


Figure 16 – Yield of extractive substances with a raw material-reagent ratio of 1:8 (maceration method)

For the comparative study of component yield, ultrasonic treatment was used. Extraction was carried out at different ultrasonic frequencies, durations, and temperatures. The duration of ultrasonic exposure depends on the particle size of the extracted raw material. For example, particles of 0.5 mm are recommended to be treated for no more than 15–30 minutes, 1 mm – no more than 60 minutes, and 2 mm – no more than 120 minutes. It is generally not recommended to carry out ultrasonic treatment of raw materials for too long, as the turbidity of the extract may increase due to the accompanying dispersion process. Based on the above, dried plant material, crushed to a particle size of 1 mm, was used for extraction.

To select the optimal extraction conditions, 10% ethyl alcohol was used as an extractant with a constant extraction time and ultrasound frequency. Extraction was performed at two ultrasound frequencies, 20 and 35 kHz, respectively. The temperature varied from 25 to 40°C, with a slight change in the yield of extractive substances observed. The results of ultrasonic extraction of components from *Tamarix Hispida Willd.* are shown in Figures 17 and 18. It follows from the data that the optimal condition for the extraction of extractive substances is 25 minutes at a frequency of 35 kHz. During ultrasonic treatment, stirring is also necessary to ensure proper contact of the extractant with each particle of the raw material and to allow effective exposure.

Thus, the optimal conditions for the extraction of extractive substances from *Tamarix Hispida Willd.* were determined as follows: 10% ethanol with a raw material to solvent ratio of 1:6, extraction duration of 25 minutes at room temperature, and an ultrasonic frequency of 35 kHz. Under these conditions, the maximum extraction of extractive substances was observed, reaching up to 37.34%. Comparative results indicate that the yield of extractive substances from *Tamarix Hispida Willd*. increases by 3–4 times at an ultrasonic power range of 20 to 35 kHz, while the extraction time is significantly reduced from 48 hours to 30 minutes.

The obtained extract, conventionally named THU-10, was filtered after extraction and concentrated to a dry residue using a rotary evaporator. The substance is a dark brown crystalline powder with a pleasant, characteristic taste.

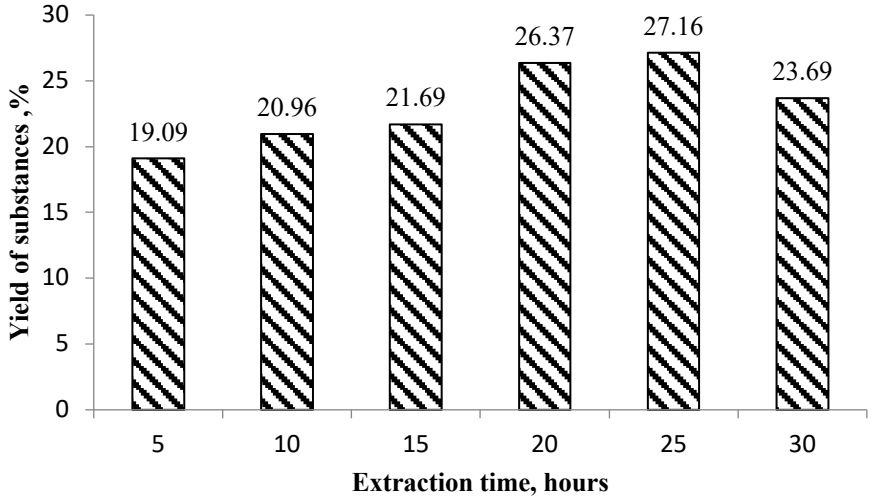


Figure 17 – Yield of extractive substances at an ultrasonic frequency of 20 kHz

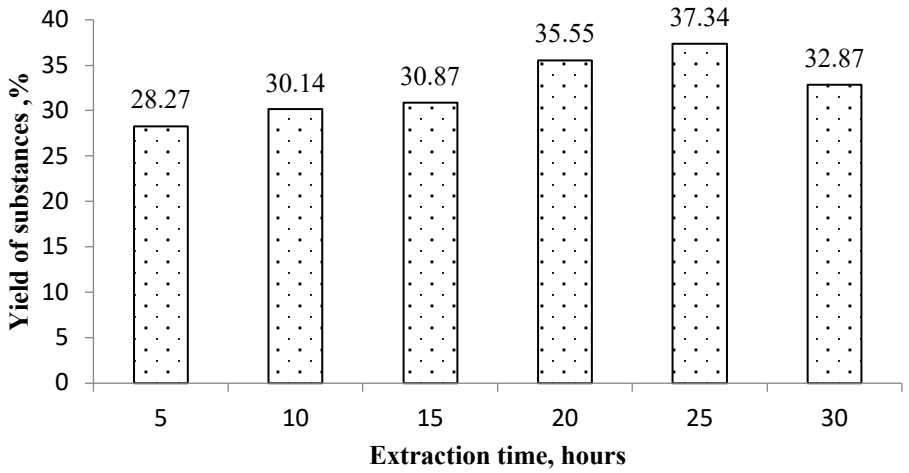


Figure 18 – Yield of extractive substances at an ultrasonic frequency of 35 kHz

The content of active substances (hydrolysable tannins) in the substances obtained by ultrasound and maceration is 10% and 8%, respectively. For the quantitative determination of hydrolysable tannins, the permanganatometry method described in the State Pharmacopoeia of the Republic of Kazakhstan [153] was used.

Based on the obtained extracts, the following conclusion was made: the ultrasonic extraction method should be used for *Tamarix Hispida* in future studies, as it allows for faster extraction compared to the traditional method. The results of the study of the plant extract THU-10 (Figure 18) obtained by ultrasonic extraction showed that the most effective solvent for separating biologically active compounds from the raw material is an aqueous-alcoholic solution. Therefore, extraction was carried out using an aqueous-alcoholic solvent.

The obtained dried extract is a brown crystalline powder. The extract contains flavonoids and tannins. The tannin content in the extract is 10%, while flavonoids are present at approximately 1%. Kaempferol and quercetin derivatives act as flavonoids. The extract also contains components such as rhamnose and glucose.

3.2.1 Results of the qualitative analysis of the extract from *Tamarix Hispida* obtained by the ultrasonic extraction method.

A qualitative analysis was performed on plant raw material extracts obtained by the ultrasonic extraction method. The key biologically active components of the extract include tannins and flavonoids. Both qualitative and quantitative analyses were performed for the specified compounds. The qualitative and quantitative characteristics of the dry extract from the aerial parts of *Tamarix Hispida*, obtained by ultrasonic extraction, are presented in Table 4.

Table 4 – Quality indicators of the *Tamarix Hispid*a plant extract obtained by the ultrasonic extraction method (The data are presented as the Mean values ± standard deviation (n=3))

Quality indicators	THU-10 extract, %	TH-50 extract,%	TH-70 extract,%
Humidity	1,94±0,12	1,54±0,25	3,17±0,11
Ash content	5,86±0,34	7,29±0,24	8,10±0,19
Extractive substances	37,34±0,24	47,83±0,09	41,23±0,16
Tannins, %	10,0±0,14	13,00±0,20	14,33±0,11
Flavonoids, %	2,70±0,31	3,70±0,26	5,14±0,17

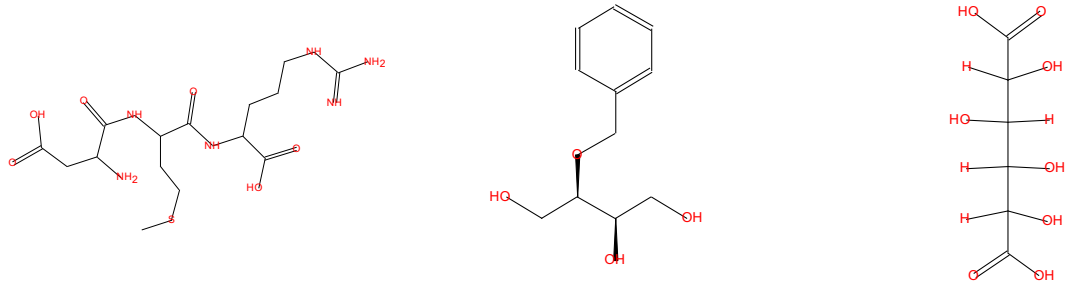
The plant extract THU-10 from Tamarix Hispida is a complex containing flavonoids, tannins, carbohydrates, and amino acids. The active components are tannins represented by derivatives of gallotannins and ellagitannins, and flavonoids such as quercetin, kaempferol, and tamarixetin glycoside. The THU-10 substance exhibits antimicrobial, antioxidant, antifungal, fungicidal, and antidiabetic activities [25].

The chemical composition of a water-ethanolic extract of Tamarix Hispida is determined by chromatography-mass spectrometric method (LCMS). The chemical composition, retention time and formulas are shown in the table below (Table 5).

Table 5 – The chemical composition of a 10% alcoholic extract of Tamarix Hispida is determined by chromatography-mass spectrometric method (LCMS)

№	Name of compound	Molecular formula	Retention time, min	m/z
1	2	3	4	5
1	Aspartylmethionylarginine (Asp-Met-Arg)	$C_{15}H_{28}N_6O_6S$	0.32	149.0
2	(Z, E)-Tetradeca-9,12-dienol	$C_{14}H_{26}O$	0.47	313.4
3	His-His-Lys (histidylhistidyllysine)	$C_{18}H_{28}N_8O_4$	22.46	295.2
4	D-Saccharic acid (D-Glucaric acid)	$C_6H_{10}O_8$	22.43	149.1
5	(2R,3R)-(-)-2-Benzyloxy-1,3,4-butanetriol	$C_{11}H_{16}O_4$	23.34	317.2
6	Sinapyl alcohol	$C_{11}H_{14}O_4$	25.76	279.2

As a result of LC-MS analysis, approximately 6 compounds were identified. The composition of the THU-10 extract included chemical substances such as tripeptides, alcohols, organic acids, and sugars. Identified tripeptides included aspartylmethionylarginine, histidylhistidyllysine (Figure 19).



Aspartylmethionylarginine (Asp-Met-Arg) | (2R,3R)-(-)-2-Benzyloxy-1,3,4-butanetriol | D-Saccharic acid (D-Glucaric acid)

Figure 19. Structural formulas of some compounds of the THU-10 extract determined by chromatography-mass spectrometric method (LCMS)

3.2.2 VLC fractionation of THU-10 extract

The ethanolic extract of the *Tamarix Hispida* (125 g) was subjected to vacuum liquid chromatography (VLC) using silica gel 60 PF 254 (187 g) as stationary phase and eluted via gradient mix elution system with increasing polarity including n-hexane, DCM (100%) DCM/Methanol (85:15, 50:50 v/v), Methanol (100%) (Figure 20).

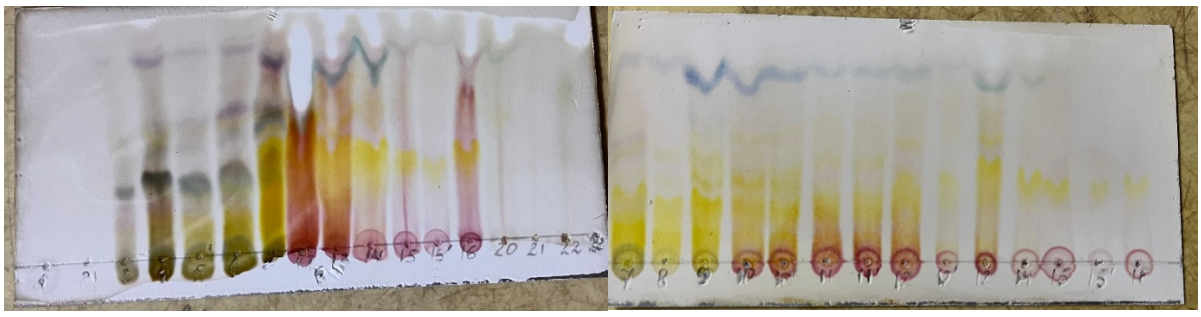


Figure 20 – TLC of the VLC fractionated THU-10 extract.

After performing VLC (Vacuum Liquid Column Chromatography), fractions were obtained, weighed, and numbered as shown in the table below (Table 6). The obtained fractions were then subjected to biological activity evaluation (anti-inflammatory and antioxidant).

Table 6 – Masses of fractions obtained by the VLC method and their solubility.

S. No	Sample code	Mass of the fraction, mg	Solubility	If Fraction (Which Solvent)

1	THU-10-1	63.3	methanol	100% Hexane
2	THU-10-2	438.2	methanol	100% DCM
3	THU-10-3	277.2	methanol	100% DCM
4	THU-10-4	688.4	methanol	15% MeOH/85% DCM
5	THU-10-5	981.7	methanol	15% MeOH/85% DCM
6	THU-10-6	12214.45	methanol	50% MeOH/50% DCM
7	THU-10-7	6843.05	Methanol + water	50% MeOH/50% DCM
8	THU-10-8	5215,3	Methanol + water	50% MeOH/50% DCM
9	THU-10-9	3405.9	Methanol + water	50% MeOH/50% DCM
10	THU-10-10	2315,8	Methanol + water	100% MeOH
11	THU-10-11	1862.2	Methanol + water	100% MeOH
12	THU-10-12	2839.0	Methanol + water	100% MeOH
13	THU-10-13	1980	Methanol + water	100% MeOH
14	THU-10-14	713.4	Methanol + water	100% MeOH

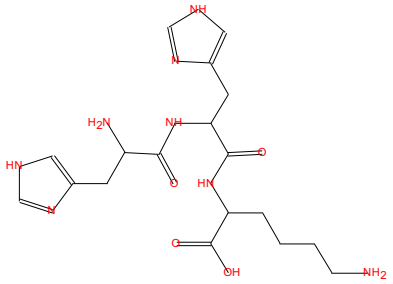
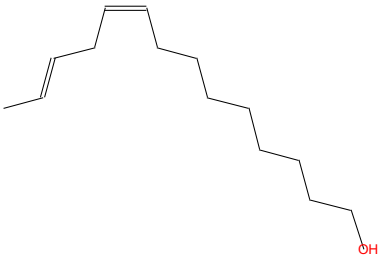
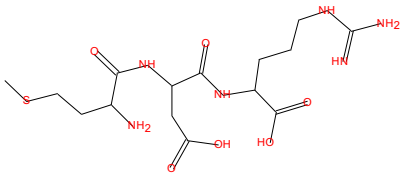
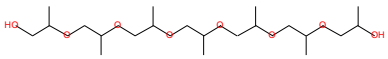
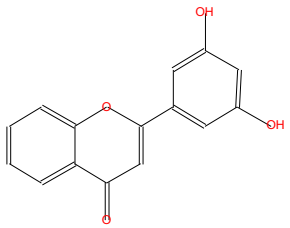
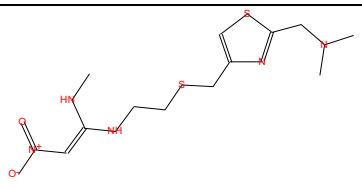
Based on the results of biological activity on fractions at different concentrations (50 μr/ml, 100 μr/ml), the following results were obtained (Table 7). Fractions numbered 2, 3, 4 were the most active in anti-inflammatory activity. Which were then sent to LCMS for further determination of the chemical composition of each fraction.

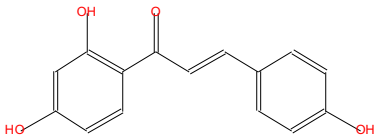
Table 7 – Anti-inflammatory activity of Tamarix Hispida extracts (10, 50, 70%) at concentrations of 50 μg/ml and 100 μg/ml extracts

S. No	Sample code	Anti-inflammatory Inhibition 50 μg/ml, %	IC_{50}±SD, mg/ml	Anti-inflammatory Inhibition 100 μg/ml, %	IC_{50}±SD, mg/ml
1	THU-10-2	28.9	-	84.1	65.0±2.7
2	THU-10-3	87,8	12.0±1.6	98.4	12.0±1.6
3	THU-10-4	63.8	27.3±1.8	81.7	27.3±1.8
Ibuprofen is proposed as implication of Anti-inflammatory activity (Ibuprofen IC_{50}=11.2±1.9 mg/ml)					

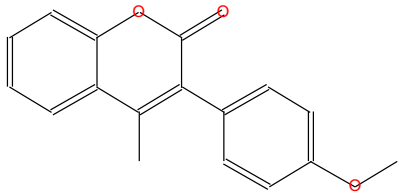
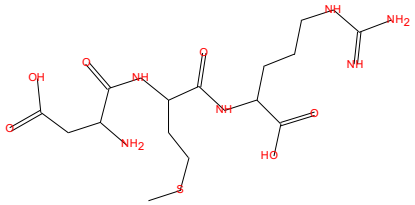
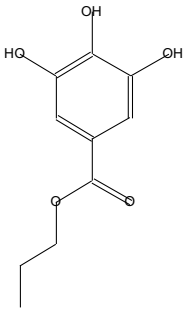
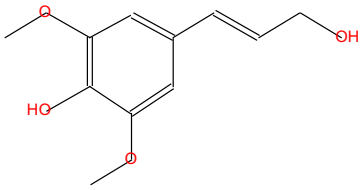
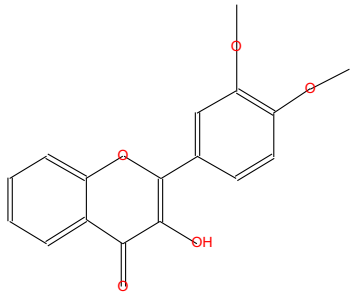
From the above data, it can be seen that fractions 2, 3, 4 showed very high anti-inflammatory activity. Based on these data, these fractions were analyzed by LCMS to determine the chemical composition of each fraction. The results are given in the tables below, separately for each fraction (Tables 8). The remaining fractions showed insignificant anti-inflammatory activity.

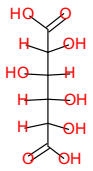
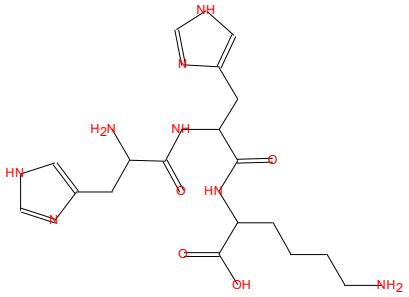
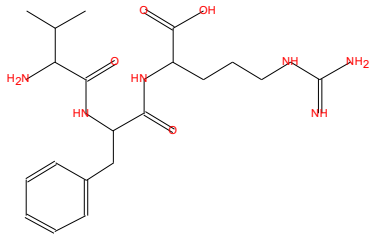
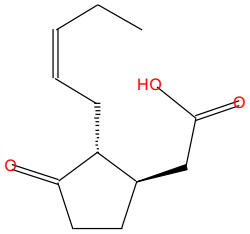
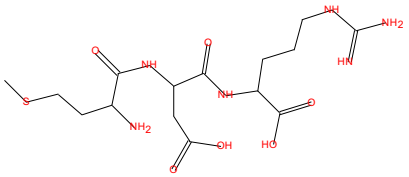
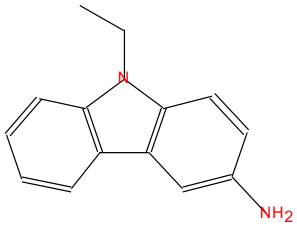
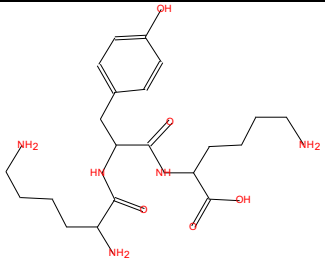
Table 8 – The chemical composition of 2^{nd}, 3^{rd}, 4^{th} fraction of a 10% alcoholic extract of Tamarix Hispida is determined by chromatography-mass spectrometric method (LCMS)

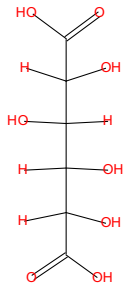
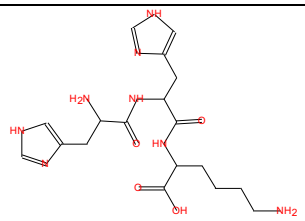
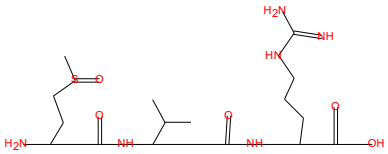
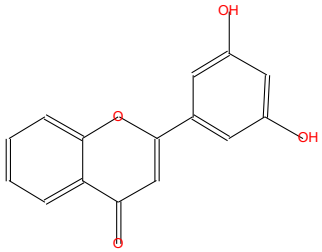
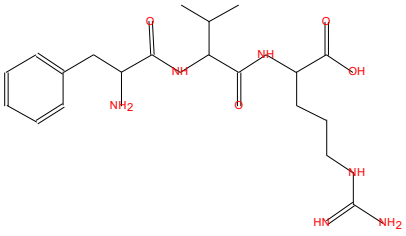
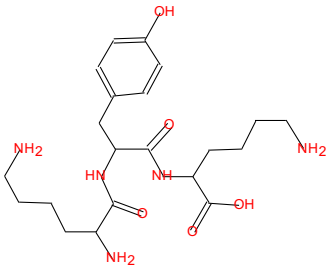
№	Name of compound	Molecular formula	Retention time, min	Structural formula
	1	**2**	**3**	**4**
	Fraction №2			
1	His-His-Lys	$C_{18}H_{28}N_8O_4$	5.00	
2	2-Pyridin-3-yl-thiazolidine-4-carboxylic acid	$C_9H_{10}N_2O_2S$	9.53	$C_9H_{10}N_2O_2S$
3	Mucic acid	$C_6H_{10}O_8$	11.78	$C_6H_{10}O_8$
4	(Z,E)-Tetradeca-9,12-dienol	$C_{14}H_{26}O$	11.21	
5	Met-Asp-Arg	$C_{15}H_{28}N_6O_6S$	10.19	
6	Heptapropylene glycol	$C_{21}H_{44}O_8$	11.36	
7	3',5'-Dihydroxyflavone	$C_{15}H_{10}O_4$	11.10	
8	Nizatidine	$C_{12}H_{21}N_5O_2S_2$	12.98	

9	Isoliquiritigenin	$C_{15}H_{12}O_4$	16.54	
10	D-SACCHARIC ACID	$C_6H_{10}O_8$	23.11	$C_6H_{10}O_8$

Fraction №3

№	Name of compound	Molecular formula	Retention time, min	Structural formula
	1	**2**	**3**	**4**
1	3(4-Methoxyphenyl)-4-methylcoumarin	$C_{17}H_{14}O_3$	2.58	
2	Asp-Met-Arg	$C_{15}H_{28}N_6O_6S$	7.44	
3	n-Propyl gallate	$C_{10}H_{12}O_5$	7.95	
4	Sinapyl alcohol	$C_{11}H_{14}O_4$	12.59	
5	3-Hydroxy-3',4'-dimethoxyflavone	$C_{17}H_{14}O_5$	8.35	

6	D-Saccharic acid	$C_6H_{10}O_8$	11.92	
7	His-His-Lys	$C_{18}H_{28}N_8O_4$	13.33	
8	Val-Phe-Arg	$C_{20}H_{32}N_6O_4$	13.48	
9	Mucic acid	$C_6H_{10}O_8$	14.02	$C_6H_{10}O_8$
10	Jasmonic acid	$C_{12}H_{18}O_3$	15.21	
11	Met-Asp-Arg	$C_{15}H_{28}N_6O_6S$	17.34	
12	9-Ethyl-9H-carbazol-3-ylamine	$C_{14}H_{14}N_2$	18.03	
13	Lys-Tyr-Lys	$C_{21}H_{35}N_5O_5$	16.18	
Fraction №4				

№	Name of compound	Molecular formula	Retention time, min	Structural formula
	1	**2**	**3**	**4**
1	D-Saccharic acid	$C_6H_{10}O_8$	5.15	
2	His-His-Lys	$C_{18}H_{28}N_8O_4$	5.59	
3	Nonic Acid	$C_9H_{16}O_4$	10.31	$C_9H_{16}O_4$
4	Met(O)-Val-Arg	$C_{16}H_{32}N_6O_5S$	13.70	
5	3',5'-Dihydroxyflavone	$C_{15}H_{10}O_4$	14.67	
6	Phe-Val-Arg	$C_{20}H_{32}N_6O_4$	17.51	
7	Lys-Tyr-Lys	$C_{21}H_{35}N_5O_5$	16.79	

The following organic substances were identified in the composition of fraction No. 2 of the THU-10 extract: tripeptides, such as His-His-Lys, Phe-Val-Arg, Met-Asp-Arg, Asp-Met-Arg, His-His-Lys, alcohols (Heptapropylene glycol, (Z,E)-Tetradeca-9,12-dienol), organic acids (2-Pyridin-3-yl-thiazolidine-4-carboxylic

acid, Mucic acid, D-SACCHARIC ACID), flavonoids (3',5'-Dihydroxyflavone) and others.

3.3 Determination of the Phenolic composition of the extracts THU-10, 50, 70 by HPLC-DAD.

The phenolic composition of the extracts THU-10, 50, 70 by HPLC-DAD method. The data are presented in Table 9 and Figures 21–23.

Table 9. Phenolic composition of the extracts THU-10, 50, 70 by HPLC-DAD (mg/g extract) [156].

Compound Name	**RT**	THU-10	THU-50	THU-70
Gallic acid	15,225	1,38	-	-
Protocatechic acid	24,625	-	-	0,08
Theophlline	29,449	0,09	1,09	0,87
Catechine	30,274	-	*tr*	*tr*
4-Hydroxy benzaldehid	33,367	1,21	2,35	-
Vanillic acid	34,758	-	-	0,20
Epicatechin	35,278	0,57	0,94	1,66
p-Coumaric acid	40,874	*tr*	-	-
Ferrulic acid	42,564	-	-	0,07
Hesperedin	47,381	*tr*	*tr*	-
Rutin	47,527	2,78	3,88	4,42
Fisetin	51,243	0,59	0,52	0,93
Genistein	57,739	-	-	-

tr: trace amount, *-: Not tested*

According to the results of the study, the composition of the THU-10 extract contains Gallic acid, Theophlline, 4-Hydroxy benzaldehid, Epicatechin, Rutin, Fisetin and *p*-Coumaric acid, Hesperedin in trace amount. The composition of THU-50 also contains these compounds, but in amounts differing from those in THU-10. However, compounds such as gallic acid and *p*-coumaric acid are absent in its composition. In contrast to the previous extracts, TH-70 was found to contain protocatechuic acid, vanillic acid, and ferulic acid, while gallic acid, 4-hydroxybenzaldehyde, *p*-coumaric acid, and hesperidin were absent.

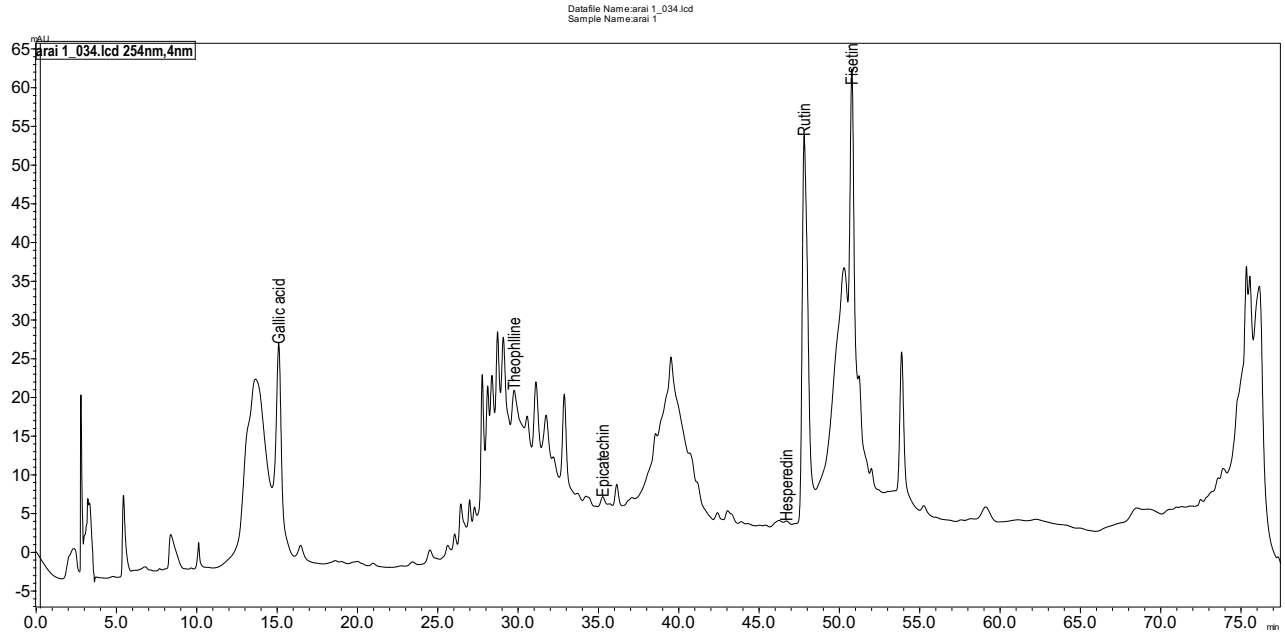


Figure 21 – HPLC–DAD chromatogram of THU-10 at 254 nm (Inertsil ODS-3 column (4 µm, 4 mm x 150 mm). Mobile phase 0.1% acetic acid-methanol (gradient elution). Flow rate 1mL/min. Diode array detection 254 nm.)

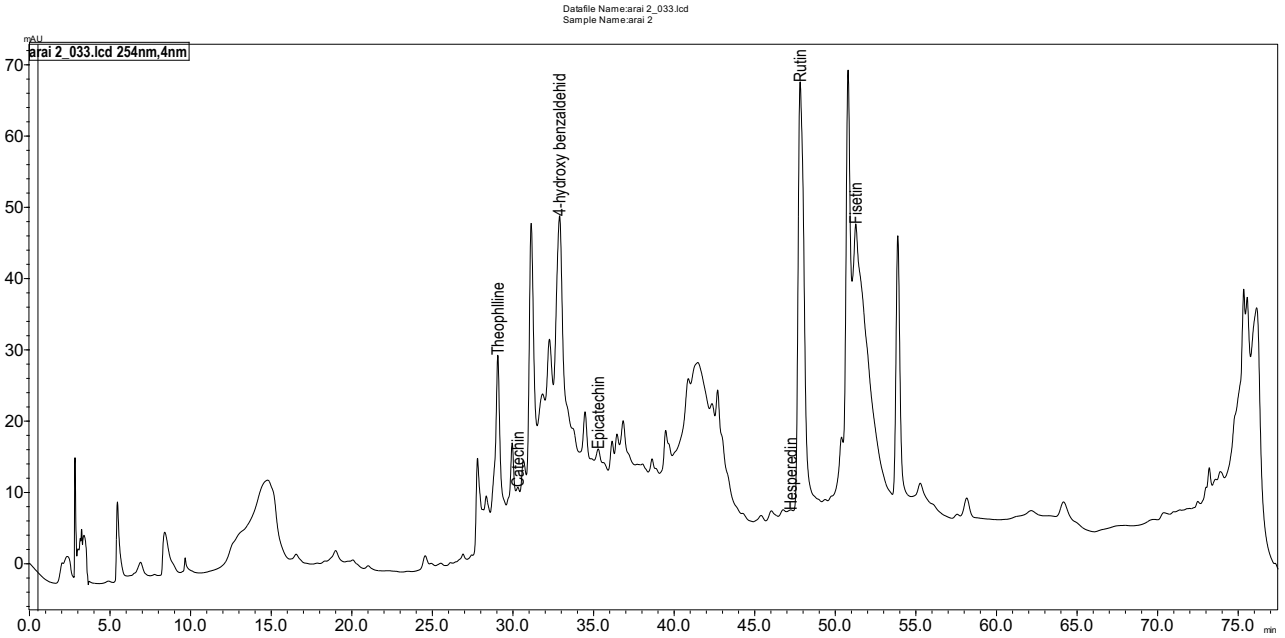


Figure 22 – HPLC–DAD chromatogram of THU-50 at 254 nm (Inertsil ODS-3 column (4 µm, 4 mm x 150 mm). Mobile phase 0.1% acetic acid-methanol (gradient elution). Flow rate 1mL/min. Diode array detection 254 nm.)

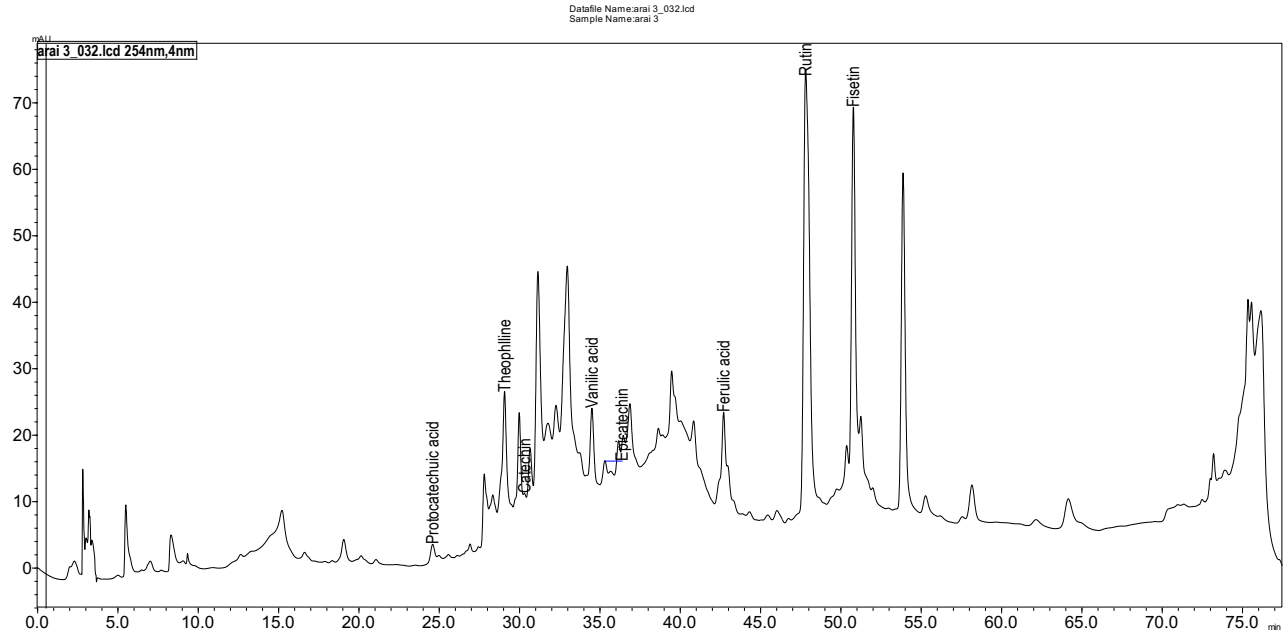


Figure 23 – HPLC–DAD chromatogram of THU-70 at 254 nm (Inertsil ODS-3 column (4 μm, 4 mm x 150 mm). Mobile phase 0.1% acetic acid-methanol (gradient elution). Flow rate 1mL/min. Diode array detection 254 nm.)

3.4 Determination of biological activity of Tamarix Hispida extract

The biological activities were investigated in the laboratories of the H.E.J. Research Institute of Chemistry and Biological Sciences of the University of Karachi, Pakistan.

3.4.1 *In vivo* studies of cytotoxicity and anti-inflammatory activity

The results of the biological activity on the plant extract THU-10 at a concentration of 50 μg/ml showed the following result (Table 10). The anti-inflammatory activity of the 10% ethanol extract showed 42.4% of the value.

Table 10. Cytotoxicity, anti-inflammatory activity and solubility of Tamarix Hispida extracts at different ethanol:water extraction concentrations (10%, 50%, 70%) by ultrasonic extraction method.

Activity / Sample	Cytotoxicity	study of immunomodulators / cytotoxicity/Anti-inflammatory	Solubility in water
THU-10	-	42.4%	+
THU-50	-	32.6%	Partially
THU-70	-	71.3%	Partially

As the results of cytotoxicity show, the obtained extracts do not exhibit cytotoxicity, while anti-inflammatory activity is shown at a level of 40–71% THU-10 compared to other extracts (THU-50, THU-70) is characterized by complete solubility.

3.4.2 Antioxidant activity studies (DPPH assay)

The antioxidant activity of the THU-10 was studied by the method of reducing the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl). The experiment was carried out at different concentrations of the extract in the range from 100 to 1000 μg/ml. Measurements were carried out spectrophotometrically at a wavelength of 517 nm. For each value, the average value of the percentage of inhibition and the standard deviation were calculated (Table 11). Based on the obtained data, a curve of the dependence of the percentage of inhibition of the DPPH radical on the concentration of the studied substance was constructed (Figure 24).

Table 11. Comparative table of antioxidant activity (DPPH method) of THU-10 extract (The data are presented as the Mean values ± standard deviation (n=3))

№	Sample concentration (μg/mL)	% inhibition DPPH (mean ± SD)	% inhibition DPPH (mean ± SD)	% inhibition DPPH (mean ± SD)
		THU-10	Gallic acid	Quercetin
1	100	13,14±1,10	89,21±0,88	-
2	200	75,77±1,79	96,56±0,19	-
3	400	96,08±0,48	96,70±0,23	95,56±0,55
4	600	96,33±0,24	96,81±0,18	94,82±0,35
5	800	96,53±0,26	96,70±0,09	94,79±0,21
6	1000	96,87±0,14	-	93,88±0,13
IC_{50} QUERCETIN ≤ 400 μg /mL IC_{50} GALLIC ACID ≤ 100 μg /mL IC_{50} THU-10 = 200 μg /mL				

The antioxidant activity of the studied THU-10 sample was compared with reference antioxidants – quercetin and gallic acid. When compared with quercetin and gallic acid, the THU-10 extract demonstrates a similar level of DPPH radical inhibition at concentrations of 400 μg/mL and higher (Figure 25).

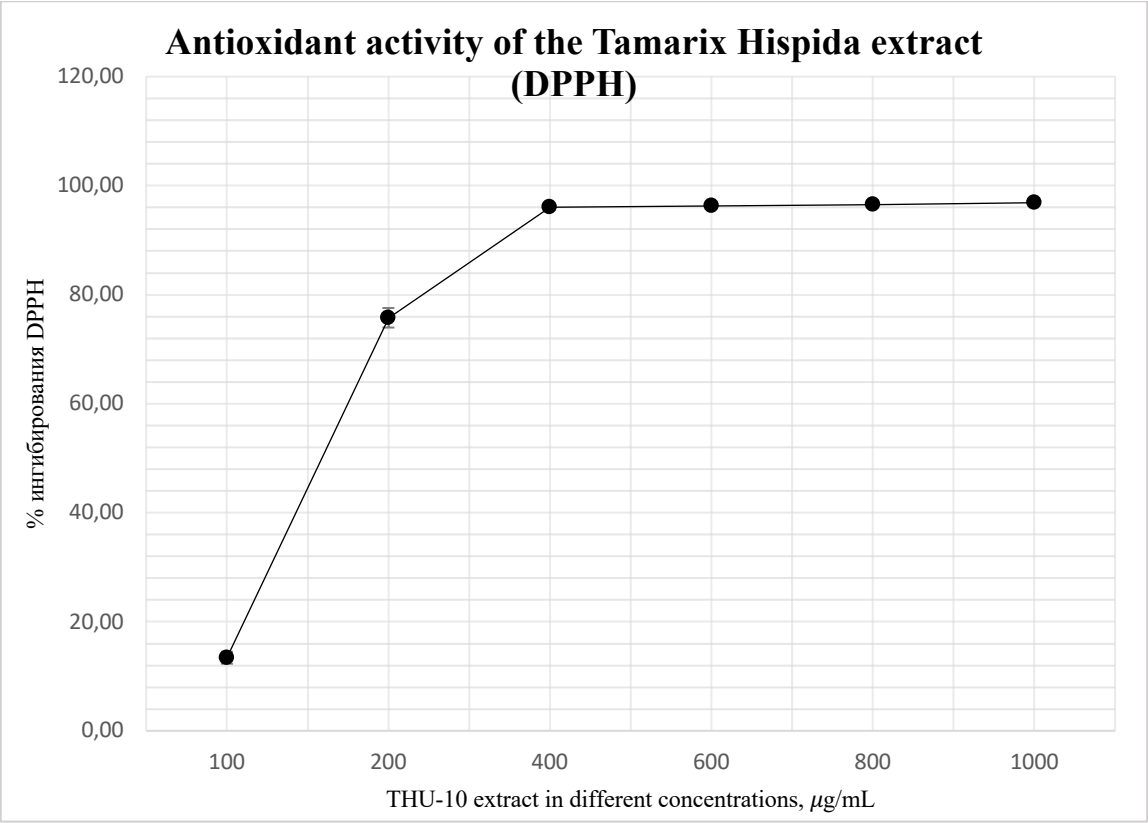
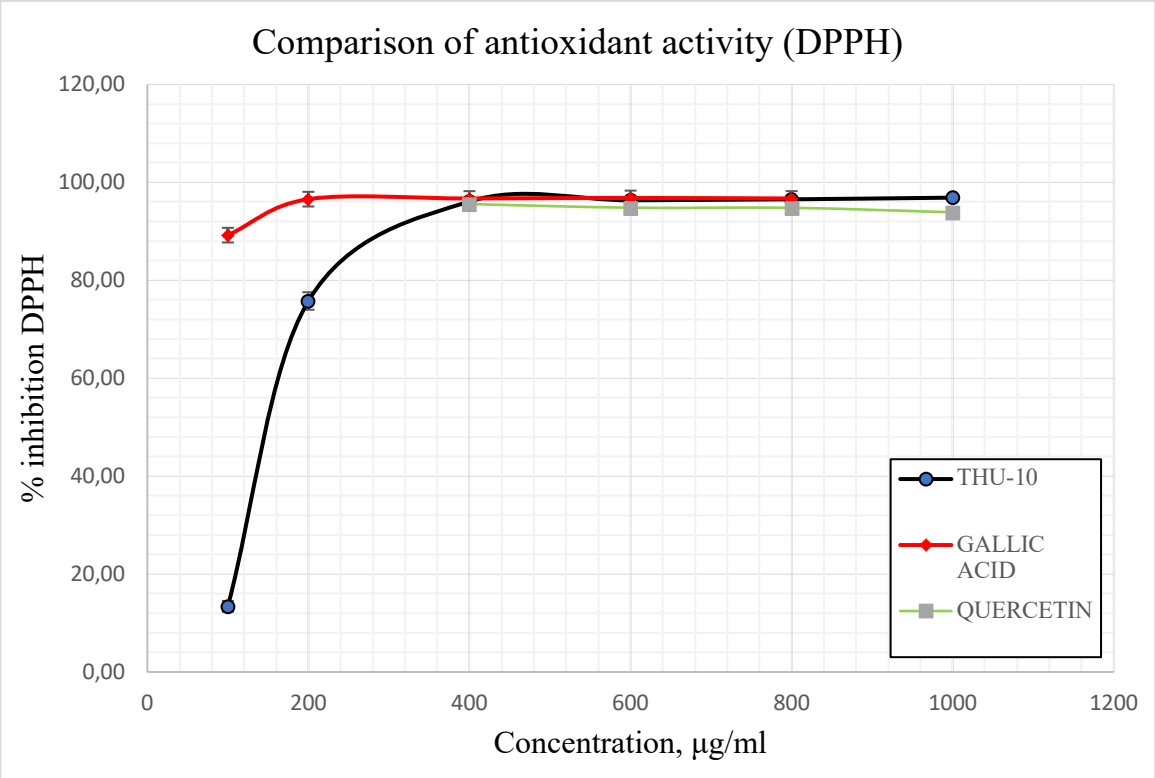
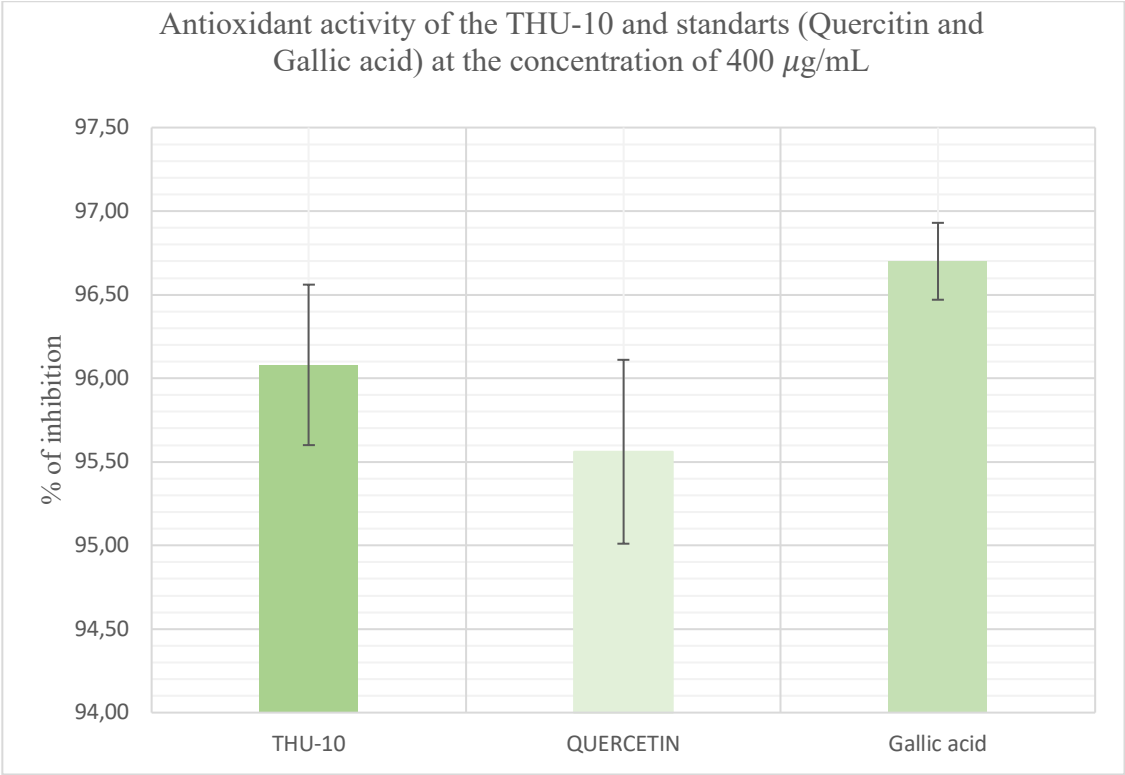


Figure 24 – Antioxidant activity (DPPH method) of THU-10 extract. Mean values ± standard deviation are shown (n=3)



a



b

Figure 25 – Antioxidant activity (DPPH method) of THU-10 extract compared to Quercetin and Gallic acid (a). The data are presented as the Mean values ± standard deviation (n=3, p > 0,05) (b)

A comparative statistical analysis of the activity of the THU-10 samples and the standards quercetin and gallic acid (400 μg/mL), performed using a two-tailed Student's t-test, revealed no significant differences between the groups. These data indicate that the antioxidant activity of the THU-10 extract is statistically comparable to that of quercetin and gallic acid.

3.4.3 Rat paw edema animal studies

The Tamarix Hispida plant is well known for its antioxidant and anti-inflammatory properties *in vitro*. However, its anti-inflammatory activity in vivo has not been studied to date. Previously, 50, 70, and 96% ethanol extracts of the plant were studied for their anti-inflammatory activity, but due to their high viscosity, they were found to be insoluble in water.

In this study, we tested a water-ethanol extract (hereinafter referred to as THU-10), which has better water solubility compared to more concentrated extracts studied earlier. The THU-10 extract also demonstrates antioxidant and anti-inflammatory activity *in vitro*, which served as the basis for its immobilization in hydrogels. New dosage forms based on the extract were evaluated for enhanced anti-inflammatory effect in *in vivo* models.

Chronic inflammatory processes can lead to the development of diseases associated with long-term inflammation, including cardiovascular, neurodegenerative pathologies, and inflammatory bowel diseases. Moreover,

chronic inflammation plays an important role in the physiological and pathological processes in various diseases, such as atherosclerosis (AS), rheumatoid arthritis (RA), depression, obesity, gout, Alzheimer's disease, and cancer.

Currently, standard anti-inflammatory drugs (NSAIDs) have anti-inflammatory, analgesic and other therapeutic effects. However, long-term use of these drugs can cause various side effects, including gastrointestinal tract lesions (stomach ulcers, bleeding, etc.), liver and kidney dysfunction, and skin diseases. In this regard, when using medications, preference should be given to safer drugs whenever possible.

Thus, the search for natural plant compounds with high therapeutic efficacy and no toxic side effects to replace traditional anti-inflammatory drugs is an urgent task in the clinical treatment of inflammatory diseases.

In the search of such plant product, we have selected *Tamarix Hispida* due to its reported antioxidant and anti-inflammatory activity. Previously, its 50, 70, 96 % ethanol extracts were reported for *in vitro* anti-inflammatory activity. However due to its high viscosity and lower water solubility, it was not feasible for animal study. Therefore, in the present study, water-ethanol extract will be prepared due to its water solubility and *in vitro* antioxidant and anti-inflammatory activity (Figure 26, Table 12).



Rat #1 THU-10 treated (a) Rat #3 Indo treated (b)

Figure 26 – Rat paw edema animal studies

Table 12 – Average paw volumes of rats before and after administration of carrageenan

Average paw volume, ml (Control)					
Measurement time	Rat 1	Rat 2	Rat 3	Rat 4	Average value
Before injection (Control)	4,34±0,07	4,54±0,09	4,30±0,06	-	4,39±0,13
1 hour	4,86±0,05	4,55±0,12	4,62±0,18	-	4,68±0,16
2 hour	5,20±0,11	5,01±0,10	4,92±0,09	-	
3 hour	5,48±0,16	5,79±0,05	5,20±0,19	-	5,49±0,30
4 hour	5,59±0,17	6,71±0,09	5,93±0,19	-	6,08±0,57
5 hour	5,75±0,18	7,05±0,16	6,26±0,20	-	6,35±0,66
24 hour	5,85±0,18	6,48±0,05	6,24±0,39	-	6,19±0,32
Average paw volume, ml (THU-10)					
Before injection (THU-10)	3,35±0,04	3,68±0,05	3,81±0,11	3,56±0,09	3,61±0,24
1 hour	3,60±0,10	4,00±0,06	4,17±0,14	3,48±0,09	3,92±0,29
2 hour	3,84±0,09	4,10±0,04	4,35±0,14	3,78±0,20	4,10±0,26
3 hour	4,56±0,09	4,23±0,06	5,17±0,17	4,12±0,10	4,65±0,48
4 hour	4,74±0,09	4,23±0,06	5,21±0,28	4,56±0,05	4,73±0,49
5 hour	4,91±0,14	4,98±0,11	6,13±0,15	4,59±0,07	5,34±0,69
24 hour	4,31±0,11	5,09±0,11	5,17±0,17	4,07±0,49	4,86±0,48
Average paw volume, ml (Control (DMSO))					
Before injection	2,66±0,14	4,41±0,05	3,71±0,12	2,9±0,05	3,42±0,80
1 hour	3,04±0,18	5,16±0,13	4,36±0,14	3,15±0,11	3,93±1,02
2 hour	3,28±0,06	5,04±0,14	4,66±0,08	3,32±0,06	4,08±0,91
3 hour	3,67±0,07	5,96±0,1	5,42±0,05	3,51±0,08	4,64±1,23
4 hour	4,00±0,07	5,99±0,07	6,27±0,21	3,54±0,02	4,95±1,38
5 hour	4,16±0,07	6,63±0,04	7,45±0,12	3,59±0,11	5,46±1,87
24 hour	3,94±0,09	5,82±0,11	6,02±0,09	3,61±0,05	4,85±1,25
Indomethacin					
Before injection (Indo)	4,28±0,03	4,2±0,05	5,14±0,08	4,37±0,05	4,54±0,52
1 hour	5,25±0,11	5,53±0,05	5,52±0,11	4,45±0,06	5,43±0,16

2 hour	5,08±0,09	5,06±0,05	5,34±0,12	4,34±0,03	5,16±0,16
3 hour	5,35±0,76	5,19±0,19	6,14±0,14	4,40±0,07	5,27±0,21
4 hour	5,85±0,03	5,85±0,35	6,31±0,15	5,09±0,23	5,75±0,19
5 hour	5,83±0,27	5,95±0,74	5,90±0,55	5,80±0,24	5,87±0,45
24 hour	6,03±0,15	6,25±0,07	5,00±0,05	6,64±0,10	5,76±0,67

Figure 27 shows the curves of the change in the volume of the right paw of rats at different time intervals after carrageenan injection (after 30 minutes). The study included four groups: control (no treatment), a group with the introduction of a solvent (DMSO), a study group with the drug THU-10 and a group with indomethacin, used as an anti-inflammatory drug as a positive control.

In the control group, a stable and significant increase in paw volume was observed, reflecting the normal course of the inflammatory process. The maximum value of edema was recorded in the interval from 4 to 24 hours after injection.

The DMSO group showed a profile similar to the control group, but with slightly lower values, which may indicate either little intrinsic solvent action or an effect on tissue permeability.

In the THU-10 group, paw volume was significantly lower at all-time points compared to the control. A particularly pronounced reduction in edema was observed in the 2–5-hour period, indicating active development of the anti-inflammatory effect. Standard deviations were within the acceptable values, confirming the reproducibility of the results.

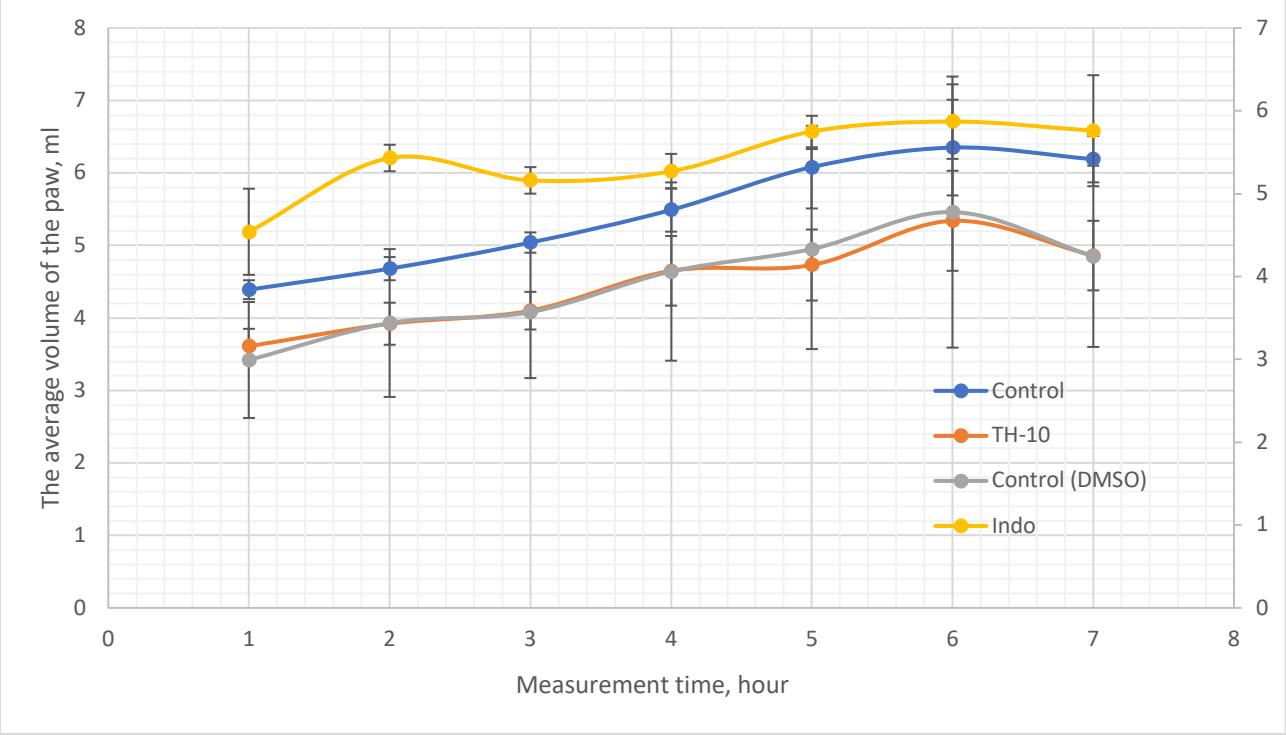


Figure 27 – Comparison of average paw volumes of rats. The data are presented as the Mean values ± standard deviation (n=3)

The positive control, indomethacin, demonstrated minimal paw volume values, especially in the first 1–3 hours after injection, which is consistent with the known pharmacodynamics of this nonsteroidal anti-inflammatory drug.

Thus, the drug THU-10 showed pronounced anti-inflammatory activity comparable to the action of indomethacin and can be considered as a promising agent for further preclinical studies.

In recent years, biopolymers have been increasingly used as carriers in pharmaceutical formulations. Medical polymers are employed for the delivery of active substances within the human body. The active substance may be of plant origin and possess therapeutic properties. One of the cellulose ethers, such as hypromellose, can serve as a polymer carrier. Hypromellose is widely used in medicine—for example, in tablet manufacturing as a carrier for the active pharmaceutical ingredient (API). This polymer can be considered an ideal drug delivery carrier, as it exhibits all the essential properties required for polymers used in pharmaceutical applications. Hypromellose demonstrates excellent mechanical, sorption, and physicochemical properties when used in the preparation of hydrogels.

The research explored the potential for gel formation through interaction with the plant extract *Tamarix Hispida* (THU-10). When preparing gels for medical use, it is essential to consider a number of requirements, as medical products undergo more rigorous testing. By meeting all the necessary criteria for the formulation of therapeutic ointments, the resulting product can exhibit the desired medicinal properties. As a result, various rheological properties of the ointments were studied and determined, including the percentage of drug release, the swelling kinetics of the gels, the surface morphology of the gels, and their antimicrobial properties.

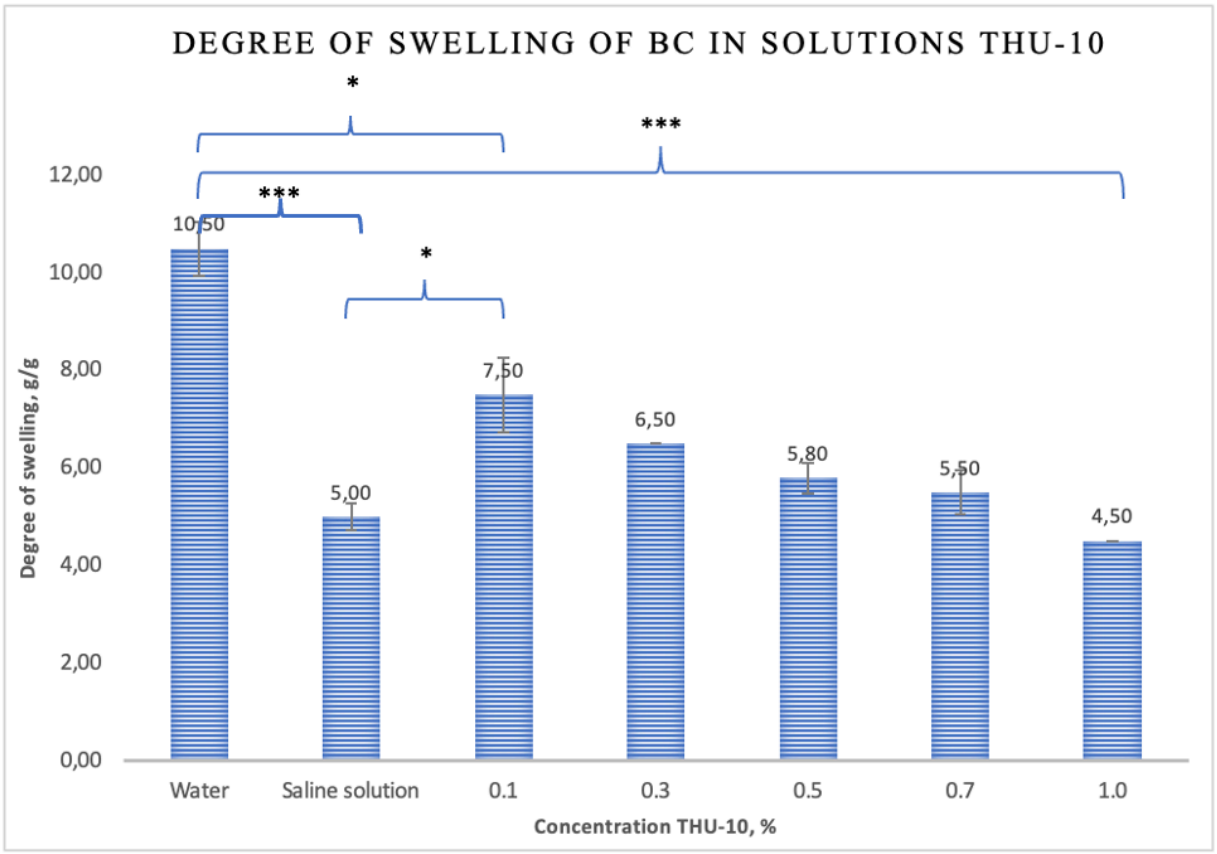
3.5 Study of the properties of bentonite clay compositions with plant extract THU-10

Bentonite clays (BC), due to their ability to undergo cation exchange, swelling, and non-toxicity, are perceived as a defining aspect in the synthesis of new materials. Promising areas include the creation of polymer composite materials, prolonged drug systems, materials with improved sorption properties and nanostructured structure [164-181]. This can be noted for a number of food products and endosorbents [182-187]. When creating materials, especially for medical purposes, it is important to know the patterns of interaction of components with each other and the behavior of the system as a whole in various environments. In order to create medical materials based on bentonite clay with a plant component, the patterns of interaction of bentonite clay from the Manrak deposit in the East Kazakhstan region were considered. Dry extract from the *Tamarix Hispida* plant, which has antimicrobial, antioxidant, antifungal, fungicidal and antidiabetic activities, was selected as the active principle (Appendix B). The establishment of interaction patterns was carried out using the methods of equilibrium swelling, sorption,

desorption, rheology, sedimentation, X-ray structural analysis, UV and IR spectroscopy.

3.5.1 Determination of the degree of swelling and sedimentation analysis of BC in THU-10 solutions.

The ability of mineral carriers to swell is an important property in the immobilization of physiologically active substances (FAS) by the sorption method. According to the data on determining the degree of swelling (Figure 28) of BC in water and THU-10 solutions, a decrease in the degree of swelling is visible due to the binding of THU-10 components to the carrier. In this case, the more concentrated the FAS solution, the greater the decrease in the degree of swelling. The physiological environment has an additional compressive effect, but weaker than the plant extract THU-10, which may indicate specific bonds, possibly due to the formation of hydrogen bonds between the hydroxyl groups of BC and the components of THU-10 - tannins and flavonoids, and also under the action of ionic strength, compression of the double electric layer occurs with the expulsion of water from the layers of montmorillonite [188].



*- 0,01<p<0,05
***p<0,001

Figure 28 - Equilibrium swelling degree of bentonite clay in aqueous solutions at 6 hours. The data are presented as the Mean values ± standard deviation (n=3)

According to a comparative analysis, the swelling degree of bentonite clay in water is significantly higher than in saline. In THU-10 solutions at concentrations of 0.1 to 0.3%, the swelling degree of bentonite clay decreases, but remains higher than in saline. At a THU-10 concentration of 1.0%, the swelling degree parameter also significantly differs from that in water ($p < 0.001$).

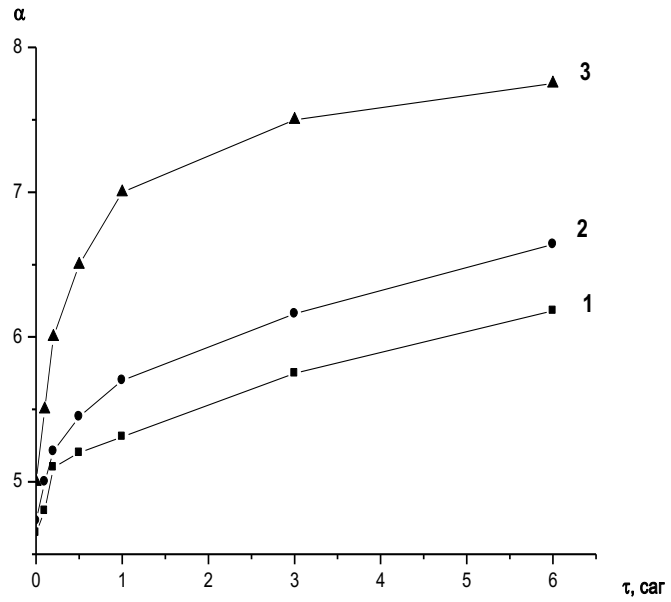
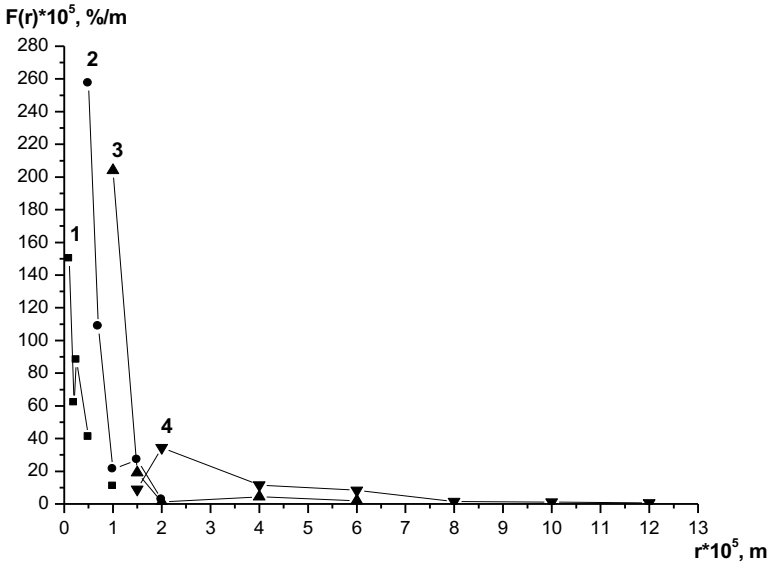


Figure 29 – The effect of temperature on the swelling kinetics of bentonite clay with THU-10

The dependence of the swelling degree of bentonite clays on temperature is shown in Figure 29. As the temperature increased, the swelling degree of the clay also increased. This can be explained by the weakening of hydrogen bonds between the clay and THU-10, as well as between the layers of montmorillonite with rising temperature, leading to enhanced swelling of the bentonite clay.

To establish the dispersion of BC, a sedimentation analysis of bentonite clay in water and aqueous solutions of *Tamarixidin* was carried out using the method of N.A. Figurovsky. The results are in good agreement and complement the swelling behavior of bentonite clay. Bentonite clay, both in water and in *Tamarixidin* solutions, is monodisperse and the particle sizes are within the range of 10^{-6}-10^{-5} m, that is, it is a coarse colloidal system. The average size of bentonite clay particles in water is $0.5 \cdot 10^{-5}$ m. In 0.01%, 0.05%, 0.1% aqueous solutions of *Tamarixidin*, the particle sizes are $1.4 \cdot 10^{-5}$ m, $4.55 \cdot 10^{-5}$ m, $5 \cdot 10^{-5}$ m, respectively. Comparing the data of sedimentation analysis and determination of the degree of swelling of bentonite clay in aqueous solutions of Tamarixidin, the decrease in the aggregate stability of clay particles can be explained as follows: as noted above, due to the hydrophobization of bentonite clay particles, lyophobic areas appear in them. Due

to the influence of these lyophobic areas, in accordance with research works [151], aggregation (sticking together) of clay particles occurs.



Water (1), [THU-10] =0,01 % (2), 0,05 % (3), 0,1 % (4)

Figure 30 - Differential distribution curves of bentonite clays in water and in aqueous solutions of tamarixidine

Table 13 – Sedimentation analysis of bentonite clays

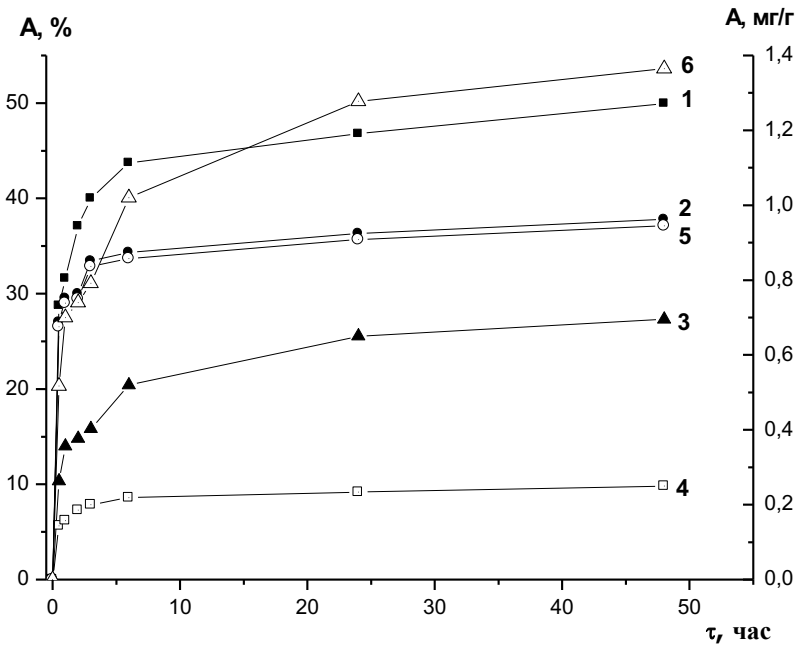
		Fractions						
Water	r*10^{-5}, m	4,8	1,42	0,628	0,3775	0,163	0,123	-
	F, %	4,4	36,6	32,3	22	12	6	-
	F(r) 10^5, %/m		10,8	40,78	88	61,9	150	
[THU-10]=0,01 %	r*10^{-5}, m	4,529	2,02	1,4	1,01	0,826	0,716	-
	F, %	1,66	6,67	16,67	8,3	20	28,3	-
	F(r) 10^5, %/m		2,65	26,88	21,28	108,69	257,27	-
[THU-10] =0,05 %	r*10^{-5}, m	11,17	6,44	4,55	3,22	2,84	2,63	-
	F, %	8,3	8,33	8,34	1,5	7,25	42,85	-
	F(r) 10^5, %/m		1,92	4,4127	1,127	19,1	204,05	-
[THU-10] =0,1 %	r*10^{-5}, m	24,9	11,9	7,9	5,538	4,56	3,95	3,22
	F, %	2	8	6	19	12	21	6,5
	F(r) 10^5, %/m		0,615	1,5	8,25	11,56	34,42	8,99
Note: r is the particle radius, F is the integral distribution function, F(r) is the differential distribution function								

In the process of immobilization of medicinal substances on inorganic carriers, it is important to know the interaction of the carrier with the medicinal substance, that is, the formation of a clay-drug composition, and the significance of quantitative indicators of the sorption of these compositions is of particular interest. Numerical values of sorption allow us to determine the composition of the dosage form, as well as regulate and control the dose of the active principle.

In this regard, in this work, the sorption indices of bentonite clay relative to Tamarixidin and the sorption kinetics were determined using the UV spectroscopy method. The numerical values of sorption are presented in g/g and percent. Based on the results of the study of sorption kinetics, it was shown that during the first 6 hours there is intensive sorption of the main plant extract (Figure 31). Based on literature data [189], the interaction between bentonite clays and organic molecules is possible due to processes such as adsorption, cation exchange and esterification. It was found that the energy of water sorption by clays is much lower than the energy of sorption of dipole organic molecules. Organic compounds with active groups are sorbed on bentonite clays, displacing water from the clay. This circumstance proves the high sorption capacity of bentonite clay and explains the compression of clay particles in the presence of a biologically active complex.

As is known, up to three molecules of an organic compound can be sorbed between layers of bentonite clays [189, 190]. Analyzing the obtained results, it can be noted that the degree of sorption of the biologically active complex, per 1 g of carrier, increases with an increase in the concentration of the plant extract. If these data are presented in percentage terms, the opposite picture can be seen. This picture of sorption is explained, on the one hand, by the flow of the sorption process by the sorbate, i.e. clay, to an equilibrium value corresponding to the exchange volume of clay.

On the other hand, the reason for this phenomenon is associated with the achievement of an equilibrium value of osmotic pressure. Let us pay attention to the numerical values of sorption, which, depending on the concentration of tamarixidine, are from 25 to 45% during the first 6 hours, which indicates a high sorption capacity of bentonite clay relative to tamarixidine.



[THU-10]=0.1% (1,4), [THU-10]=0.5% (2,5), [THU-10]=1% (3,6)
1-3 – in %, 4-6 mg/g

Figure 31 – Kinetics of THU-10 sorption by bentonite clay

The conducted X-ray diffractometric analysis of clay samples after sorption of Tamarixidin showed that during the sorption process, the introduction of molecules of compounds included in the composition of THU-10 occurs (Table 14-15, Figure 32), as a result of which we can observe an increase in the interlayer space to 1.48 nm (for pure bentonite it is 1.3 nm).

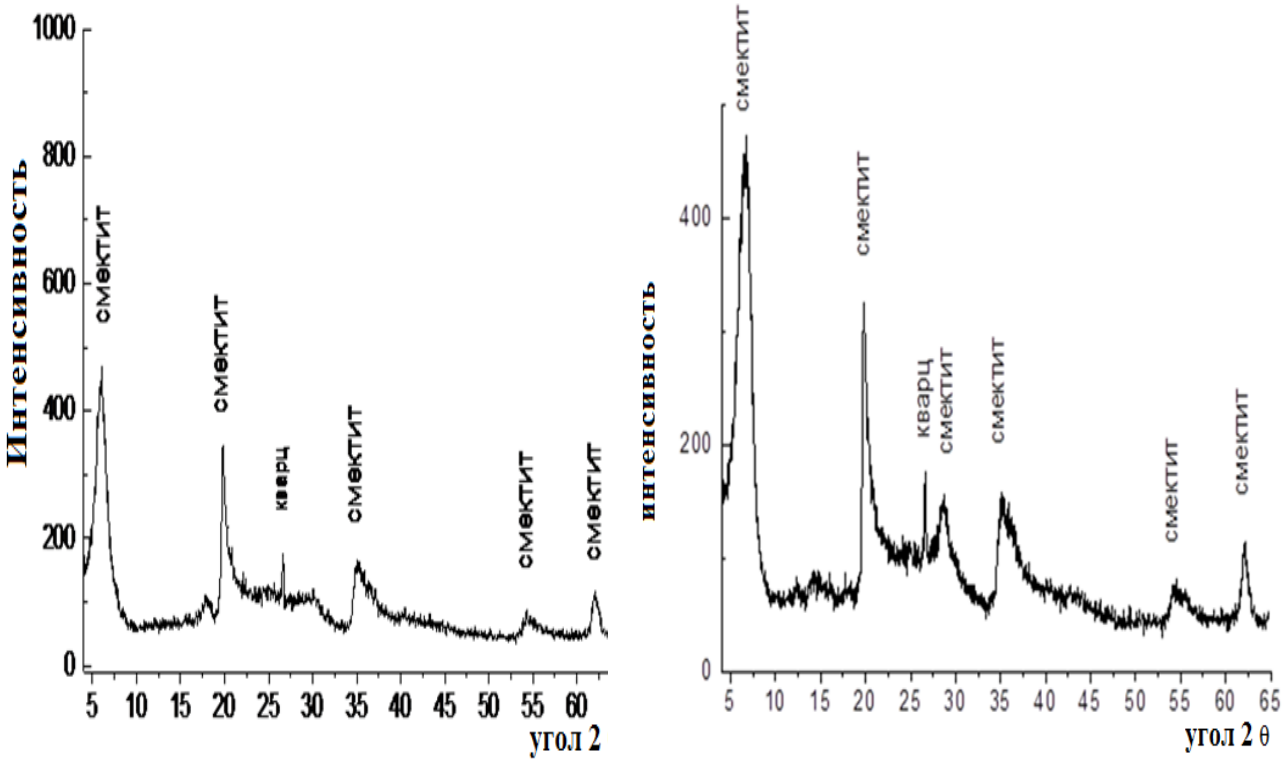
Table 14. Interlayer spacing of minerals, phase composition (purified bentonite clay).

Distance between layers, (*d*) Å	*I* %	Phase composition, mineral
13.349	94	Smectite
4.476	69	Smectite
3.346	37	Quartz
3.119	31	Smectite
2.535	33	Smectite
1.682	16	Smectite
1.494	24	Smectite

Table 15 - Distance between mineral layers (bentonite clay after sorption of THU-10)

Distance between layers, (*d*) Å	*I* %	Phase composition, mineral
14.817	95	Smectite
4.946	23	Smectite
4.48	74	Smectite
4.257	40	Quartz
2.552	35	Smectite
1.686	16	Smectite
1.494	24	Smectite

The process of sorption of organic molecules on the carrier is accompanied by a change in the electrokinetic potential (ζ potential). To clarify the mechanism of sorption of THU-10 on BC, an electrophoretic study of BC suspensions with biologically active substances was carried out.

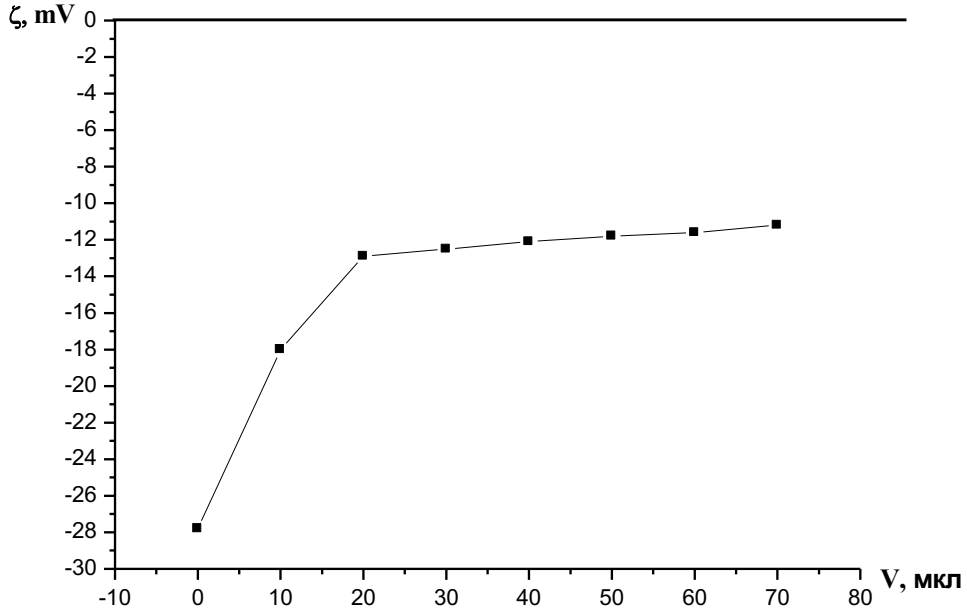


1 2

Figure 32 - Diffraction pattern of bentonite clay after sorption of tamarixidine (1) and pure clay (2)

The process of sorption of organic molecules on the carrier is accompanied by a change in the electrokinetic potential (ζ potential). To clarify the mechanism of sorption of THU-10 on BC, an electrophoretic study of BC suspensions with biologically active substances was carried out. The data of electrophoretic studies

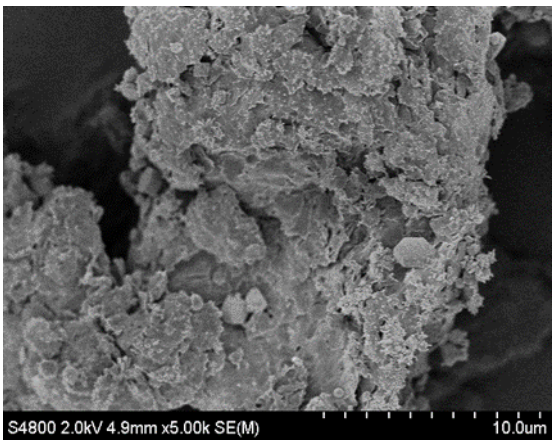
(Figure 33) show that the negative values of the ζ -potential of clay particles decrease with the introduction of tamarixidin, but it should be noted that the negative charge, in general, is preserved. This confirms the proposed mechanism of complex formation between clay and tamarixidin components due to hydrogen bonds. With another mechanism, one could expect a more noticeable change in the electrokinetic potential. A decrease in the negative charge at the initial stage of interaction is possible due to the availability of internal cavities of bentonite particles for amino acids, which, when introduced, interact with negative groups on the surface of the particles, thereby reducing the charge. The complexes formed due to ionic strength are then stabilized by hydrogen bonds between the polymer components of the plant extract and the hydroxyl groups of bentonite clay.

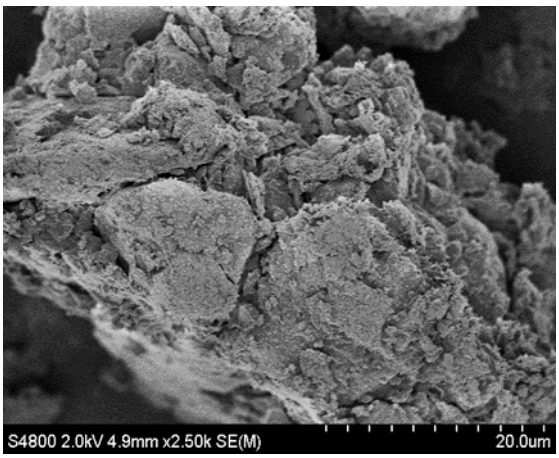


[BC]=0,025 %

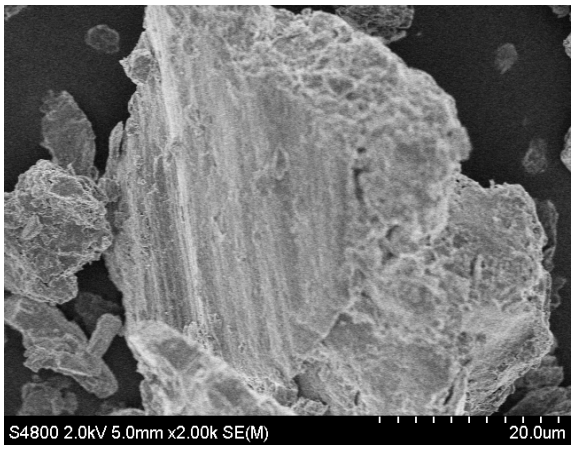
Figure 33 - Values of electrokinetic potential of BC and its mixtures with THU-10

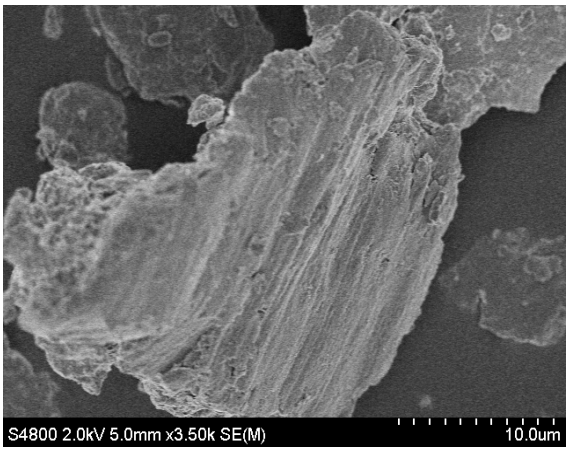
SEM images were taken for bentonite clay gels after THU-10 sorption (Figure 34), in which the formation of Tamarixidin-clay complexes on the surface can be clearly seen. As can be seen from the images, the surface of the clay particles is leveled due to the introduction of THU-10 molecules into the interlayer space and a change in the pattern characteristic of bentonite clay itself. Aggregation of clay particles under the influence of the formation of the BC-THU-10 complex can also be seen.





a



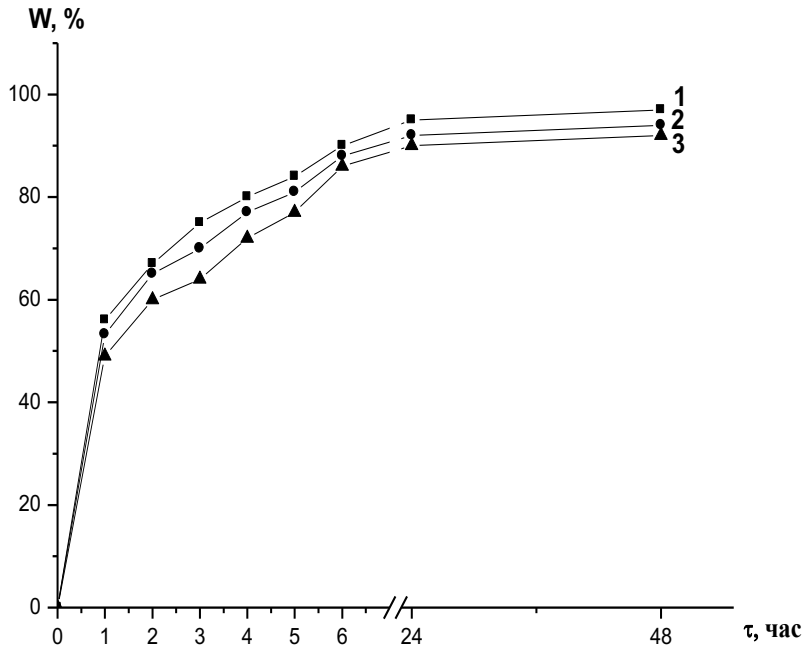


b

Figure 34 – SEM images of bentonite clay before (a) and after (b) sorption of THU-10

3.5.2 Determination of the release rate of a drug from bentonite clay

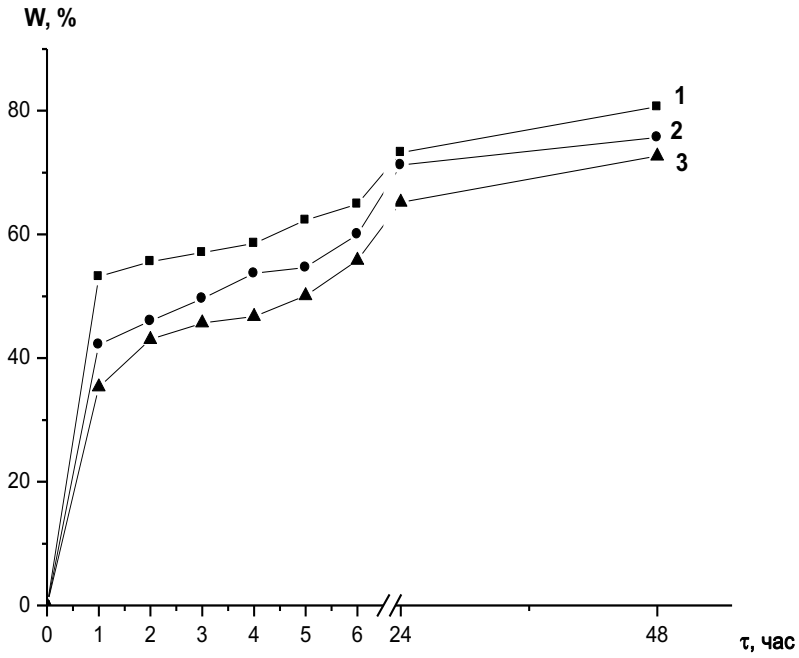
In order to evaluate the prolongation effect and to know the numerical values of the release of the biological complex from the bentonite base, the kinetics of tamarixidine desorption from bentonite clay was studied using the spectrophotometric method. For the studies, gels based on BC with a concentration in the range from 10 to 30% with a THU-10 content from 0.1 to 1% were obtained. It is necessary to note the formation of homogeneous gels, the structuring of which requires time from 24 to 48 hours. Visual observations also showed that these gels are resistant to microbial spoilage at room temperature for up to 7 days, while an aqueous solution of THU-10 is prone to microbial spoilage after two days of storage. To increase stability, it is enough to store in a refrigerator, and when developing a dosage form - ointments and gels, optimal stabilizers will be selected. Figure 35 shows data on the kinetics of the release of the active substance from the dosage form into water. As can be seen from the figure, the concentration of the clay base has a significant effect on the release of the plant complex: with an increase in the content of bentonite clay, the degree of release of the active principle decreases, which is explained by an increase in the density of the gel, which complicates the diffusion of the drug.



[THU-10]=1 %, [BC]=20 % (1), 25 % (2), 30 % (3)

Figure 35 - Kinetics of tamarixidine release from bentonite clay into water

Considering that in the future, in terms of practical application, the drug system will work in biological and physiological environments, it is important to know the behavior of the drug form in these conditions. Drug systems, entering the human body, find themselves in a solution of low-molecular strong electrolytes, in a physiological environment. Therefore, it is very important to study the behavior of the clay-drug complex in electrolyte solutions. In this regard, a study was conducted on the kinetics of tamarixidine release into a physiological solution (0.86% NaCl solution in water) (Figure 36). The influence of bentonite clay content on the kinetic behavior during the desorption process is similar to its release into water. However, it should be noted that in physiological saline, the numerical values of active substance release are lower compared to water. This behavior may be attributed to the osmotic pressure of the saline solution, which leads to the compression of bentonite clay particles and hinders release, as reflected in the desorption data. In physiological saline, equilibrium is reached after 24 hours, whereas in water, equilibrium is established within 6 hours. The delayed and gradual release of the active substance into the surrounding environment from the base is of great importance, and the fact that the physiological medium promotes a prolonged release of tamarixidin from bentonite clay is favorable for the further practical application of the system.



[THU-10]=1 %, [BC]=20 % (1), 25 % (2), 30 % (3)

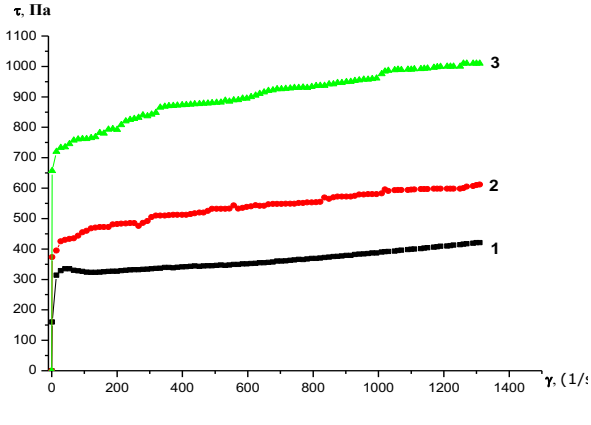
Figure 36 – Release kinetics of tamarixidin from bentonite clay into physiological saline

The release kinetics curves of THU-10 into physiological saline from gels with higher tamarixidin content also draw attention. As shown in the figure, the curves exhibit a stepwise pattern, which indicates polymolecular desorption of the active substance. A similar pattern is observed during sorption onto bentonite clay from solutions with a high concentration of the active compound. This behavior suggests that the sorption of THU-10 onto the bentonite carrier follows a "relay" mechanism. The desorption values range from 60% to 80% within 24 hours, depending on the bentonite clay content.

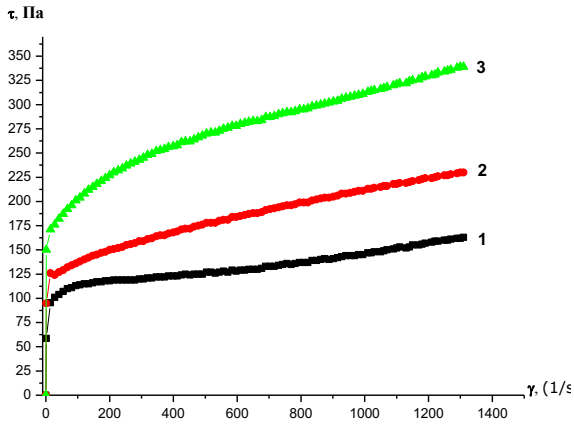
3.5.3 Study of rheological parameters of obtained dosage forms based on bentonite clay

At present, the rheological characteristics (rheological consistency and optimal spreadability) of soft medicinal systems, including hydrophilic and lipophilic ointments, have been experimentally established [191]. To evaluate the consistency, rheograms are constructed at a storage temperature of 20 °C and in the shear rate range of 1.5-1312 s^{-1}. Under these conditions, the rheological optimum consistency should have the following values: yield strength of 45-160 Pa and effective viscosity of 0.34–108 Pa·s for hydrophilic ointments, for lipophilic ointments - 35-140 Pa and 0.32–93.3 Pa·s, respectively [192]. Rheograms of the developed ointment samples were obtained using a rotational viscometer Anton Paar MCR 102 (Austria). As shown by the data of the rheological studies (Table 16), the ointment bases with bentonite exhibit high yield stress values, which do not

correspond to the proposed optima. In the hydrogel samples with THU-10, a decrease in yield stress was observed, which is associated with the incorporation of THU-10 into the bentonite suspension. The obtained data also show that as the concentration of THU-10 increases, the viscosity of the formulations decreases, while the base component, bentonite clay, on the contrary, contributes to an increase in viscosity. According to the obtained yield stress and viscosity data, the hydrogels containing 25% bentonite and THU-10 at concentrations of 1–3% were considered to meet the rheological consistency optima. Their yield stress values were 60.21, 123.79, and 159.87 Pa, respectively (Figure 37).



BC=20% (1), BC=25% (2), BC=30% (3), T=20 °C



THU-10=1% (1), THU-10=2% (2), THU-10=3% (3), T=20 °C, BC=25%

Figure 37 – Flow rheograms of hydrogels based on bentonite clay

Many soft drugs are classified as plastic-flowing systems, therefore, for them, not only the dependence of rheological characteristics on the shear rate is determined, but also the time dependence of the duration of the viscous-flowing state. Such systems are classified as thixotropic [193]. Changes in thixotropic systems are called hysteresis, and rheograms have the form of a hysteresis loop, in turn, these rheograms serve as an assessment of the system's recovery capacity. Soft dosage forms should possess thixotropic properties; therefore, the thixotropic properties and thixotropy energy of the obtained hydrogels were determined. Understanding the resistance of hydrogels to mechanical stress is important for transportation and use. Research results show that all bases exhibit thixotropic properties (Table 17). Samples of hydrogels with THU-10 also exhibit thixotropic properties, and with an increase in the BC content, the thixotropy energy increases several times due to an increase in the viscosity of the system. Based on the calculation of thixotropy energy, ointments with different THU-10 contents with BC of 25 and 30% were selected. A hydrogel based on 20% BC, while having satisfactory thixotropic properties, does not meet the requirements due to low thixotropy energy values.

Table 16– Yield stress limits of ointment samples based on bentonite clay and THU-10

Sample	BC=20%, THU-10 = 0%	BC=25%, THU-10 = 0%	BC=30%, THU-10 = 0%	BC=20%, THU-10 = 1%	BC=20%, THU-10 = 2%	BC=20%, THU-10 = 3%
Yield stress	304 Pa	416,35 Pa	739,5 Pa	15,3 Pa	9,33 Pa	5,91 Pa
Sample	BC=25%, THU-10=1%	BC=25%, THU-10=2%	BC=25%, THU-10=3%	BC=30%, THU-10=1%	BC=30%, THU-10=2%	BC=30%, THU-10=3%
Yield stress	60,217 Pa	123,79 Pa	159,87 Pa	494,6 Pa	553 Pa	1110 Pa

Table 17 – Thixotropic energy indicators of ointments based on bentonite clay and THU-10

Sample	BC=20%, THU-10=0%	BC=25%, THU-10=0%	BC=30%, THU-10=0%	BC=20%, THU-10=1%	BC=20 %, THU-10=2%	BC=20% , THU-10=3%
Thixotropic energy	4 133,57 Pa/(s·ml)	24 120,95 Pa/(s·ml)	322 927,23 Pa/(s·ml)	2 75,03 Pa/(s·ml)	1 12,179 Pa/(s·ml)	31,633 Pa/(s·ml)
Sample	BC=25%, THU-10=1%	BC=25%, THU-10=2%	BC=25%, THU-10=3%	BC=30%, THU-10=1%	BC=30 %, THU-10=2%	BC=30% , THU-10=3%
Thixotropic energy	1132 Pa/(s·ml)	876,45 Pa/(s·ml)	548,18 Pa/(s·ml)	12 057,37 Pa/(s·ml)	8 995,36 Pa/(s·ml)	3262,84 Pa/(s·ml)

Thus, rheograms of the BC-THU-10 base and ointment samples were obtained, yield stress, viscosity, and thixotropic properties were determined, and thixotropic energies were calculated. Ointments based on 25% and 30% BC were considered to meet the requirements for ointments in terms of rheological properties.

3.6 Optimal ratios of components for obtaining hydrogels based on hydroxypropylmethylcellulose and THU-10

In order to prepare the hydrogels, it was necessary to consider the properties and characteristics of the intended formulation. For this purpose, optimal components and desired properties of the new ointment were selected. The main components of the formulation were HPMC and distilled water. Additional ingredients were included, such as glycerin and petrolatum for skin hydration, Tween-80 to ensure the stability and uniformity of the ointment, as well as for its

skin-softening properties. Potassium sorbate was also added to enhance the antimicrobial stability of the final product. The high hygroscopicity of glycerin prevents it from drying out or crystallizing and also helps prevent drying of the formulation when mixed with other substances. In this study, glycerin acts as a plasticizer in the preparation of hydrogels. The optimal component ratios are presented in Table 19 and Table 20.

Table 18 – Composition of Ointment Bases

Composition, %	**1**	**2**	**3**
HPMC	2	3	4
Glycerol	5	5	5
Sodium sorbate	0,1	0,1	0,1
Water	86,9	85,9	84,9
Twin-80	1	1	1
Vaseline	5	5	5

An additional series of experiments was conducted using a rheometer, focusing only on the complete formulations of the prepared ointments. The obtained hysteresis loops (Figures 38–43) confirm that the resulting gels exhibit thixotropic properties, which is highly beneficial for the performance of the final ointments. Thixotropy is essential for gel-ointment systems, as it allows the formulation to become more fluid under mechanical stress and effectively cover the wound. The flow behavior of the gel is non-Newtonian, since the application of shear rate or time affects the viscosity of the system. Polymer-based systems are characterized by pronounced flowability.

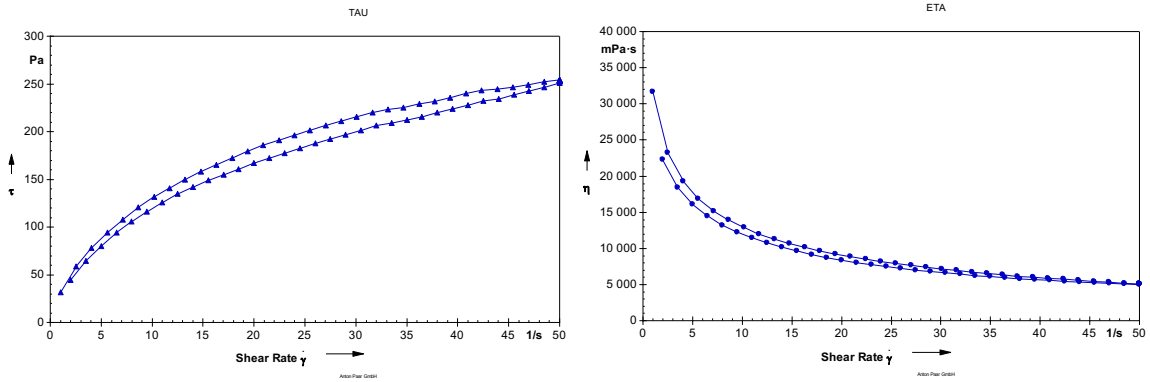
Table 19 - Composition of ointment bases and THU-10

Composition, %	**1**	**2**	**3**
HPMC	2	3	4
Glycerol	5	5	5
Sodium sorbate	0,1	0,1	0,1
Water	85,9	84,9	83,9
Twin-80	1	1	1
THU-10	1	1	1
Vaseline	5	5	5

Based on the rheological data of gels with and without the extract, it can be concluded that the viscosity of the gels containing the extract is lower than that of the gels without it. These observations are justified by the fact that when the polymer carrier interacts with the medicinal extract, it exhibits reduced viscosity

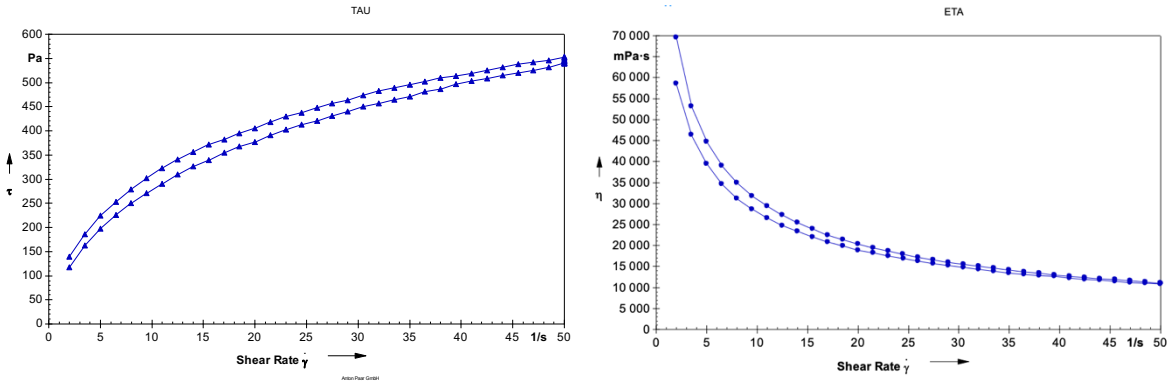
characteristics. The aqueous-alcoholic extract binds to the functional groups of the polymer, which leads to decreased viscosity.

The polymer ratio in the formulation affects the viscosity of the gel. As the amount of polymer in the gels increases, the viscosity of the system also increases.



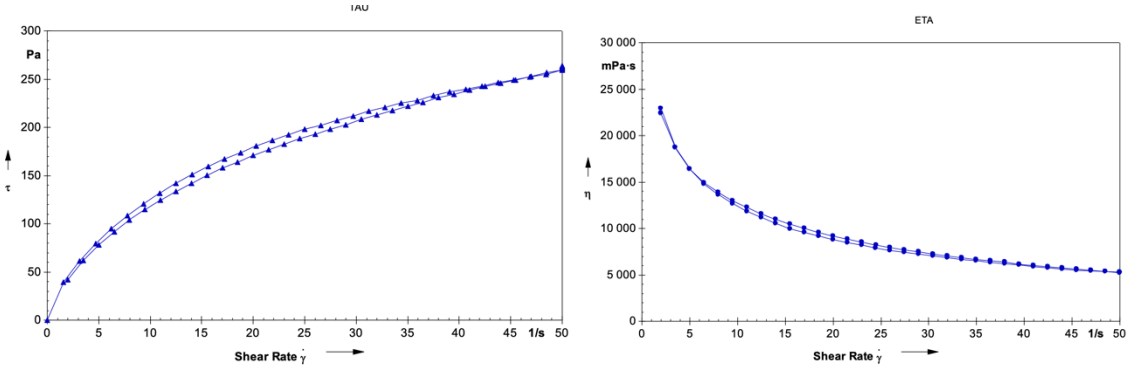
[HPMC] = 2%; [H2O] = 86,9%; [glycerol] = 5%; [Tween-80] = 1%; [Potassium sorbate] = 0,1%; [Vaseline] = 5%; t=25◦C

Figure 38 – Hysteresis of the hydrogel base



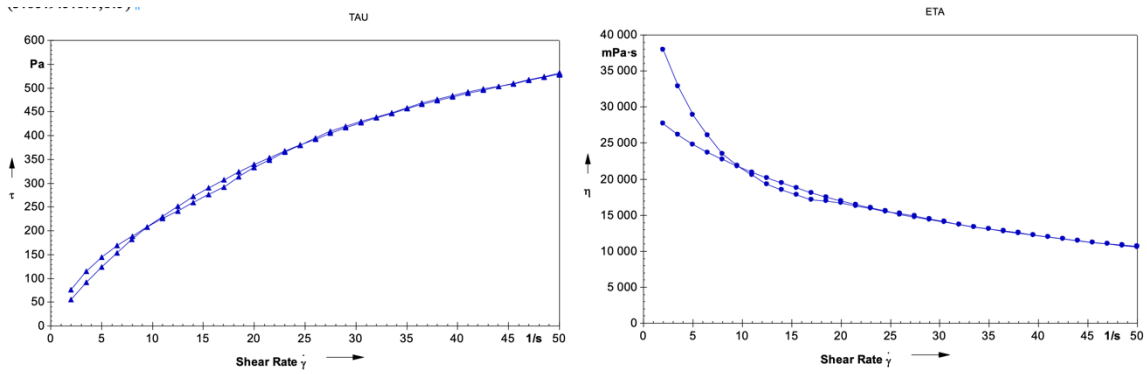
[HPMC] = 4%; [H2O] = 84,9%; [glycerol] = 5%; [Tween-80] = 1%; [Potassium sorbate] = 0,1%; [Vaseline] = 5%; t=25◦C

Figure 39 –Hysteresis of the hydrogel base



[HPMC] = 2%; [H2O] = 85,9%; [glycerol] = 5%; [Tween-80] = 1%; [Potassium sorbate] = 0,1%; [Vaseline] = 5%; [THU-10]=1% t=25◦C

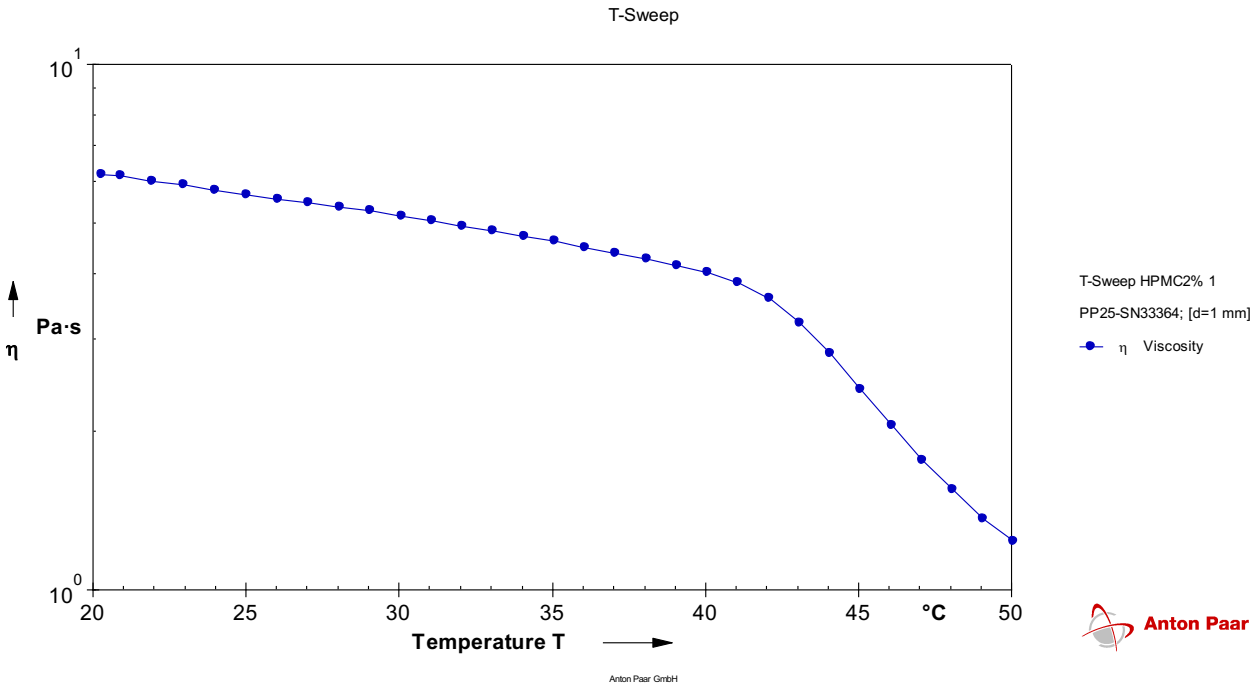
Figure 40 – Hysteresis of the hydrogel Base with THU-10



[HPMC] = 4%; [H2O] = 83,9%; [glycerol] = 5%; [Tween-80] = 1%; [Potassium sorbate] = 0,1%; [Vaseline] = 5%; [THU-10]=1% t=25◦C

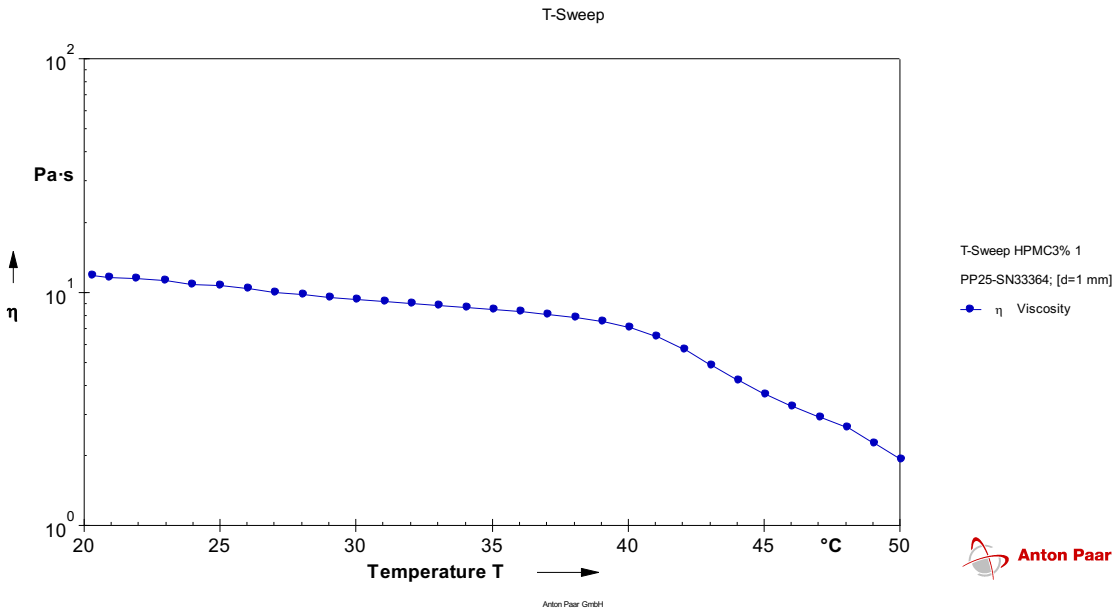
Figure 41 – Hysteresis of the hydrogel Base with THU-10

The obtained gels were studied, and the dependence of the viscosity of the polymer systems on temperature was determined. Based on the resulting graphs, it can be concluded that as the temperature increases, the viscosity of the polymer gel decreases, and it transitions into a liquid state at different gel ratios.



[HPMC] = 2%; [H2O] = 86,9%; [glycerol] = 5%; [Tween-80] = 1%; [Potassium sorbate] = 0,1%; [Vaseline] = 5%; t=25◦C

Figure 42 – Determining the dependence of viscosity on temperature



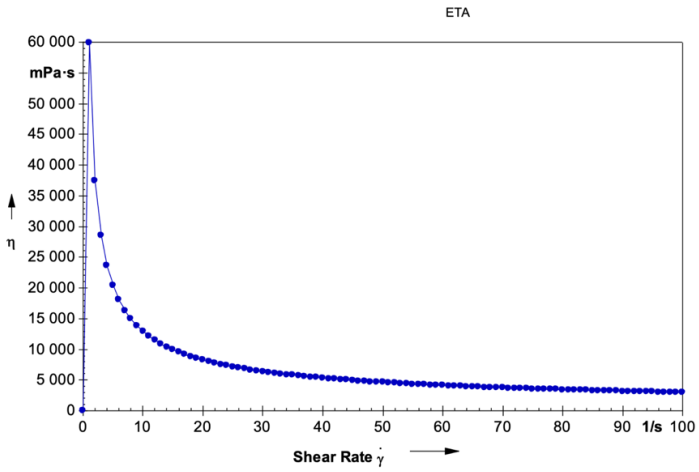
[HPMC] = 3%; [H2O] = 85,9%; [glycerol] = 5%; [Tween-80] = 1%; [Potassium sorbate] = 0,1%; [Vaseline] = 5%; t=25◦C

Figure 43 – Determining the dependence of viscosity on temperature

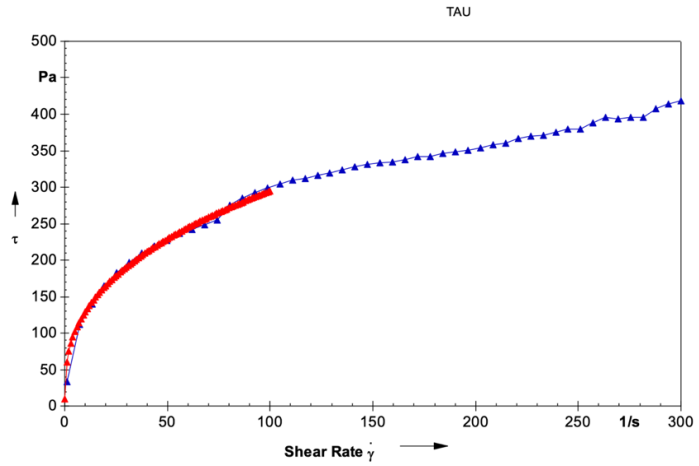
3.6.1 Rheological studies

The rheological characteristics of hydrogel ointments were studied to determine their viscosity and thixotropy in accordance with the quality requirements for soft dosage forms. Samples of hydrogel ointments based on hypromellose without medicinal substances were presented for the study. Before the study, to obtain correct data, the measuring systems were calibrated in accordance with the operating instructions for the rheometric device MCR 102 Anton Paar (Germany).

The most important rheological characteristic of a dispersed system is viscosity – a measure of resistance to the movement of one layer of liquid relative to another under the action of external forces. Consequently, the dependence of viscosity and stress rate on the shear rate at a constant temperature of 20°C was determined for hydrogel ointments based on hydroxypropyl methylcellulose (HPMC) without a medicinal substance in an amount of 2, 3, 4, 5, 7%. total weight of the ointment. The obtained data are presented in the form of graphs of the dependence of viscosity and stress rate on the shear rate – rheograms (Figures 44-47).

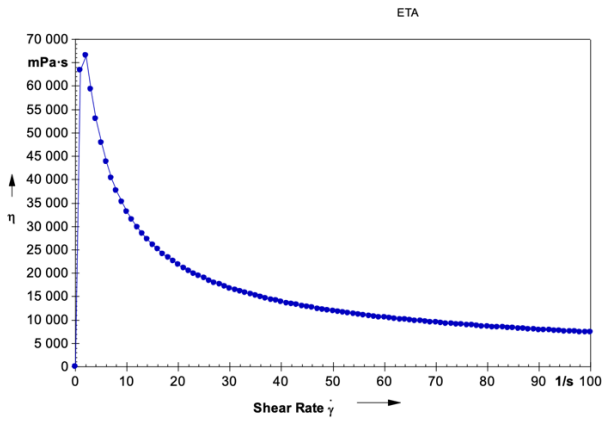


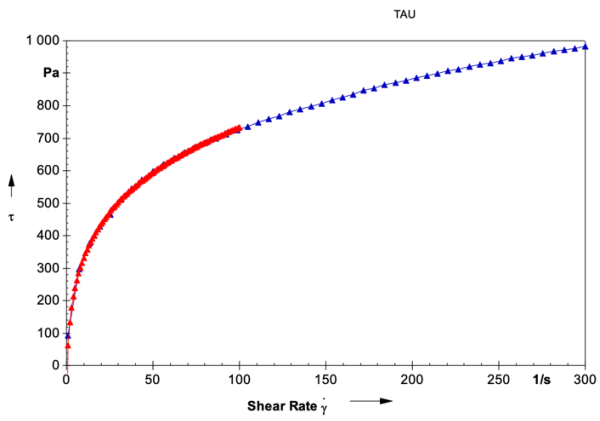
(a)



(b)

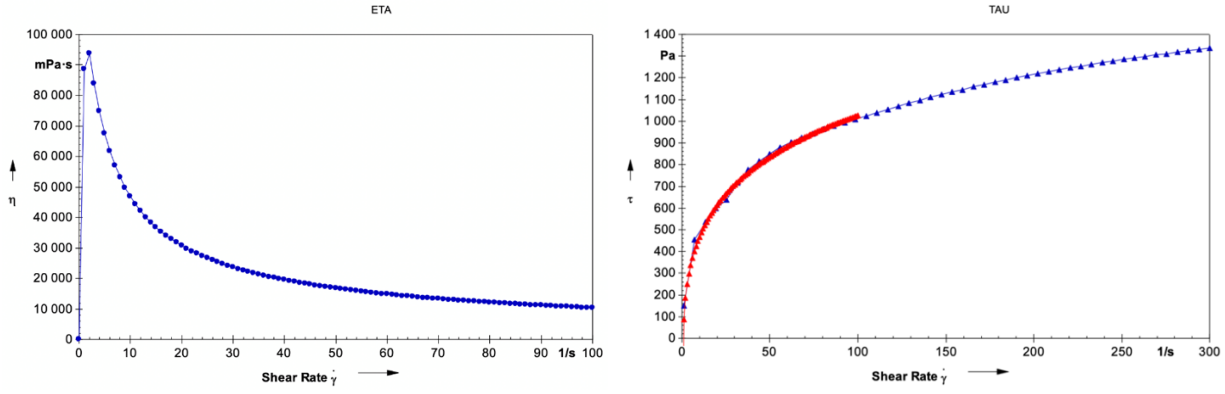
Figure 44 (a, b) – Deformation from shear stress (HPMC 2%)





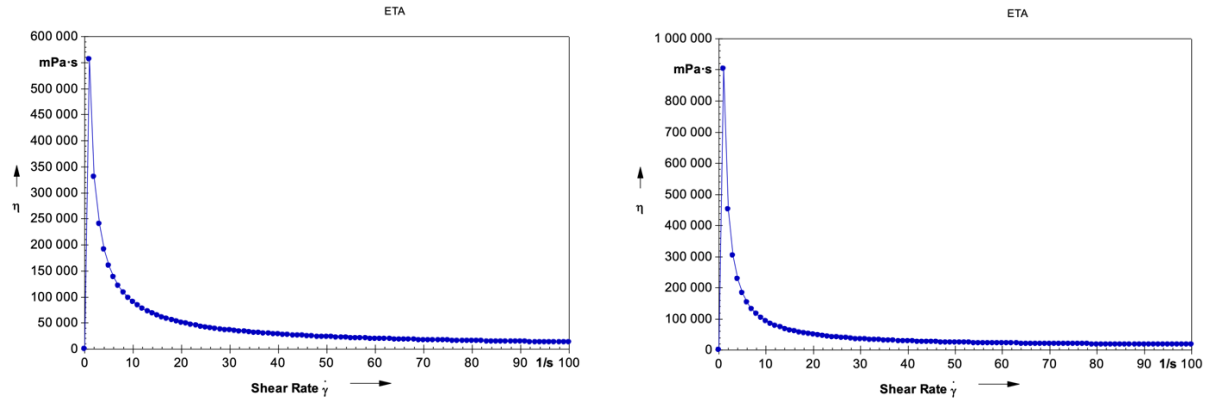
(a) (b)

Figure 45 (a, b) – Deformation from shear stress (HPMC 3%)



a b

Figure 46 (a, b) – Deformation from shear stress (HPMC 4%)



a b

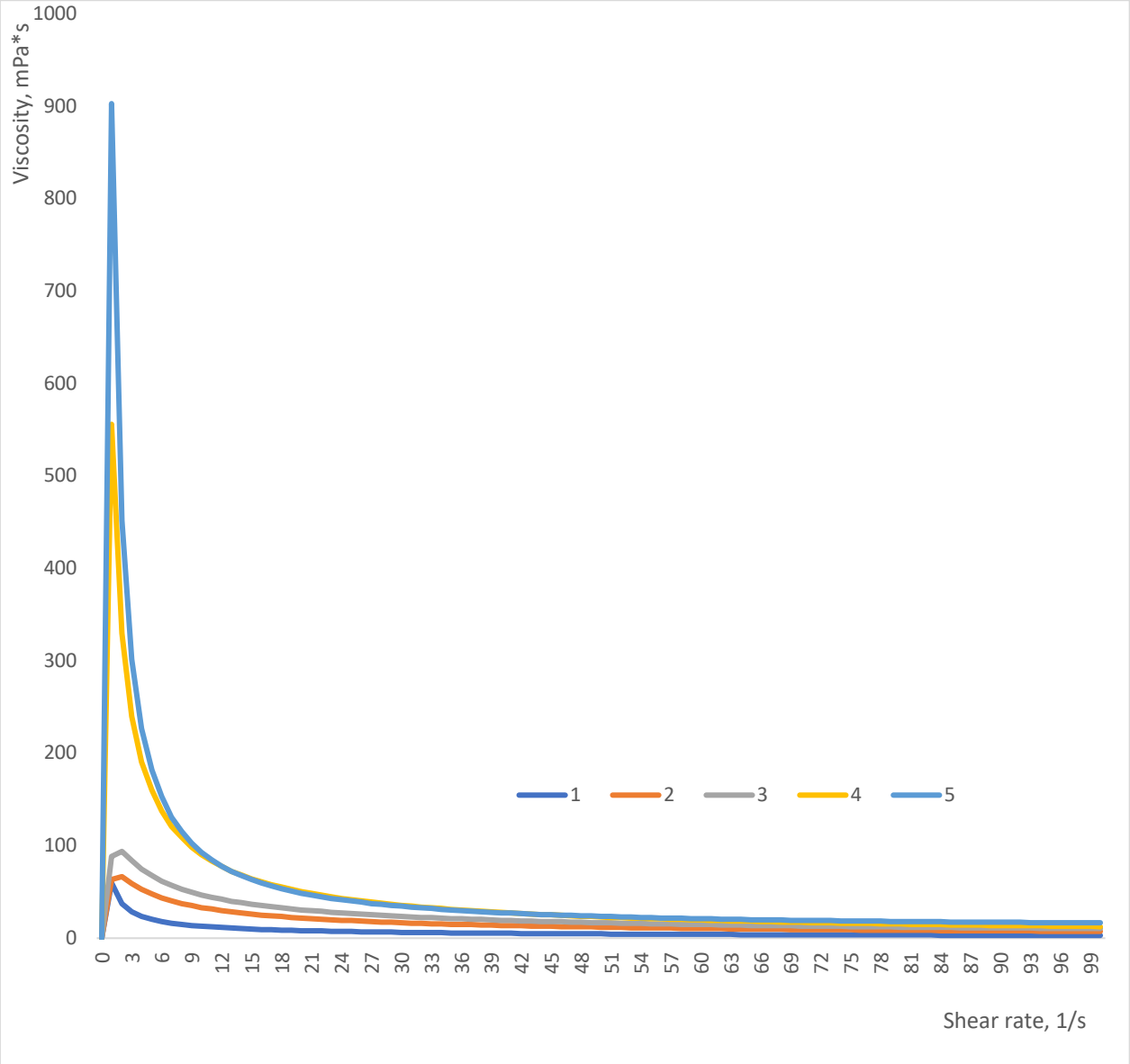
Figure 47 (a, b) – Deformation from shear stress (HPMC 5, 7%)

At present, various primary rheological properties (rheological optimums of consistency and spreadability) of hydrophilic and lipophilic ointments have been experimentally determined, which determine their optimal consistency from a consumer point of view. To assess the consistency of the ointment, rheograms of its fluidity are constructed at shear rates in the range from 1.5 to 1312 s^{-1} at 20°C (the estimated storage temperature of the ointment). The rheological optimum of the consistency of hydrophilic ointments in this range of shear rates is determined by the yield point of 45–160 Pa and the effective viscosity of 0.34–108 Pa·s. The rheological optimum consistency of lipophilic ointments is characterized by a yield point of 35–140 Pa and an effective viscosity of 0.32–93.3 Pa·s.

For comparison and evaluation of the obtained results, the data and graphs of the viscosity and shear rate dependence of the obtained hydrogel ointments based on hydroxypropyl methylcellulose (HPMC) without a medicinal substance in the amount of 2, 3, 4, 5, 7. % of the total mass of the ointment are given and are indicated in the general table and on the general graph. In the table (Table 20), the maximum deformation values of each obtained hydrogel ointment are taken at the corresponding ratios of hypromellose to water and glycerin.

Table 20 – Maximum deformation values at different ratios of HPMC to the total mass of the ointment.

Mass (HPMC), %	2	3	4	5	7
Viscosity, mPa·s	59,9	66,6	93,9	556	903



(1) [HPMC]-2%; (2) [HPMC]-3%; (3) [HPMC]-4%; (4) [HPMC]-5%; (5) [HPMC]-7%;

Figure 48 – Dependence of viscosity on shear rate at different ratios of HPMC mass to total mass

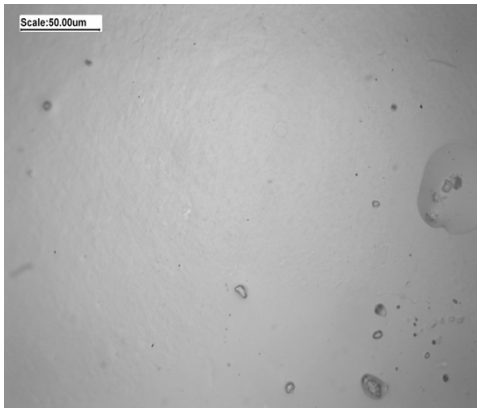
Of the five hydrogel ointments obtained, the most viscous was the sample with a hypromellose mass of 0.7 grams per 8.3 milliliters of water and 1 milliliter of

glycerin. At the same time, the sample in the amount of 0.2 grams to the total mass of the ointment showed low viscosity. These observations show that the viscosity of the obtained hydrogel ointment depends on the ratio of the components used in the preparation of the hydrogel ointment. The greater the mass of HPMC and the smaller the volume of water, the more viscous the hydrogel ointment is. Hydrogel graphs show the properties of non-Newtonian fluids because their nonlinear nature is visible from the graphs. When gels are studied at low shear rates, the structure quickly breaks down and reforms, and this is when the gels exhibit high viscosity. Viscosity decreases as the shear rate increases. When the shear rate reaches high values, the polymer system begins to flow, i.e., the structure is completely destroyed.

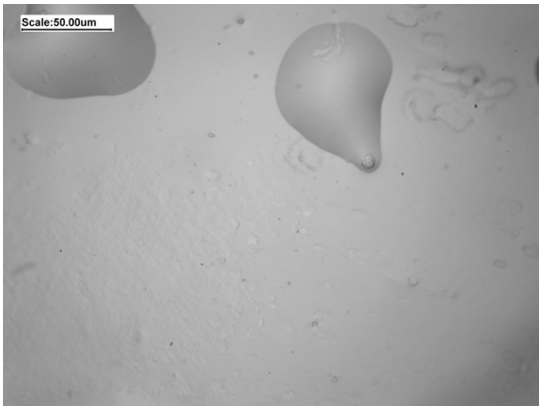
3.6.2 Morphological description of structures

To determine the morphological structure of the obtained gels and determine the density, studies were carried out under an optical and scanning electron microscope. Based on the images of the studied gels through an optical microscope, it can be concluded that the surface of the gels is smooth and non-porous.

Optical microscope

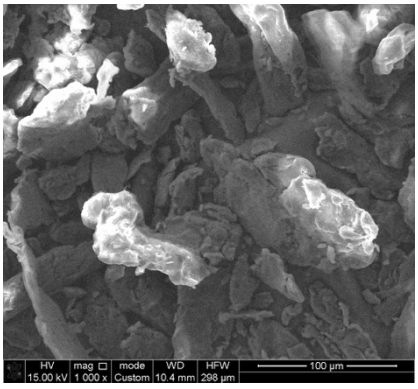


Hydrogel HPMC 3% (1)

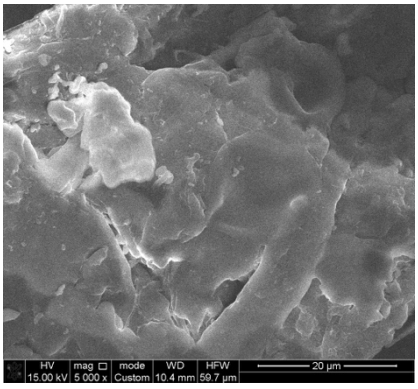


Hydrogel HPMC 3% (2)

SEM



HPMC



Hydrogel HPMC 3%

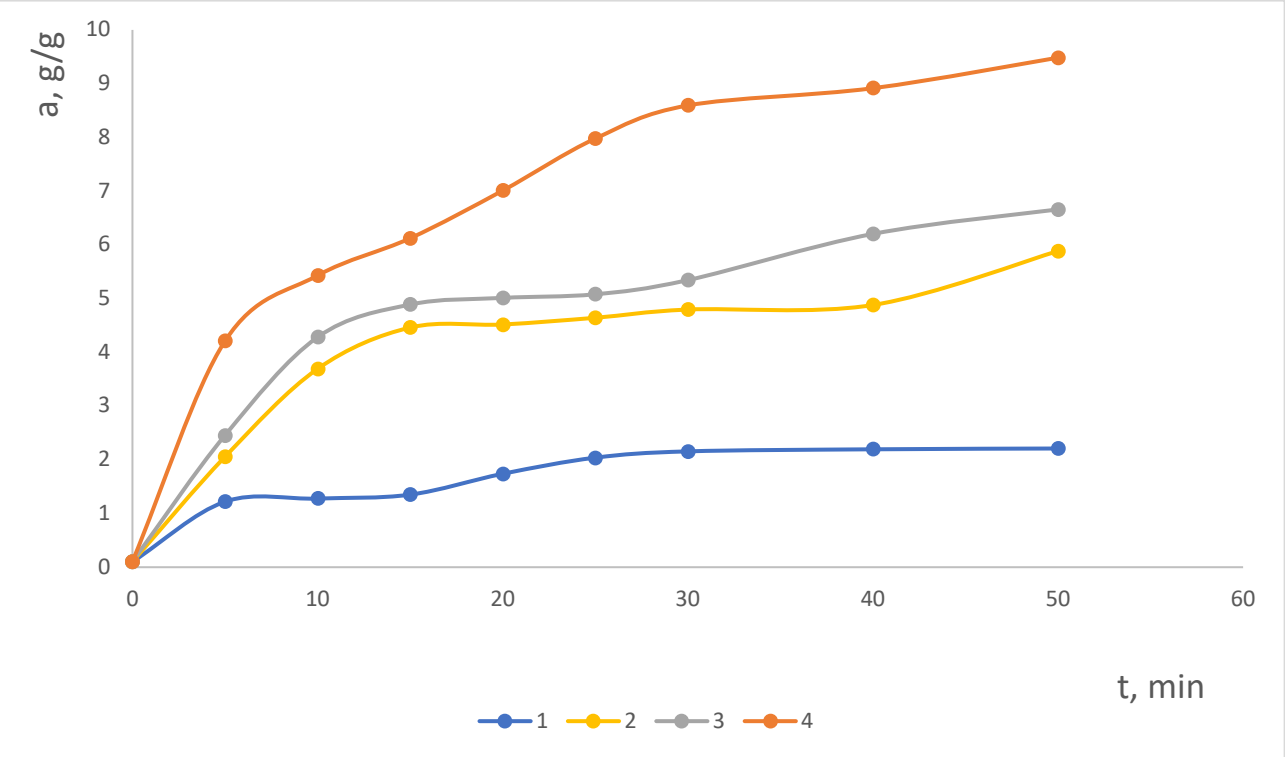
Figure 49 – Images of gels examined by SEM and optical microscope.

Compared with the results obtained using a scanning electron microscope, it can be said that the gel sample has a non-uniform, weakly porous structure. Based on the images of the gels, it can be assumed that the low porosity of the obtained samples indicates that the gels release the drug better (Figure 49).

3.6.3 Study of swelling of gels

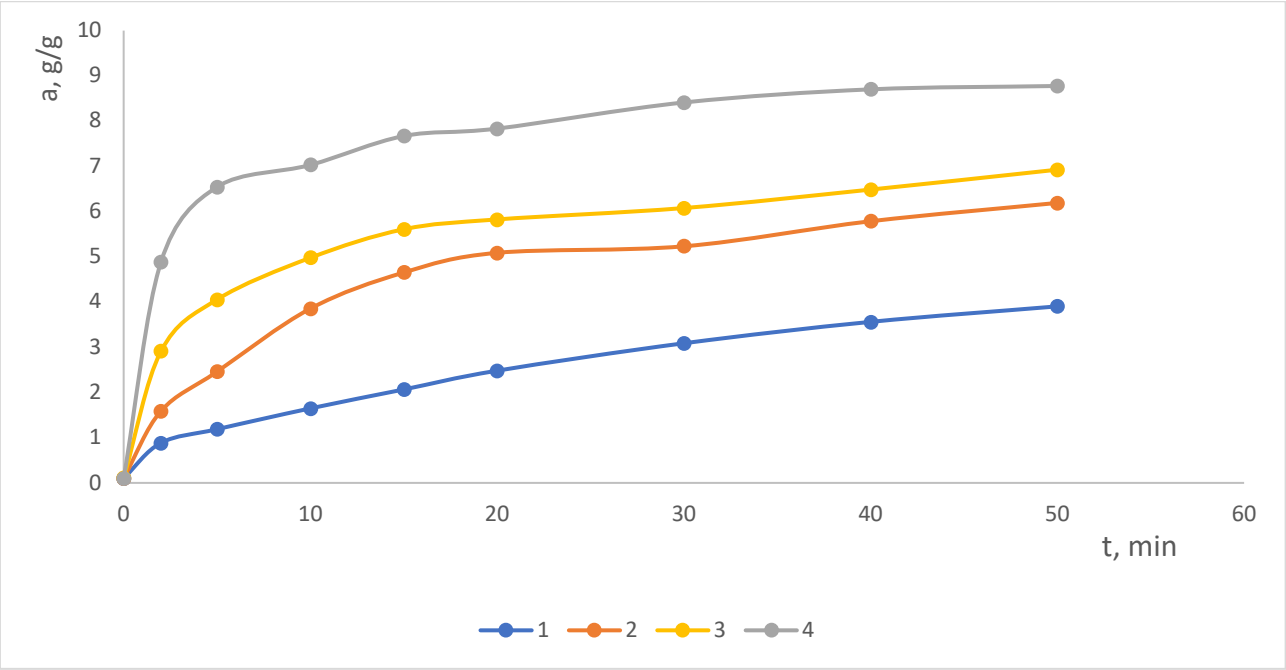
The swelling kinetics of the obtained gels is necessary to obtain information about the absorption properties of the gels. The swelling degree, expressed as a percentage, shows the ability of the gel to absorb wound exudate from the affected area, since our ointments will be used for open wounds and burns. Based on the results obtained, it can be concluded that with an increase in the amount of polymer in the composition, the swelling ability increases (Fig. 50, 51, 52). However, when using ointments, it should be borne in mind that there is no need for too high swelling and wound exudate absorption ability, since this can hinder rapid healing.

The ointment should create a favorable environment during treatment, so it is desirable that the ointment moisturizes the wound and can absorb unfavorable fluids from the wound, such as pus or blood. Gels contain glycerin, which helps to impart moisturizing properties to wounds and injuries. The wound healing process is influenced by many factors.



[HPMC]=1%(1), [HPMC]-2%(2), [HPMC]-3%(3), [HPMC]-4%(4).

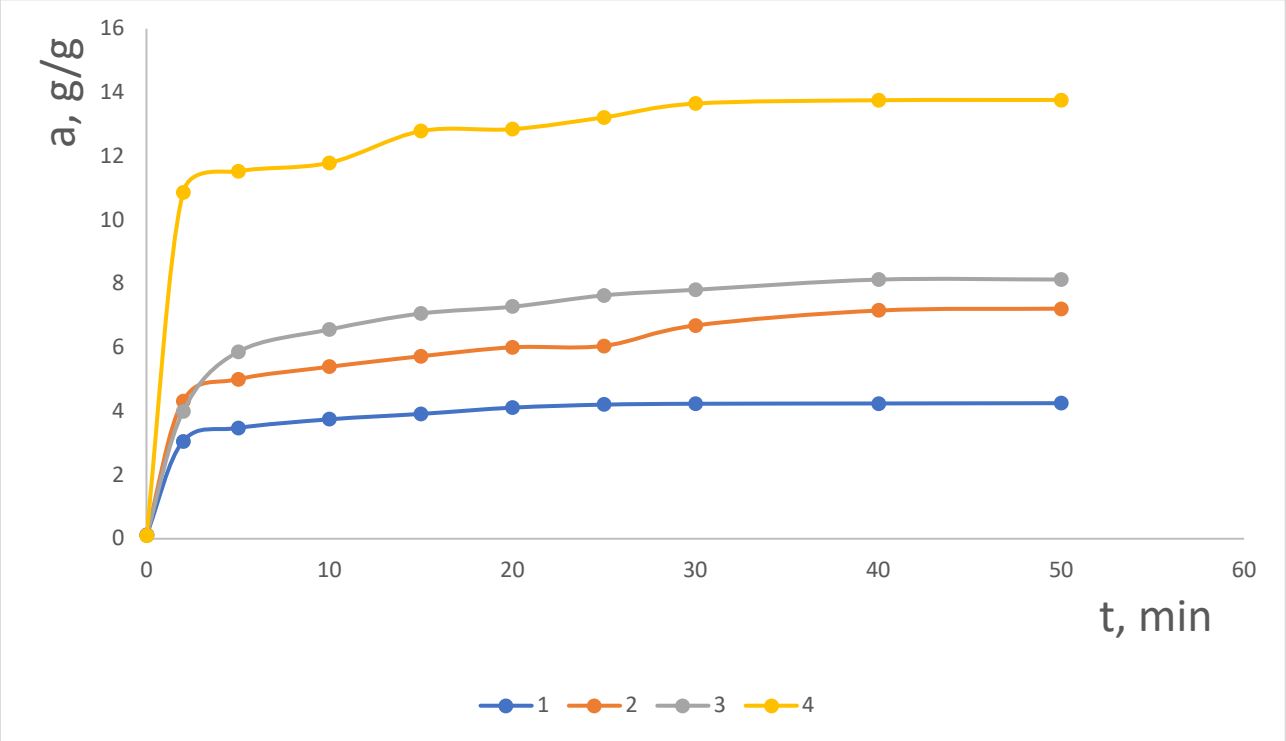
Figure 50 – Kinetics of swelling of gels without extract in water



[THU-10]=1%;

[HPMC]=1%(1), [HPMC]-2%(2), [HPMC]-3%(3), [HPMC]-4%(4).

Figure 51 – Kinetics of swelling of gels with extract in water



[THU-10] =1%;

[HPMC]=1%(1), [HPMC]-2%(2), [HPMC]-3%(3), [HPMC]-4%(4).

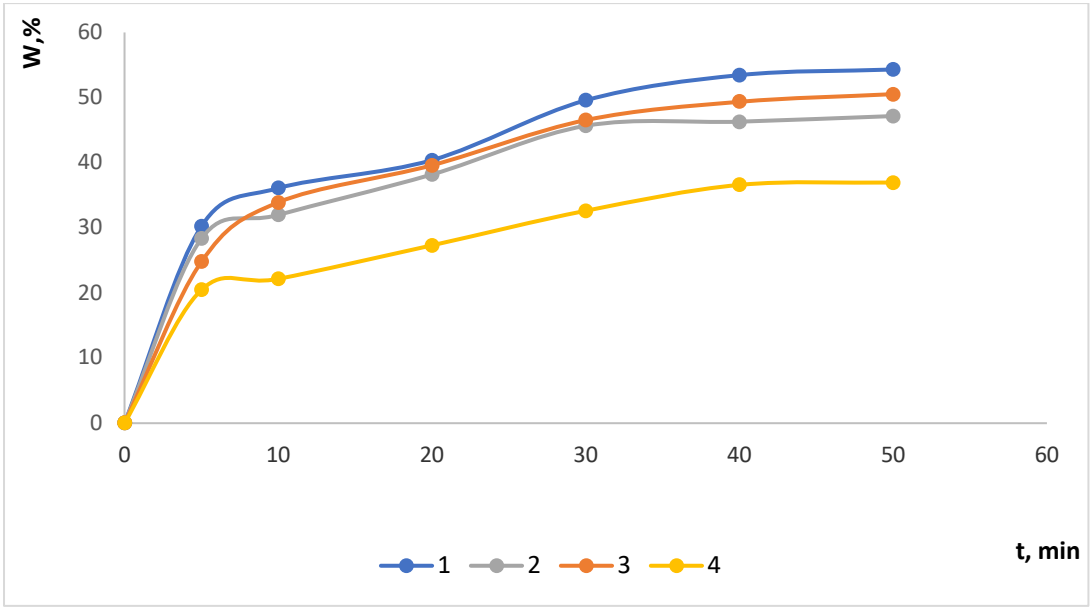
Figure 52 – Kinetics of swelling of gels with extract in physical solution

Based on the data obtained during swelling of gels without and with extract in an aqueous medium, it can be said that the swelling degree of gels with extract is somewhat lower. This indicator is very small and not critical. This may mean that gels with extract swell less due to the fact that when the gels are immersed in water, the plant extract is released into the water, so the swelling degree is somewhat reduced.

In order to test the interaction of the obtained gels with the human body, namely with the wound, it was decided to test the swelling of the gels in a physical solution. According to the data obtained when comparing the results of the behavior of the gels in two environments, it can be concluded that the swelling of gels with an extract in the composition in a physical environment is higher than in an aqueous one. This process can be explained by the fact that in the presence of an electrolyte, in our case NaCl, cations are introduced between the dissociated functional groups of hypromellose, which allows them to linger and exhibit strong swelling of the gel. Gels in an aqueous environment begin to dissolve over time, since dissociation occurs in an aqueous environment.

3.6.4 Drug release

In order to estimate the amount of the drug released into the environment, it was decided to study the kinetics of THU-10 desorption in aqueous and physical environments. As can be seen from the graph (Fig. 53), the percentage of release depends on the polymer content in the gel. With an increase in the composition of Hypromellose, the release of the extract decreases. This observation can be characterized by the fact that with an increase in the amount of polymer in the composition, the interaction of its functional groups with groups in the plant extract increases, which indicates a greater interaction between the polymer carrier and the plant. extract. The lower the concentration of the polymer in the gel, the greater the release of the drug and vice versa.

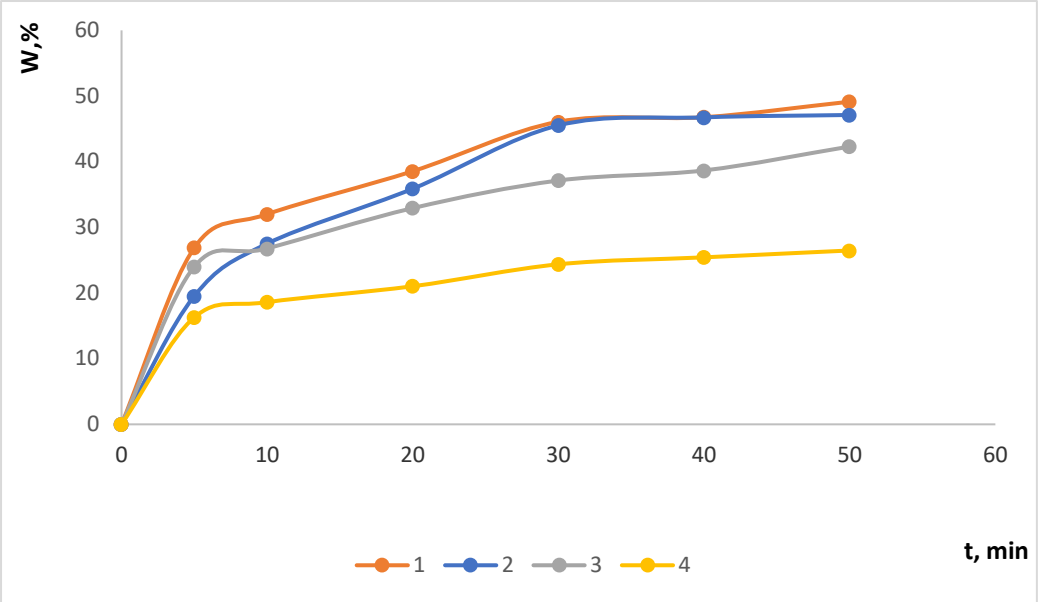


[THU-10]=1%;

[HPMC]=1%(1), [HPMC]-2%(2), [HPMC]-3%(3), [HPMC]-4%(4).

Figure 53 – Quantitative calculation of the yield of THU-10 from gels in water

NaCl is a strong electrolyte. In solution with water, the strong electrolyte dissociates into ions. When the extract was released from the gel into a physical solution, it was observed that the percentage of release decreased (Figure 54, 55). This observation suggests that the process is influenced by osmotic pressure, which causes the polymer network to contract, and the ions in the solution prevent the release.



[Glycerol] =5%; [THU-10]=1%;
[HPMC]=1%(1), [HPMC]-2%(2), [HPMC]-3%(3), [HPMC]-4%(4).

Figure 54 – Quantitative calculation of the yield of THU-10 from gels in physical solution

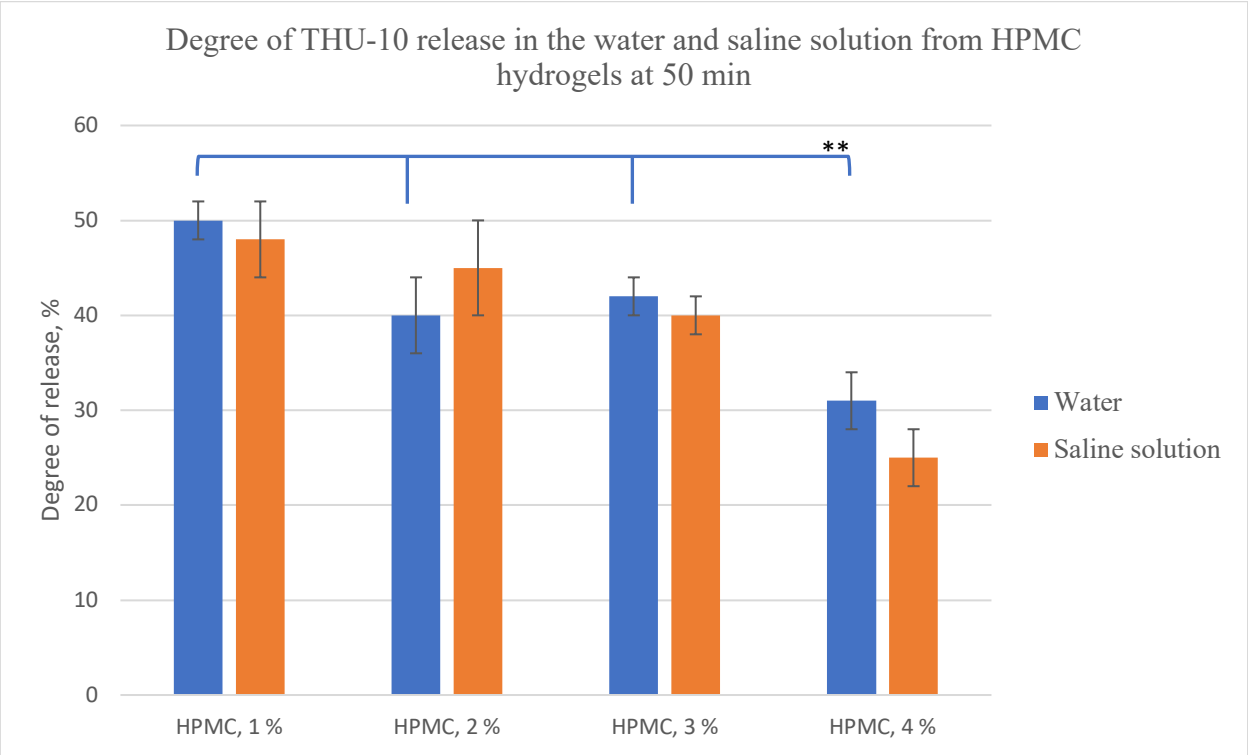


Figure 55 – Yield of the THU-10 release in the water and saline solution from HPMC hydrogels in different concentrations. The data are presented as the Mean values ± standard deviation (n=3, p<0,01)

Based on the work performed and the results obtained, the following conclusions can be made:

1. For the first time, an ointment based on hypromellose with an extract of the *Tamarix Hispida* plant was developed and their interaction was studied.

2. The interaction between hypromellose and the extract of Tamarix Hispida was studied.

3. The rheological parameters of the obtained gels were studied, which showed thixotropic, non-Newtonian and pseudoplastic properties. The swelling capacity of the gel forms in an aqueous medium and a physiological solution (0.9%) was studied, it was found that the degree of swelling of the gel depends on the concentration of hypromellose.

A study was conducted on the desorption (release) of *Tamarix Hispida* extract molecules from a polymer base (hypromellose).

The maximum release of THU-10 was:

- in water: 56% (at 1% HPMC)
- in saline: 50% (at 1% HPMC)

Based on all the data obtained, the optimal composition of the ointment was determined in the following ratio of components:

- Hypromellose (HPMC) – 4%
- THU-10 - 1%
- Glycerin - 5%
- Potassium sorbate - 0.1%

• Tween-80 - 1%
• Vaseline - 5%
• Water - 83.9%

This composition demonstrated optimal rheological properties, release kinetics and swelling.

3.6 **Possibility of obtaining composite hydrogels based on natural polymers HPMC and BC with THU-10 extract**

Figure 56 shows the results of IR spectroscopy analysis of a hydrogel based on 4% HPMC. Figure 57 shows the results of X-ray phase analysis of a gel based on 4% HPMC. The obtained sample has a completely amorphous structure. The absence of sharp peaks characteristic of clear crystalline phases in the graph and the observation of a broad diffraction maximum confirm that the gel is X-ray amorphous.

As a result of quantitative evaluation of the phase composition, it was determined that the amorphous phase of the sample is 100%. This is due to the polymeric nature of HPMC and its disordered molecular structure. The presence of an amorphous structure increases the water absorption capacity, flexibility and stability of the gel, allowing it to be used in medicine.

In the IR spectrum of bentonite clay, absorption bands observed in the 3627–3431 cm^{-1} regions indicate the presence of structural and adsorbed water molecules. Strong absorption bands at around 1036 cm^{-1} correspond to tetrahedral Si–O–Si and Si–O–Al bonds. In addition, vibrations characteristic of octahedral Al–Al–OH or Al–OH–Al bonds are observed in the 914 cm^{-1} region (Figure 58).

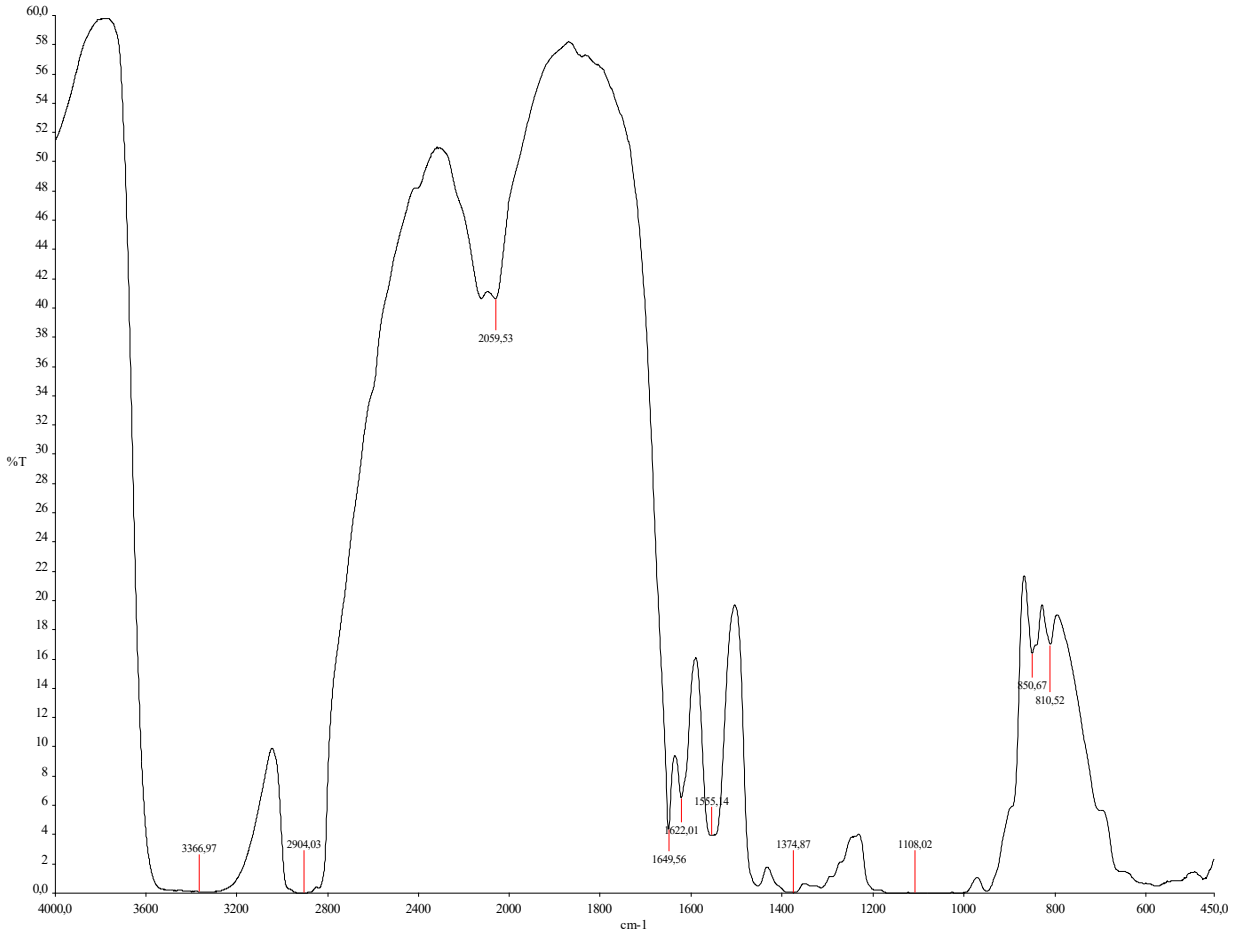


Figure 56. IR spectroscopy results of a 4% HPMC-based gel

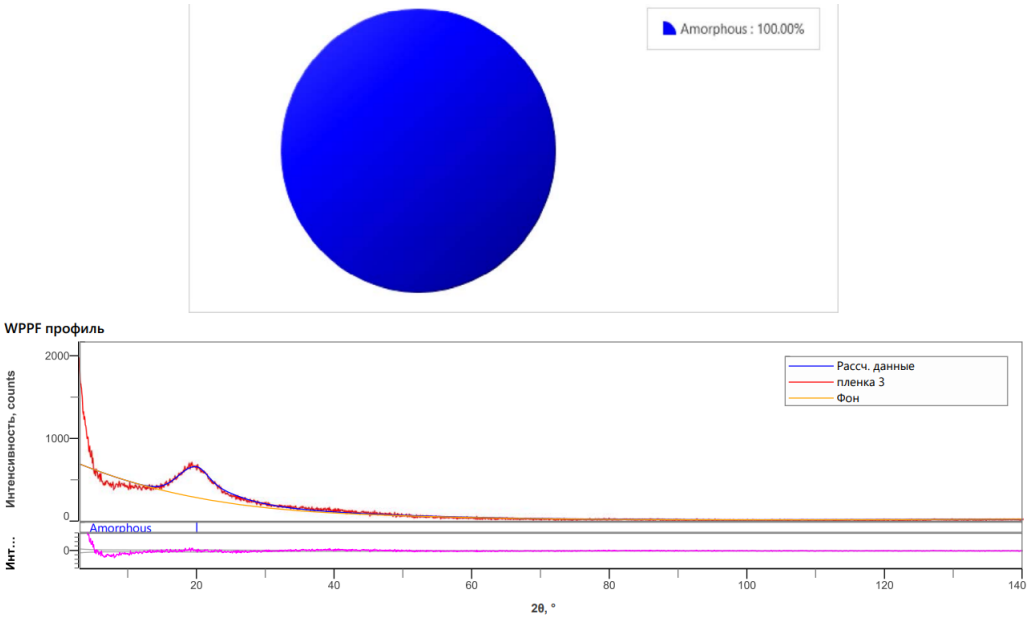


Figure 57. X-ray phase analysis of a gel based on 4% HPMC

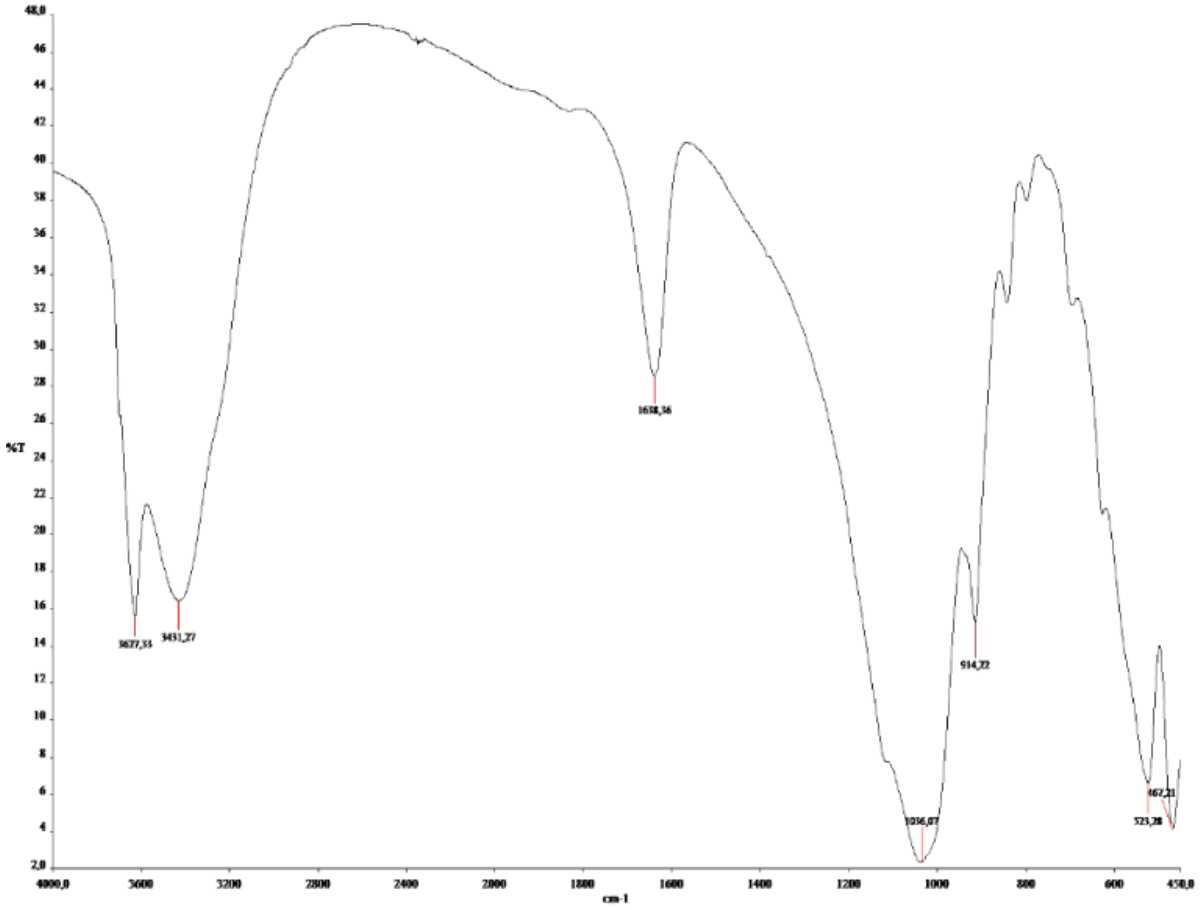


Figure 58 – IR spectra of bentonite clay

The IR spectrum of the THU-10 extract obtained from the plant material of *Tamarix Hispida* using an ultrasonic bath was recorded (Figure 59). It is known that the THU-10 extract contains biologically active substances such as polyphenols, terpenoids, a complex of steroids, and tannins. According to the research results, the IR spectrum absorption region at 3400 cm^{-1} corresponds to the –OH functional groups of flavonoids and hydrolysable tannins. In addition, the absorption bands at 3400 cm^{-1} and 1622 cm^{-1} correspond to O–H, C=O, and C=C bonds, specifically indicating the presence of terpenoids. Intermolecular hydrogen bonds are observed in the absorption region at 1433 cm^{-1}.

In Figure 60, the IR spectroscopic analysis of the composite gel was carried out, and absorption bands characteristic of functional groups were recorded. The main absorption bands in the spectrum were observed in the following wavenumber regions:

3467.13 cm^{-1} – this broad absorption band indicates the presence of phenolic or alcoholic –OH groups, broadened due to hydrogen bonding.

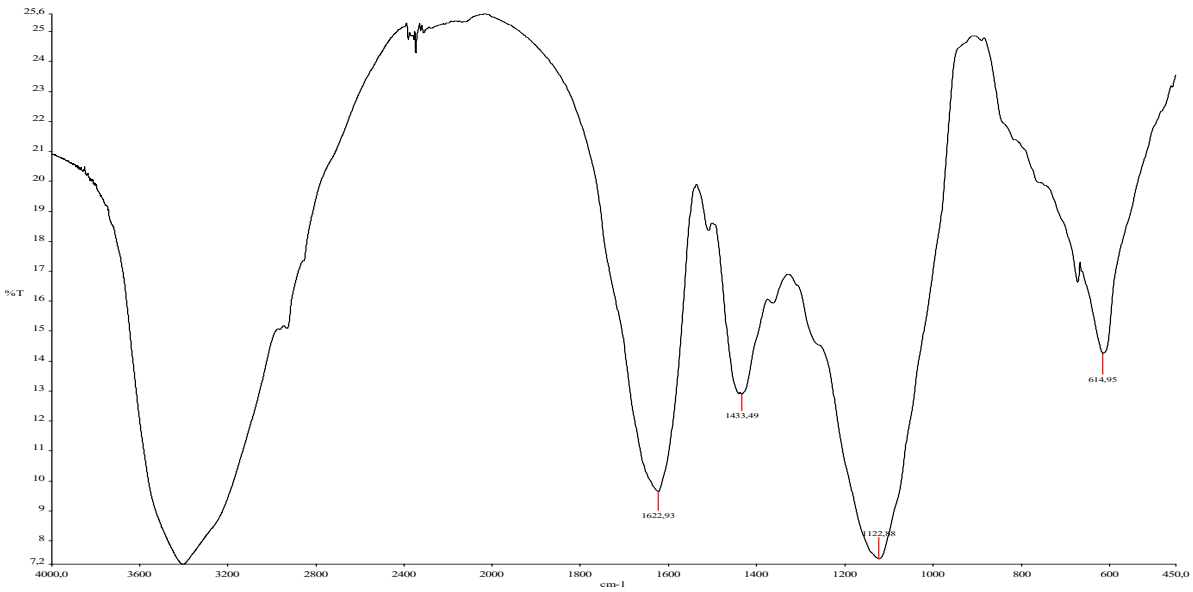


Figure 59 – IR spectrum of THU-10 extract

2931.14 cm^{-1} – this band is characteristic of $–CH_2$ and $–CH_3$ groups, indicating the presence of aliphatic hydrocarbon chains

2059.21 cm^{-1} – this region usually corresponds to cumulative systems or triple bonds, such as alkynes or isocyanates.

1641.79 cm^{-1} – these absorption bands are characteristic of C=C or C=O groups, indicating the presence of an unsaturated compound or a carbonyl group.

1455.47 cm^{-1} and 1374.57 cm^{-1} – these bands correspond to the deformation vibrations of CH_2/CH_3 groups.

1315.52 cm^{-1} and 1123.48 cm^{-1} – characteristic of C–O or C–N bonds, which may indicate the presence of ether or amine groups in the extract.

1123.48 cm^{-1} – also corresponds to C–O–C bonds or vibrations typical of amines.

949.24 cm^{-1} – this frequency usually corresponds to =C–H deformation vibrations, commonly found in alkenes or aromatic systems. In some cases, it may also be observed in heterocyclic compounds such as furan or pyran.

850.67 cm^{-1} – vibration of the aromatic ring, indicating the presence of cyclic structures.

571.44 cm^{-1} and 471.05 cm^{-1} – these absorption bands are mainly characteristic of structures bonded with heavy atoms or may represent ring vibrations. As a result of IR spectroscopic analysis, it was determined that the TH-10 extract consists of organic compounds containing hydroxyl (–OH) groups, aliphatic hydrocarbon chains ($–CH_2$, $–CH_3$), aromatic rings, carbonyl (C=O) groups, and possibly ether (C–O) or amine (C–N) groups.

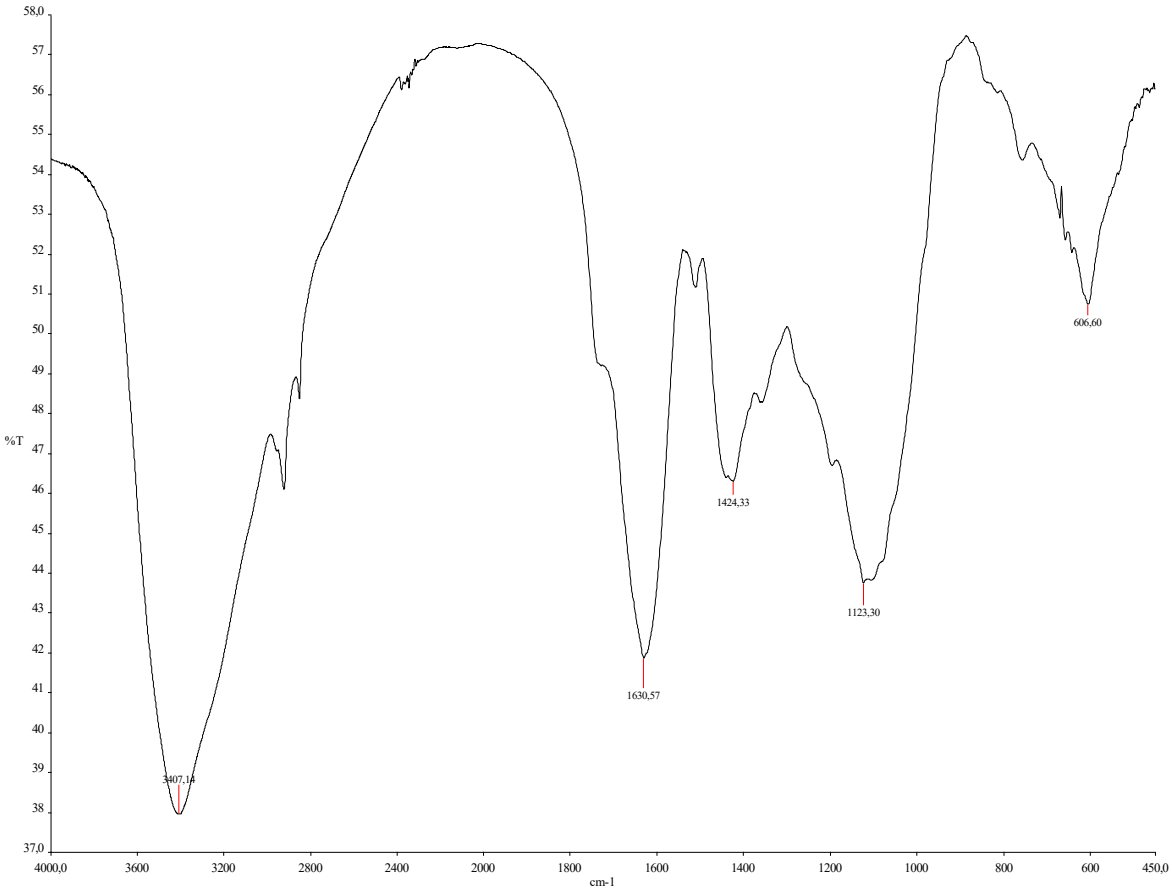


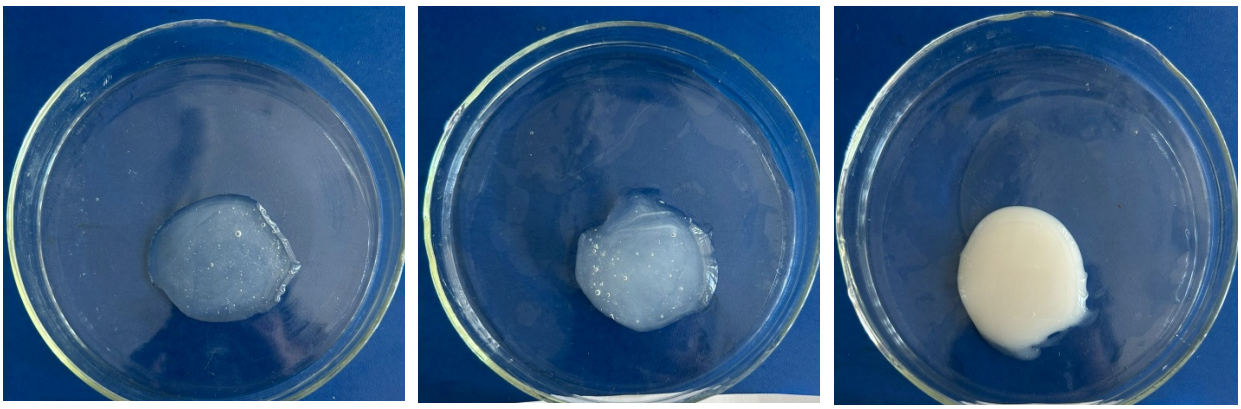
Figure 60 – IR spectroscopic analysis of the composite gel

As for the method of obtaining HPMC gel in different ratios, first it is necessary to dissolve HPMC by the “hot-cold” method.

The required amount of bentonite clay was taken and mixed with water for 1 hour on a magnetic stirrer. After 1 hour, the two suspensions were combined and kept on a magnetic stirrer for another 1 hour. The most effective consistency was 1:9, 2:8. The gel in the 3:7 ratio did not mix properly due to the high content of bentonite clay (Figure 61, Table 21).

Table 21. Composition of HPMC gel at different ratios

Content	HPMC: BC 1:9, g	HPMC: BC 2:8, g	HPMC: BC 3:7, g
Bentonite clay	0,4	0,8	1,2
HPMC	3,6	3,2	1,2
H_2O	96	96	96



a b c

Figure 61. HPMC:BC gels (a – HPMC: BC 1:9, b – HPMC: BC 2:8, c – HPMC: BC 3:7)

Figure 62 shows the results of IR spectroscopy of a gel based on bentonite clay and HPMC. The data obtained by IR spectroscopy allow us to identify the main functional groups in the gel. The absorption bands recorded in the spectrum are interpreted as follows:

The broad band in this region at 3467.13 cm^{-1} corresponds to O-H stretching vibrations, indicating that the gel contains hydroxyl groups and that they give rise to the broad band by forming hydrogen bonds.

This band at 2931.14 cm^{-1} is characteristic of the C-H stretching vibrations of methyl ($-CH_3$) and methylene ($-CH_2$) groups, which characterizes the organic structure of the HPMC polymer.

The weak absorption bands in this specific region at 2059.21 cm^{-1} may indicate the presence of weak interactions or complex formations between HPMC and bentonite.

This band at 1641.79 cm^{-1} corresponds to the H-O-H bending vibrations of water molecules, confirming that the gel is in a hydrated state.

The absorption in this region at 1455.47 cm^{-1} may correspond to bending vibrations of methyl groups or signals characteristic of the aluminosilicate structure in bentonite.

This band at 1315.22 cm^{-1} is characteristic of –CH bending vibrations, which characterizes the structure of the HPMC polymer.

The absorption in this region at 1123.48 cm^{-1} corresponds to the vibrations of the C-O-C bond, which indicates the presence of ester groups in the HPMC structure.

These bands at 850.67 cm^{-1}, 571.44 cm^{-1} and 470.95 cm^{-1} correspond to the bending vibrations of the Si-O-Si and Al-O-Si bonds in bentonite, which indicates that the natural structure of bentonite is preserved. In general, the obtained IR spectroscopic data indicate the presence of hydroxyl, methyl, methylene, ether and aluminosilicate structures in the gel. This characterizes the structural stability of the gel and the interaction of the components in it.

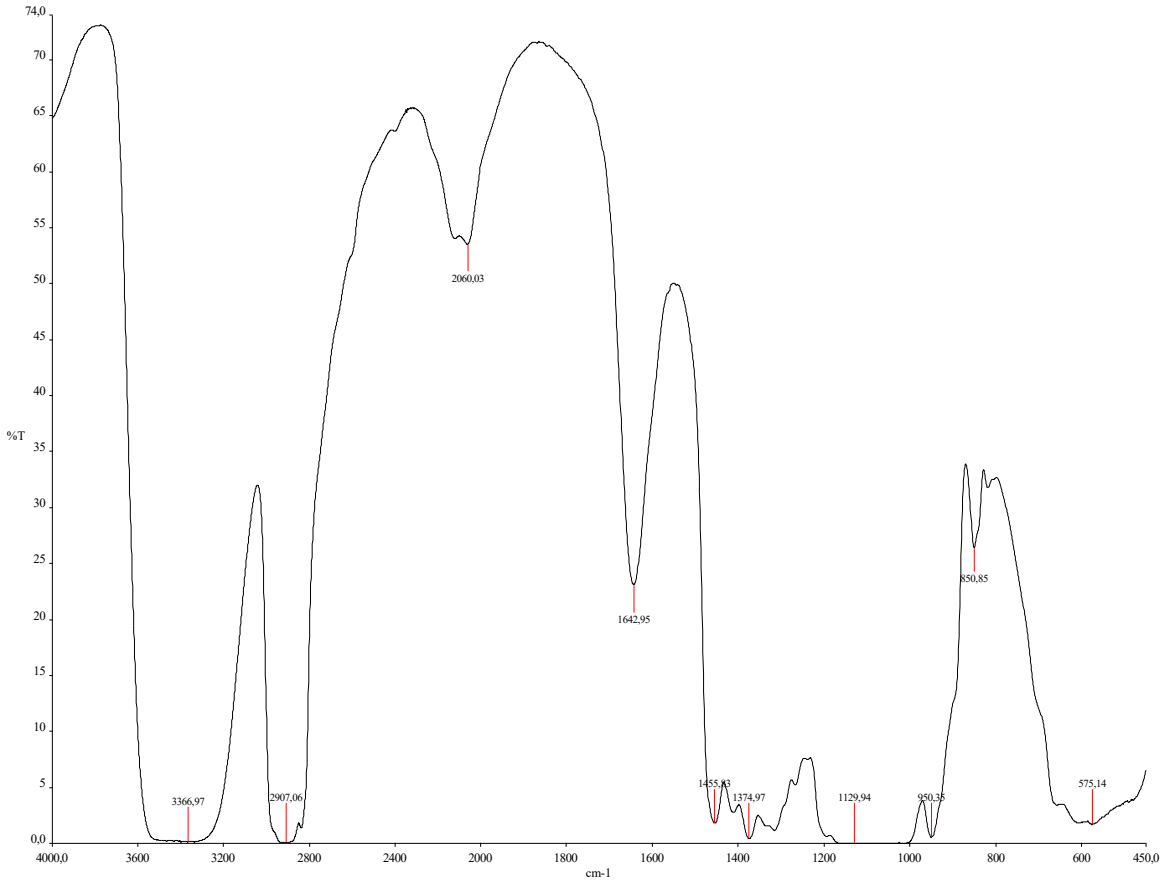


Figure 62. IR spectroscopic study of a gel based on bentonite clay and HPMC

Figure 63 shows the results of X-ray phase analysis of a gel based on bentonite clay and HPMC. The results of the X-ray diffraction analysis show that the composite gel based on bentonite clay and HPMC has an amorphous structure. The spectrum does not show any diffraction peaks characteristic of clear crystalline phases, but instead a broad diffraction maximum is recorded. This proves that the sample has a disordered atomic structure and is X-ray amorphous. According to the results of quantitative phase analysis, the sample consists of 100% amorphous phase. This indicates that the crystal structures are disrupted as a result of the interaction between bentonite clay and polymer HPMC. Such an amorphous structure can increase the stability, dispersion properties and water absorption capacity of the composite gel, which makes it suitable for medical purposes.

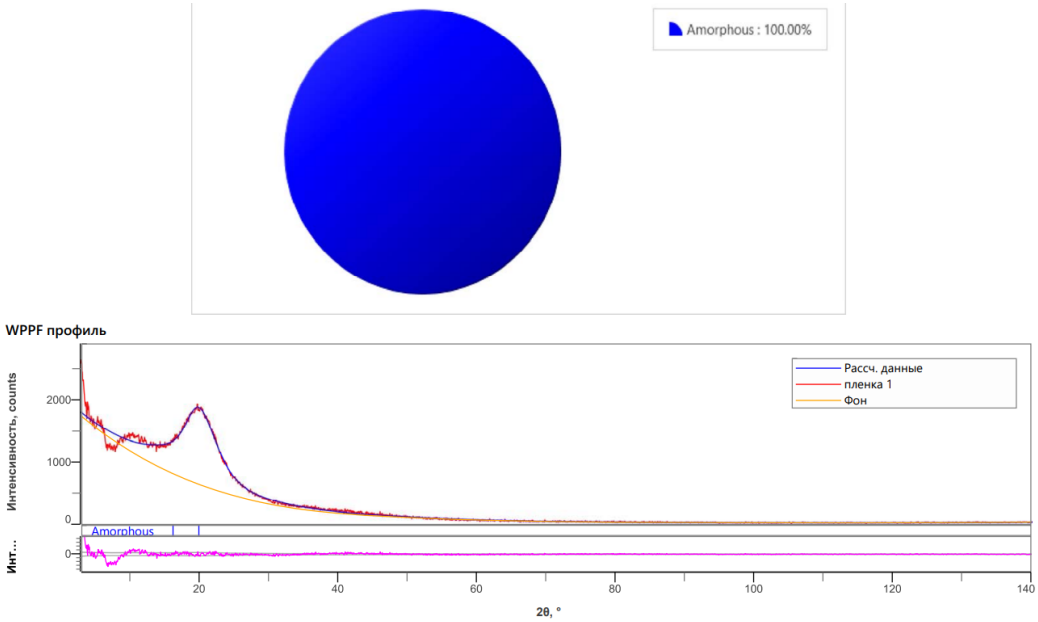


Figure 63. Results of X-ray phase analysis of a gel based on bentonite clay and HPMC

The rheological behavior of the gel based on bentonite clay and HPMC—specifically, the change in shear stress is shown in Figure 64. Initially, the shear stress is high, but as time or shear rate increases, it decreases and approaches a steady value. This indicates that the gel exhibits pseudoplastic properties, meaning that its viscosity decreases and its flowability increases as its structure is disrupted. Such behavior demonstrates that the gel is initially structured but softens in response to mechanical impact, allowing it to spread easily. This makes it suitable for medical use—ensuring ease of application and formulation stability.

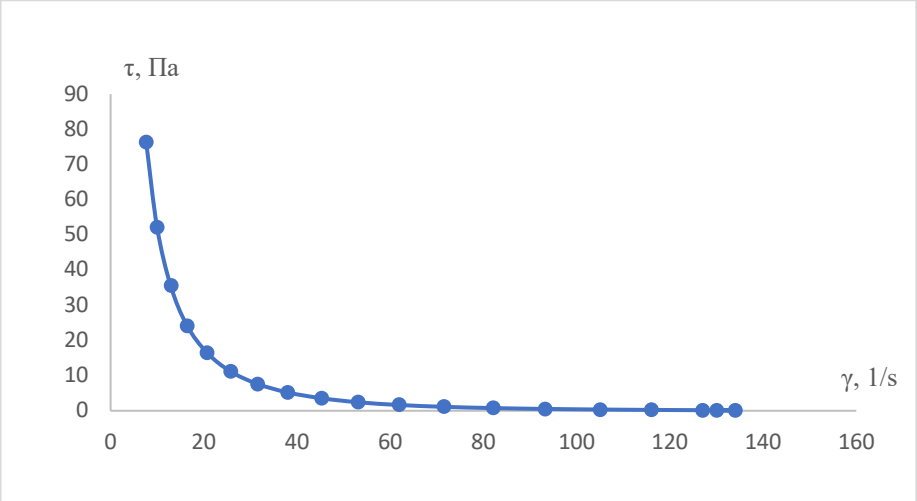


Figure 64. Shear stress in the rheological behavior of the gel based on bentonite clay and HPMC

Figure 65 shows the viscosity in the rheological behavior of the gel based on bentonite clay and HPMC. The dependency curve indicates that the viscosity starts

off very low and increases as the shear rate rises. This phenomenon is characteristic of shear-thickening systems. The increase in viscosity of the gel composed of hydroxypropyl methylcellulose and bentonite under shear suggests that the system's structure becomes more compact and the interactions between particles intensify. In conclusion, the gel exhibits a thickening behavior, meaning it becomes more viscous and structurally reinforced under mechanical stress.

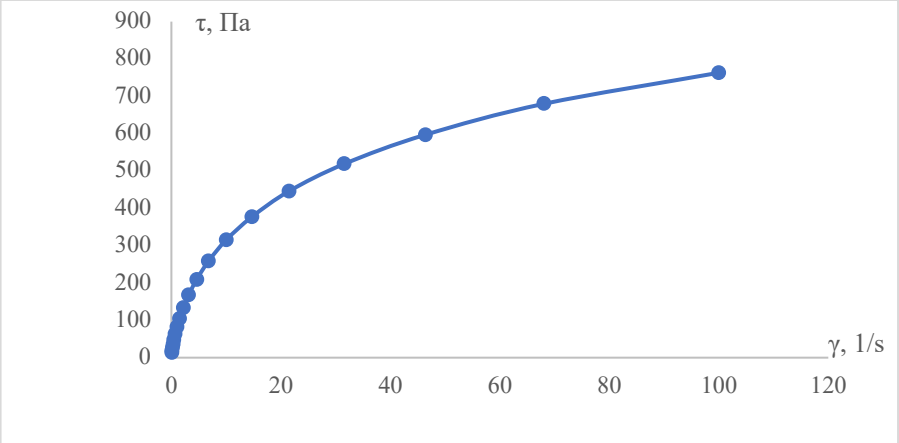


Figure 65. Viscosity in the rheological behavior of the gel based on bentonite clay and HPMC

Figure 66 illustrates the shear stress hysteresis observed in the rheological behavior of the gel composed of bentonite clay and HPMC. The graph displays how shear stress varies with increasing shear rate. The presence of hysteresis in the curve indicates the gel's ability to recover its structure after deformation. As the shear rate rises, the gel progressively breaks down its internal structure, demonstrating typical pseudoplastic behavior.

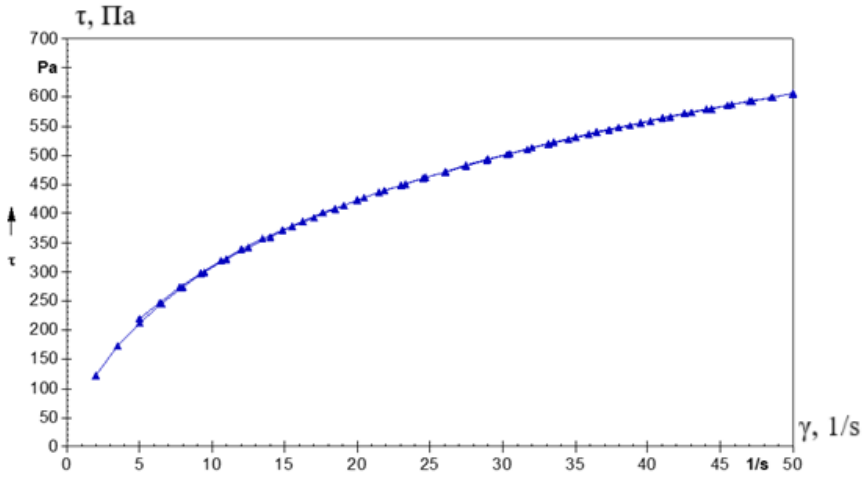


Figure 66. Hysteresis of shear stress in the rheological behavior of the gel based on bentonite clay and HPMC

The viscosity hysteresis in the rheological behavior of the gel based on bentonite clay and HPMC shown in Figure 67. In the graph, viscosity is plotted on the vertical axis and shear rate on the horizontal axis. As the shear rate increases, the viscosity of the gel sharply decreases. This demonstrates its pseudoplastic nature—meaning that the internal structure of the gel is disrupted under mechanical stress, allowing it to flow more easily. In the reverse direction, as the shear rate decreases, the viscosity does not immediately recover. This is due to the hysteresis effect, indicating that the system requires time for its internal structure to rebuild. Such behavior is characteristic of thixotropy—systems that lose and recover their structure over time. These types of gels become more fluid during application, making them easy to spread.

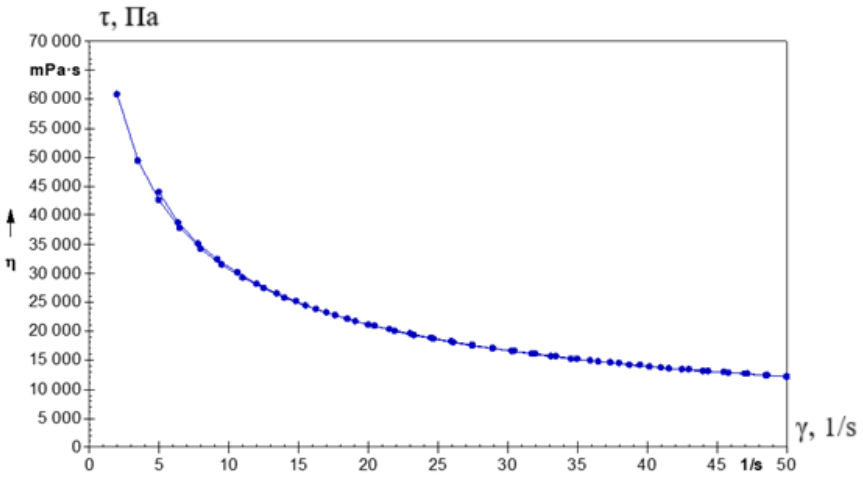


Figure 67. Viscosity hysteresis in the rheological behavior of the gel based on bentonite clay and HPMC

Figure 68 presents an SEM micrograph of the dried sample of the gel based on bentonite clay and HPMC. The analysis was conducted at an accelerating voltage of 5.00 kV, with 1000× magnification and a working distance of 13.3 mm. The horizontal field width of the image is approximately 298 µm. On the surface of the sample, particles and granules of various shapes and sizes are observed (sizes: 2.12 µm, 1.70 µm, 6.76 µm). These structures may represent bentonite particles or gel-forming residues of HPMC. The aggregation of fine particles is likely the result of interactions between bentonite and HPMC. After drying, the gel structure appears non-uniform, with cracks and textural changes visible in some areas, which may be associated with water loss and structural shrinkage during the drying process.

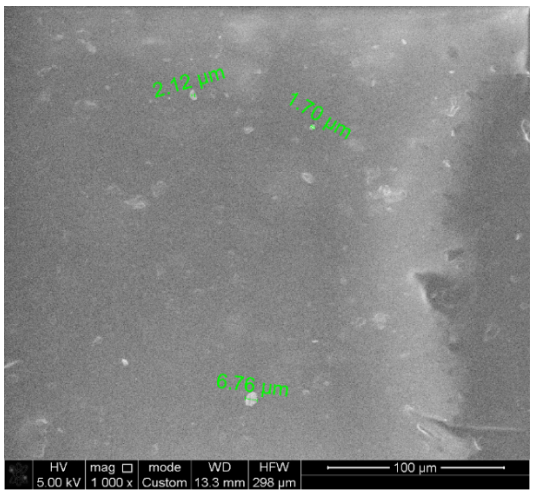


Figure 68. SEM image of the gel based on bentonite clay and HPMC

Determination of Gel pH

According to the requirements of the "Ointments" monograph of the State Pharmacopoeia of the Republic of Kazakhstan, the following parameter was determined in the gel samples: the pH value of the dosage form.

- The composition of the 4% HPMC gel is free of foreign impurities, colorless, homogeneous, and presents as a clear jelly-like mass with a pH value of 5.66.
- The BS-HPMC gel composition is white, homogeneous, and appears as an opaque viscous mass with a pH value of 5.39.
- The BS-HPMC gel with preservative is white, homogeneous, and appears as an opaque viscous mass with a pH value of 5.5.
- The BS-HPMC gel with THU-10 and preservative is pale yellow, homogeneous, and appears as an opaque viscous mass with a pH value of 5.7.

CONCLUSION

Based on the obtained results, the following conclusions can be drawn:

1. Aqueous-alcoholic extracts were obtained from the aerial parts of *Tamarix Hispida* plant material using ultrasonic treatment. The optimal conditions for the ultrasonic extraction method were determined. After optimizing the extraction methods, the use of ultrasound resulted in a 3–4-fold increase in the yield of components from the plant material and a significant reduction in extraction time (from 48 hours to 30 minutes) at an ultrasonic frequency range of 20 to 35 kHz. The qualitative and quantitative compositions of the obtained extracts were determined.

2. The biological activities of *Tamarix Hispida* plant extracts were determined. Anti-inflammatory and antioxidant activities were studied, as well as the cytotoxicity of the extracts. The THU-10 extract demonstrated a comparable level of DPPH radical inhibition to the standards gallic acid and quercetin at concentrations of 400 μg/mL and above (DPPH inhibition: 96.08 ± 0.48%).

3. Hydrogels based on hydroxypropylmethylcellulose (HPMC) were obtained, and their rheological characteristics were studied. HPMC hydrogels with various concentrations and compositions were prepared, and the most suitable formulations for external medical use in the form of hydrogel ointments were identified. Hydrogels based on hydroxypropylmethylcellulose (HPMC), bentonite clay, and its composites with THU-10 extract in various concentrations and compositions were obtained.

4. The physicochemical and rheological characteristics of hydrogels based on HPMC, bentonite clay and its composites with THU-10 extract were determined, and the optimal compositions of hydrogels were determined. The optimal hydrogel compositions are: a hydrogel based on bentonite clay (25%) with the addition of 1% THU-10 extract; a hydrogel based on hydroxypropylmethylcellulose (2-4%) with 1% THU-10; and a composite hydrogel based on HPMC:BC 4% (in a ratio of 7:3) with 1% THU-10.

Evaluation of the completeness of the solution of the assigned tasks. The objectives and tasks set in the dissertation have been fully accomplished. In accordance with the aims of the study, water-alcohol extracts from the plant *Tamarix Hispida* were obtained using ultrasonic treatment. The anti-inflammatory, antioxidant activities, and cytotoxicity of the obtained extracts were determined. It has been established that the THU-10 extract exhibits inhibition at the level of the reference standards. Hydrogel formulations based on bentonite clay, HPMC, and their composites containing the THU-10 extract have been proposed. Based on the results of rheological studies, the optimal compositions for application in medical practice have been identified.

Recommendations and baseline data for the practical application of the research results. The obtained water-ethanolic extracts can be recommended for the development of pharmaceutical formulations with antioxidant and anti-inflammatory activities. The developed gels based on bentonite clay, HPMC, and their composites with extracts from the plant *Tamarix Hispida* can serve as a basis

for the creation of wound-healing and anti-inflammatory hydrogels and ointments with prolonged action.

Evaluation of technical and economic efficiency of implementation. The results obtained in the dissertation possess high scientific and practical significance, ensured by the effective use of domestic plant and mineral raw materials and the development of prolonged-release gel formulations based on them, which can subsequently be implemented in domestic pharmaceutical production

Assessment of the scientific level of the work performed in comparison with the best achievements in this field. Based on the results of the research carried out within the dissertation, three scientific papers were published in co-authorship, including two articles in international peer-reviewed journals: *Molecules* (Switzerland), which in 2024 was ranked in Q1 in the field of "Chemistry (miscellaneous)" with an SJR of 0.865 and a percentile of 80 (according to the Scopus database), and *Chemistry of Plant Raw Material*, with a percentile of 23 (in the Scopus database), included in the list of journals recommended by the Committee for Quality Assurance in the Sphere of Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan. In addition, one patent for a utility model was obtained, and materials and abstracts were presented at international scientific conferences: the *5th International Conference on Chemical Investigation and Utilization of Natural Resources*, Ulaanbaatar, Mongolia, October 14–15, 2021: “New polymer forms of Tamarixidin and their properties”; the *XXXI Russian Youth Scientific Conference on Problems of Theoretical and Experimental Chemistry*, Yekaterinburg, April 20–23, 2021: “Synthesis and study of the properties of polymer forms of Tamarixidin”; and Galissultanova A., Amanzholkyzy A., Abilov Zh. A. “Preparation and rheological properties of HPMC gels” // *International Chemistry and Chemical Technology Congress “XI International Congress of Birimzhanov”* – Almaty, 2021. – P.142.

REFERENCES

1. Vlaia, L.; Coneac, G.; Olariu, I.; Vlaia, V.; Lupuleasa, D. Cellulose-Derivatives-Based Hydrogels as Vehicles for Dermal and Transdermal Drug Delivery. In *Emerging Concepts in Analysis and Applications of Hydrogels*; IntechOpen: London, UK, 2016.

2. Yang, Z.; Peng, H.; Wang, W.; Liu, T. Crystallization behavior of poly(ε-caprolactone)/layered double hydroxide nanocomposites. *J. Appl. Polym. Sci.* **2010**, *116*, 2658–2667.

3. Duan, J.J.; Zhang, L.N. Robust and smart hydrogels based on natural polymers. *Chin. J. Polym. Sci.* **2017**, *35*, 1165–1180.

4. Hoare, T.R.; Kohane, D.S. Hydrogels in drug delivery: Progress and challenges. *Polymer* **2008**, *49*, 1993–2007.

5. Yudaev, P.; Semenova, A.; Chistyakov, E. Gel based on modified chitosan for oil spill cleanup. *J. Appl. Polym. Sci.* **2024**, *141*, e54838.

6. Leyva-Jiménez, F.J.; Oliver-Simancas, R.; Castangia, I.; Rodríguez-García, A.M.; Alañón, M.E. Comprehensive review of natural based hydrogels as an upcoming trend for food packing. *Food Hydrocoll.* **2023**, *135*, 108124.

7. Mohammadinejad, R.; Maleki, H.; Larrañeta, E.; Fajardo, A.R.; Nik, A.B.; Shavandi, A.; Sheikhi, A.; Ghorbanpour, M.; Farokhi, M.; Govindh, P.; et al. Status and future scope of plant-based green hydrogels in biomedical engineering. *Appl. Mater. Today* **2019**, *16*, 213–246.

8. Caló, E.; Khutoryanskiy, V.V. Biomedical applications of hydrogels: A review of patents and commercial products. *Eur. Polym. J.* **2015**, *65*, 252–267.

9. Ma, S.; Yu, B.; Pei, X.; Zhou, F. Structural hydrogels. *Polymer* **2016**, *98*, 516–535.

10. Chang, C.; Zhang, L. Cellulose-based hydrogels: Present status and application prospects. *Carbohydr. Polym.* **2011**, *84*, 40–53.

11. Ito, T.; Yeo, Y.; Highley, C.B.; Bellas, E.; Benitez, C.A.; Kohane, D.S. The prevention of peritoneal adhesions by in situ cross-linking hydrogels of hyaluronic acid and cellulose derivatives. *Biomaterials* **2007**, *28*, 975–983.

12. Maulvi, F.A.; Pillai, L.V.; Patel, K.P.; Desai, A.R.; Shukla, M.R.; Desai, D.T.; Patel, H.P.; Ranch, K.M.; Shah, S.A.; Shah, D.O. Lidocaine tripotassium phosphate complex laden microemulsion for prolonged local anaesthesia: In vitro and in vivo studies. *Colloids Surf. B Biointerfaces* **2020**, *185*, 110632.

13. Zhang, X.; Dang, M.; Zhang, W.; Lei, Y.; Zhou, W. Sustained delivery of prilocaine and lidocaine using depot microemulsion system: In vitro, ex vivo and in vivo animal studies. *Drug Dev. Ind. Pharm.* **2020**, *46*, 264–271.

14. Michida, N.; Hayashi, M.; Hori, T. Comparison of event related potentials with and without hypnagogic imagery. *Psychiatry Clin. Neurosci.* **1998**, *52*, 145–147.

15. Siepmann, J.; Peppas, N.A. Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). *Adv. Drug Deliv. Rev.* **2001**, *48*, 139–157.

16. Sadeghi, E. Effect of Strong Electrolyte Containing Gelling Aids on the Sol-Gel Transition Temperature of Hypromellose 2910. Master's Thesis, University of New Mexico, Albuquerque, NM, USA, 2018.

17. Mašková, E.; Kubová, K.; Raimi-Abraham, B.T.; Vllasaliu, D.; Vohlídalová, E.; Turánek, J.; Mašek, J. Hypromellose—A traditional pharmaceutical excipient with modern applications in oral and oromucosal drug delivery. *J. Control. Release* **2020**, *324*, 695–727.

18. Kabir, S.M.F.; Sikdar, P.P.; Haque, B.; Bhuiyan, M.A.R.; Ali, A.; Islam, M.N. Cellulose-based hydrogel materials: Chemistry, properties and their prospective applications. *Prog. Biomater.* **2018**, *7*, 153–174.

19. Ciolacu, D.; Oprea, A.M.; Anghel, N.; Cazacu, G.; Cazacu, M. New cellulose-lignin hydrogels and their application in controlled release of polyphenols. *Mater. Sci. Eng. C* **2012**, *32*, 452–463.

20. De Sousa Moraes, P.R.F.; Saska, S.; Barud, H.; De Lima, L.R.; Da Conceicao Amaro Martins, V.; De Guzzi Plepis, A.M.; Ribeiro, S.J.L.; Gaspar, A.M.M. Bacterial cellulose/collagen hydrogel for wound healing. *Mater. Res.* **2016**, *19*, 106–116.

21. Zhumagalieva, S.N.; Kudaibergenova, B.M.; Beisebekov, M.K.; Abilov, Z.A. Properties of bentonite and local anesthetics composition. *J. Appl. Polym. Sci.* **2007**, *106*, 1601–1605.

22. Zhumagalieva, S.N.; Beisebekov, M.K.; Abilov, Z.A. Immobilization of 2,5-dimethyl-4-benzoyl-oxypiperidine succinate over polyacrylic acid (PAA) gels. I. Study of interaction between linear and network PAA with succinate of 2,5-dimethyl-4-benzoyl-oxypiperidine. *J. Appl. Polym. Sci.* **2005**, *96*, 1183–1186.

23. Zhumagalieva, S.N.; Beisebekov, M.K.; Abilov, Z.A. Immobilization of 2,5-dimethyl-4-benzoyl-oxypiperldine succinate over polyacrylic acid (PAA) gels. II. Study of quantitative characteristics of immobilization of succinate of 2,5-dimethyl-4-benzoyl-oxypiperidine over PAA. *J. Appl. Polym. Sci.* **2005**, *96*, 1187–1192.

24. Temirkhanova, G.; Burasheva, G.; Abilov, Z.; Irmukhametova, G.; Mun, A.; Beksultanov, Z.; Myktybaeva, Z.; Shnaukshta, V. Creation of polymer hydrogel dressings with herbal medicinal substsnce "Alkhydin" and their properties. *Eurasian Chem.-Technol. J.* **2017**, *19*, 57–62.

25. Zhumagaliyeva, S.N.; Amanzholkyzy, A.; Sultanova, N.A.; Abilov, Z.A. Use of ultrasound for extraction of biologically active substances from tamarix hispida willd. *Chem. Plant Raw Mater.* **2021**, *3*, 283–289. https://doi.org/10.14258/jcprm.2021038695.

26. Zhumagaliyeva, S.N.; Abdikarim, G.G.; Berikova, A.B.; Abilov, Z.A.; Koetz, J.; Kopbayeva, M.T. Study of the Mechanical Properties of Gelatin Films with Natural Compounds of Tamarix hispida. *Eurasian Chem.-Technol. J.* **2023**, *25*, 165–171.

27. Bahramsoltani, R.; Kalkhorani, M.; Abbas Zaidi, S.M.; Farzaei, M.H.; Rahimi, R. The genus Tamarix: Traditional uses, phytochemistry, and pharmacology. *J. Ethnopharmacol.* **2020**, *246*, 112245.

28. Drury, J.L.; Mooney, D.J. Hydrogels for tissue engineering: Scaffold design variables and applications. *Biomaterials* **2003**, *24*, 4337–4351.

29. Wang, W.; Wang, J.; Kang, Y.; Wang, A. Synthesis, swelling and responsive properties of a new composite hydrogel based on hydroxyethyl cellulose and medicinal stone. *Compos. Part B Eng.* **2011**, *42*, 809–818.

30. Kryazhev, D.V.; Smirnov, V.F.; Mochalova, A.E., Smirnova O. N., Zakharova E. A., Zotov K. A., Smirnova L. A.; et al. Stability of synthetic and natural polymer composite materials to the action of micromyccetes under natural conditions. *Bull. N.I. Lobachevsky Nizhny Novgorod Univ.* **2010**, *2*, 536–540.

31. Li, X.; Wan, C.; Tao, T.; Chai, H.; Huang, Q.; Chai, Y.; Wu, Y. An overview of the development status and applications of cellulose-based functional materials. *Cellulose* **2024**, *31*, 61–99. https://doi.org/10.1007/s10570-023-05616-8.

32. Zhao, J.; Li, S.; Zhao, Y.; Peng, Z. Effects of cellulose nanocrystal polymorphs and initial state of hydrogels on swelling and drug release behavior of alginate-based hydrogels. *Polym. Bull.* **2020**, *77*, 4401–4416. https://doi.org/10.1007/s00289-019-02972-z.

33. Gupta, Y.; Khan, M.S.; Bansal, M.; Singh, M.K.; Pragatheesh, K.; Thakur, A. A review of carboxymethyl cellulose composite-based hydrogels in drug delivery applications. *Results Chem.* **2024**, *10*, 101695.

34. Do, M.P.; Neut, C.; Metz, H.; Delcourt, E.; Siepmann, J.; Mäder, K.; Siepmann, F. Mechanistic analysis of PLGA/HPMC-based in-situ forming implants for periodontitis treatment. *Eur. J. Pharm. Biopharm.* **2015**, *94*, 273–283.

35. Shi, X.; Chen, Z.; He, Y.; Lu, Q.; Chen, R.; Zhao, C.; Dong, D.; Sun, Y.; He, H. Dual light-responsive cellulose nanofibril-based in situ hydrogel for drug-resistant bacteria infected wound healing. *Carbohydr. Polym.* **2022**, *297*, 120042.

36. Chen, Y.C.; Chen, Y.H. Thermo and pH-responsive methylcellulose and hydroxypropyl methylcellulose hydrogels containing K2SO4 for water retention and a controlled-release water-soluble fertilizer. *Sci. Total Environ.* **2019**, *655*, 958–967.

37. Selvaraj, S.; Chauhan, A.; Dutta, V.; Verma, R.; Rao, S.K.; Radhakrishnan, A.; Ghotekar, S. A state-of-the-art review on plant-derived cellulose-based green hydrogels and their multifunctional role in advanced biomedical applications. *Int. J. Biol. Macromol.* **2024**, *265 Pt 2*, 130991. https://doi.org/10.1016/j.ijbiomac.2024.130991.

38. Heinze, T.; Liebert, T. Unconventional methods in cellulose functionalization. *Prog. Polym. Sci.* **2001**, *26*, 1689–1762.

39. Li, C.L.; Martini, L.G.; Ford, J.L.; Roberts, M. The use of hypromellose in oral drug delivery. *J. Pharm. Pharmacol.* **2010**, *57*, 533–546.

40. Smith, A.M.; Moxon, S.; Morris, G.A. Biopolymers as wound healing materials. *Wound Heal. Biomater.* **2016**, *2*, 261–287.

41. Karimian, A.; Parsian, H.; Majidinia, M.; Rahimi, M.; Mir, S.M.; Samadi Kafil, H.; Shafiei-Irannejad, V.; Kheyrollah, M.; Ostadi, H.; Yousefi, B. Nanocrystalline cellulose: Preparation, physicochemical properties, and applications in drug delivery systems. *Int. J. Biol. Macromol.* **2019**, *133*, 850–859.

42. Liebner, F.; Pircher, N.; Schimper, C.; Haimer, E.; Rosenau, T. Aerogels: Cellulose-Based. In *Concise Encyclopedia of Biomedical Polymers and Polymeric Biomaterials*; CRC Press: Boca Raton, FL, USA, 2016.

43. Pan, P.; Svirskis, D.; Waterhouse, G.I.N.; Wu, Z. Hydroxypropyl Methylcellulose Bioadhesive Hydrogels for Topical Application and Sustained Drug Release: The Effect of Polyvinylpyrrolidone on the Physicomechanical Properties of Hydrogel. *Pharmaceutics* **2023**, *15*, 2360. https://doi.org/10.3390/pharmaceutics15092360.

44. dos Santos Carvalho, J.D.; Rabelo, R.S.; Hubinger, M.D. Thermo-rheological properties of chitosan hydrogels with hydroxypropyl methylcellulose and methylcellulose. *Int. J. Biol. Macromol.* **2022**, *209*, 367–375.

45. Ranjha, N.M.; Qureshi, U.F. Preparation and characterization of crosslinked acrylic acid/hydroxypropyl methyl cellulose hydrogels for drug delivery. *Int. J. Pharm. Pharm. Sci.* **2014**, *6*, 400–410.

46. Bashir, S.; Zafar, N.; Lebaz, N.; Mahmood, A.; Elaissari, A. Hydroxypropyl Methylcellulose-Based Hydrogel Copolymeric for Controlled Delivery of Galantamine Hydrobromide in Dementia. *Processes* **2020**, *8*, 1350.

47. Lamberti, G.; Cascone, S.; Cafaro, M.M.; Titomanlio, G.; d'Amore, M.; Barba, A.A. Measurements of water content in hydroxypropyl-methyl-cellulose based hydrogels via texture analysis. *Carbohydr. Polym.* **2013**, *92*, 765–768.

48. Kopeček, J. Hydrogel biomaterials: A smart future? *Biomaterials* **2007**, *28*, 5185–5192.

49. Ghorbani, S.; Eyni, H.; Bazaz, S.R.; Nazari, H.; Asl, L.S.; Zaferani, H.; Kiani, V.; Mehrizi, A.A.; Soleimani, M. Hydrogels Based on Cellulose and its Derivatives: Applications, Synthesis, and Characteristics. *Polym. Sci. Ser. A* **2018**, *60*, 707–722. https://doi.org/10.1134/S0965545X18060044.

50. Mahdavinia, G.R.; Ettehadi, S.; Amini, M.; Sabzi, M. Synthesis and characterization of hydroxypropyl methylcellulose-g-poly(acrylamide)/LAPONITE® RD nanocomposites as novel magnetic- and pH-sensitive carriers for controlled drug release. *RSC Adv.* **2015**, *5*, 44516–44523.

51. Wen, H.; Park, K. *Oral Controlled Release Formulation Design and Drug Delivery: Theory to Practice*; Wiley: Hoboken, NJ, USA, 2010; 363p.

52. Haque, A.; Morris, E.R. Thermogelation of methylcellulose. Part I: Molecular structures and processes. *Carbohydr. Polym.* **1993**, *22*, 161–173 p.

53. Mitchell, K.; Ford, J.L.; Armstrong, D.J.; Elliott, P.N.C.; Rostron, C.; Hogan, J.E. The influence of additives on the cloud point, disintegration and dissolution of hydroxypropylmethylcellulose gels and matrix tablets. *Int. J. Pharm.* **1990**, *66*, 233–242.

54. Rowe, R.C.; Sheskey, P.J.; Owen, S.C. *Handbook of Pharmaceutical Excipients*, 5th ed.; Libros Digitales-Pharmaceutical Press: London, UK, 2009.

55. Joshi, S.C. Sol-gel behavior of hydroxypropyl methylcellulose (HPMC) in ionic media including drug release. *Materials* **2011**, *4*, 1861–1905.

56. Sarkar, N. Thermal Gelation Properties of Methyl and Hydroxypropyl Methylcellulose. *J. Appl. Polym. Sci.* **1979**, *24*, 1073–1087.

57. Jani, R.; Patel, D. Hot melt extrusion: An industrially feasible approach for casting orodispersible film. *Asian J. Pharm. Sci.* **2014**, *10*, 292–305.

58. Huang, S.; O'Donnell, K.P.; Keen, J.M.; Rickard, M.A.; McGinity, J.W.; Williams, R.O. A New Extrudable Form of Hypromellose: AFFINISOL™ HPMC HME. *AAPS PharmSciTech* **2016**, *17*, 106–119.

59. Doelker, E. Cellulose derivatives. *Adv. Polym. Sci.* **1993**, *107*, 198–265.

60. Jana, P.; Shyam, M.; Singh, S.; Jayaprakash, V.; Dev, A. Biodegradable polymers in drug delivery and oral vaccination. *Eur. Polym. J.* **2021**, *142*, 110155. https://doi.org/10.1016/j.eurpolymj.2020.110155.

61. Sannino, A.; Demitri, C.; Madaghiele, M. Biodegradable Cellulose-based Hydrogels: Design and Applications. *Materials* **2009**, *2*, 353–373. https://doi.org/10.3390/ma2020353.

62. Ghiorghita, C.A.; Platon, I.V.; Lazar, M.M.; Dinu, M.V.; Aprotosoaie, A.C. Trends in polysaccharide-based hydrogels and their role in enhancing the bioavailability and bioactivity of phytocompounds. *Carbohydr. Polym.* **2024**, *334*, 122033.

63. Kim, S.; Song, M.; Lee, M.; Kwon, S. Controlled release of quercetin from HPMC/gellan gum hydrogel for inhibiting melanogenesis in murine melanoma cells. *Korean J. Chem. Eng.* **2023**, *40*, 337–343. https://doi.org/10.1007/s11814-022-1269-y.

64. Abou-Taleb, H.A.; Mohamed, M.S.; Zayed, G.M.; Abdelaty, L.N.; Makki, M.A.; Abdel-Aleem, H.L.; El-Mokhtar, M.A.; Hetta, H.F.; Abdullah, N.; Saddik, M.S. HPMC-Zein Film-forming Gel Loaded with 5-Fluorouracil Coupled with CO_2 Laser Dermabrasion for Managing Stable Vitiligo. *AAPS PharmSciTech* **2024**, *25*, 225. https://doi.org/10.1208/s12249-024-02937-0.

65. Barros, S.C.; da Silva, A.A.; Costa, D.B.; Cesarino, I.; Costa, C.M.; Lanceros-Méndez, S.; Pawlicka, A.; Silva, M.M. Thermo-sensitive chitosan–cellulose derivative hydrogels: Swelling behaviour and morphologic studies. *Cellulose* **2014**, *21*, 4531–4544. https://doi.org/10.1007/s10570-014-0442-9.

66. Mu, A.M.; Vlaia, L.; Coneac, G.; Olariu, I.; Vlaia, I.; St, C.; Mitu, M.A.; Lupuliasa, D. Chitosan/HPMC-based hydrogels containing essential oils for topical delivery of fluconazole: Preliminary studies. *Farmacia* **2018**, *66*, 248–256.

67. Agubata, C.O.; Okereke, C.; Nzekwe, I.T.; Onoja, R.I.; Obitte, N.C. Development and evaluation of wound healing hydrogels based on a quinolone, hydroxypropyl methylcellulose and biodegradable microfibres. *Eur. J. Pharm. Sci.* **2016**, *89*, 1–10.

68. He, W.; Mu, H.; Genina, N. Bespoke hydroxypropyl methylcellulose-based solid foams loaded with poorly soluble drugs by tunable modular design. *Carbohydr. Polym.* **2025**, *357*, 123397. https://doi.org/10.1016/j.carbpol.2025.123397.

69. Silva, G.D.A.; Campelo, M.D.S.; Lima, A.B.N.; Mattos, A.L.A.; De Sousa, V.C.; Dutra, P.G.P.; Leal, L.K.A.M.; Ricardo, N.M.P.S.; Ribeiro, M.E.N.P. Rheological and osteogenic effect of Agaricus blazei Murill polysaccharides on composite hydrogels based on HPMC/graphene oxide—A preliminary study. *Mater. Today Commun.* **2024**, *40*, 109986. https://doi.org/10.1016/j.mtcomm.2024.109986.

70. Knarr, M.; Rogers, T.L.; Petermann, O.; Adden, R. Investigation and rank-ordering of hydroxypropyl methylcellulose (HPMC) properties impacting controlled release performance. *J. Drug Deliv. Sci. Technol.* **2025**, *104*, 106425. https://doi.org/10.1016/j.jddst.2024.106425.

71. Ellakwa, T.E.; Abu-Khadra, A.S.; Ellakwa, D.E.S. Influence of physico-chemical properties of hydroxypropyl methylcellulose on quetiapine fumarate release from sustained release matrix tablets. *BMC Chem.* **2024**, *18*, 219. https://doi.org/10.1186/s13065-024-01311-2.

72. Liu, T.; Wang, J.; Feng, Y.; Wang, H.; Xu, Y.; Yin, T.; Zhang, Y.; He, H.; Gou, J.; Tang, X. Further enhancement of the sustained-release properties and stability of direct compression gel matrix bilayer tablets by controlling the particle size of HPMC and drug microencapsulation. *Powder Technol.* **2024**, *448*, 120256. https://doi.org/10.1016/j.powtec.2024.120256.

73. Kim, C.-J. Release kinetics of coated, donut-shaped tablets for water soluble drugs. *Eur. J. Pharm. Sci.* **1999**, *7*, 237–242.

74. Elena Campos-Aldrete, M. Influence of the viscosity grade and the particle size of HPMC on metronidazole release from matrix tablets. *Eur. J. Pharm. Biopharm.* **1997**, *43*, 173–178.

75. Melia, C.D.; Rajabi-Siahboomi, A.R.; Hodsdon, A.C.; Adler, J.; Mitchell, J.R. Structure and behaviour of hydrophilic matrix sustained release dosage forms: 1. The origin and mechanism of formation of gas bubbles in the hydrated surface layer. *Int. J. Fharmaceutics* **1993**, *100*, 263–269.

76. Hosseinzadeh, S.; Jafarikukhdan, A.; Hosseini, A.; Armand, R. The Application of Medicinal Plants in Traditional and Modern Medicine: A Review of *Thymus vulgaris*. *Int. J. Clin. Med.* **2015**, *6*, 635–642.

77. Newman, D.J.; Cragg, G.M.; Snader, K.M. The influence of natural products upon drug discovery. *Nat. Prod. Rep.* **2000**, *17*, 215–234.

78. Rasouli, H.; Farzaei, M.H.; Khodarahmi, R. Polyphenols and their benefits: A review. *Int. J. Food Prop.* **2017**, *20*, 1700–1741.

79. Sun, J.; Tan, H. Alginate-based biomaterials for regenerative medicine applications. *Materials* **2013**, *6*, 1285–1309.

80. Obradovic, J.; Wondraczek, H.; Fardim, P.; Lassila, L.; Navard, P. Preparation of three-dimensional cellulose objects previously swollen in a DMAc/LiCl solvent system. *Cellulose* **2014**, *21*, 4029–4038.

81. Klemm, D.; Heublein, B.; Fink, H.P.; Bohn, A. Cellulose: Fascinating biopolymer and sustainable raw material. *Angew. Chem. Int. Ed.* **2005**, *44*, 3358–3393.

82. Kouchak, M.; Rezaee, S.; Moshabeh, N.; Handali, S. Preparation and evaluation of matrix containing lidocaine and prilocaine for using in transdermal films. *J. Rep. Pharm. Sci.* **2019**, *8*, 270–276.

83. Bagheri, M.; Shokoohinia, Y.; Pourmanouchehri, Z.; Jalilian, F.; Khaledian, S.; Mirzaie, S.; Behbood, L. Formulation and evaluation of the novel herbal antibacterial gel to the treatment of cutaneous burn infections. *J. Rep. Pharm. Sci.* **2021**, *10*, 93–100.

84. Wu, Y.C.; Wu, G.X.; Huang, H.H.; Kuo, S.M. Liposome-encapsulated farnesol accelerated tissue repair in third-degree burns on a rat model. *Burns* **2019**, *45*, 1139–1151.

85. Chang, R.Y.K.; Morales, S.; Okamoto, Y.; Chan, H.K. Topical application of bacteriophages for treatment of wound infections. *Transl. Res.* **2020**, *220*, 153–166.

86. Saddik, M.S.; Alsharif, F.M.; El-Mokhtar, M.A.; Al-Hakkani, M.F.; El-Mahdy, M.M.; Farghaly, H.S.; Abou-Taleb, H.A. Biosynthesis, Characterization, and Wound-Healing Activity of Phenytoin-Loaded Copper Nanoparticles. *AAPS PharmSciTech* **2020**, *21*, 175.

87. Kumari, S.; Harjai, K.; Chhibber, S. Topical treatment of Klebsiella pneumoniae B5055 induced burn wound infection in mice using natural products. *J. Infect. Dev. Ctries.* **2010**, *4*, 367–377.

88. Zakaria, A.S.; Afifi, S.A.; Elkhodairy, K.A. Newly Developed Topical Cefotaxime Sodium Hydrogels: Antibacterial Activity and in Vivo Evaluation. *BioMed Res. Int.* **2016**, *2016*, 6525163.

89. Mohd Noor, A.; bin Bai, S. Mechanical properties and water vapour permeability of film from haruan (channa striatus) and fusidic acid spray for wound dressing and wound healing. *Pak. J. Pharm. Sci.* **2010**, *23*, 155–159.

90. Huang, T.W.; Lu, H.T.; Ho, Y.C.; Lu, K.Y.; Wang, P.; Mi, F.L. A smart and active film with tunable drug release and color change abilities for detection and inhibition of bacterial growth. *Mater. Sci. Eng. C* **2021**, *118*, 111396.

91. Jayaramudu, T.; Varaprasad, K.; Pyarasani, R.D.; Reddy, K.K.; Akbari-Fakhrabadi, A.; Carrasco-Sánchez, V.; Amalraj, J. Hydroxypropyl methylcellulose-copper nanoparticle and its nanocomposite hydrogel films for antibacterial application. *Carbohydr. Polym.* **2021**, *254*, 117302.

92. Verma, S.; Gokhale, R.; Burgess, D.J. A comparative study of top-down and bottom-up approaches for the preparation of micro/nanosuspensions. *Int. J. Pharm.* **2009**, *380*, 216–222.

93. Oliveira, D.M.L.; Rezende, P.S.; Barbosa, T.C.; Andrade, L.N.; Bani, C.; Tavares, D.S.; Da Silva, C.F.; Chaud, M.V.; Padilha, F.; Cano, A.; et al. Double membrane based on lidocaine-coated polymyxin-alginate nanoparticles for wound healing: In vitro characterization and in vivo tissue repair. *Int. J. Pharm.* **2020**, *591*, 120001.

94. Uslu, I.; Aytimur, A. Production and characterization of poly(vinyl alcohol)/ poly(vinylpyrrolidone) iodine/poly(ethylene glycol) electrospun fibers with (hydroxypropyl)methyl cellulose and aloe vera as promising material for wound dressing. *J. Appl. Polym. Sci.* **2012**, *124*, 3520–3524.

95. Bilbao-Sainz, C.; Chiou B sen Valenzuela-Medina, D.; Du, W.X.; Gregorski, K.S.; Williams, T.G.; Wood, D.F.; Glenn, G.M.; Orts, W.J. Solution blow spun poly(lactic acid)/hydroxypropyl methylcellulose nanofibers with antimicrobial properties. *Eur. Polym. J.* **2014**, *54*, 1–10.

96. Das, S.; De, A.; Das, B.; Mukherjee, B.; Samanta, A. Development of gum odina-gelatin based antimicrobial loaded biodegradable spongy scaffold: A promising wound care tool. *J. Appl. Polym. Sci.* **2021**, *138*, 50057.

97. Dharmalingam, K.; Anandalakshmi, R. Fabrication, characterization and drug loading efficiency of citric acid crosslinked NaCMC-HPMC hydrogel films for wound healing drug delivery applications. *Int. J. Biol. Macromol.* **2019**, *134*, 815–829.

98. Khanum, H.; Ullah, K.; Murtaza, G.; Khan, S.A. Fabrication and in vitro characterization of HPMC-g-poly(AMPS) hydrogels loaded with loxoprofen sodium. *Int. J. Biol. Macromol.* **2018**, *120*, 1624–1631.

99. Tagami, T.; Ito, E.; Kida, R.; Hirose, K.; Noda, T.; Ozeki, T. 3D printing of gummy drug formulations composed of gelatin and an HPMC-based hydrogel for pediatric use. *Int. J. Pharm.* **2021**, *594*, 120118.

100. Nochos, A.; Douroumis, D.; Bouropoulos, N. In vitro release of bovine serum albumin from alginate/HPMC hydrogel beads. *Carbohydr. Polym.* **2008**, *74*, 451–457.

101. Kundu, J.; Patra, C.; Kundu, S.C. Design, fabrication and characterization of silk fibroin-HPMC-PEG blended films as vehicle for transmucosal delivery. *Mater. Sci. Eng. C* **2008**, *28*, 1376–1380.

102. Ghosal, K.; Ray, S.D. Alginate/hydrophobic HPMC (60M) particulate systems: New matrix for site-specific and controlled drug delivery. *Braz. J. Pharm. Sci.* **2011**, *47*, 833–844.

103. Natori, N.; Shibano, Y.; Hiroki, A.; Taguchi, M.; Miyajima, A.; Yoshizawa, K.; Kawano, Y.; Hanawa, T. Preparation and Evaluation of Hydrogel Film Containing Tramadol for Reduction of Peripheral Neuropathic Pain. *J. Pharm. Sci.* **2023**, *112*, 132–137.

104. Shi, Y.; Xue, J.; Sang, Y.; Xu, X.; Shang, Q. Insulin-loaded hydroxypropyl methyl cellulose-co-polyacrylamide-co-methacrylic acid hydrogels used as rectal suppositories to regulate the blood glucose of diabetic rats. *Int. J. Biol. Macromol.* **2019**, *121*, 1346–1353.

105. Corredor-Chaparro, M.Y.; Vargas-Riveros, D.; Mora-Huertas, C.E. Hypromellose–Collagen hydrogels/sesame oil organogel based bigels as controlled drug delivery systems. *J. Drug Deliv. Sci. Technol.* **2022**, *75*, 103637.

106. Abazari, M.; Akbari, T.; Hasani, M.; Sharifikolouei, E.; Raoufi, M.; Foroumadi, A.; Sharifzadeh, M.; Firoozpour, L.; Khoobi, M. Polysaccharide-based hydrogels containing herbal extracts for wound healing applications. *Carbohydr. Polym.* **2022**, *294*, 119808.

107. Yazarlu, O.; Iranshahi, M.; Kashani, H.R.K.; Reshadat, S.; Habtemariam, S.; Iranshahy, M.; Hasanpour, M. Perspective on the application of medicinal plants and natural products in wound healing: A mechanistic review. *Pharmacol. Res.* **2021**, *174*, 105841.

108. Nishadani, D.K.S.; Gunathilake, T.M.S.U.; Ching, Y.C.; Noothalapati, H. Carboxymethyl cellulose hydrogel for pH-responsive drug release of curcumin. *Iran. Polym. J.* **2024**, *33*, 1449–1467. https://doi.org/10.1007/s13726-024-01331-8.

109. Younis, N.A.; Hemdan, A.; Zafer, M.M.; Abd-Elsalam, W.H.; Abouelatta, S.M. Standardization and quantitative analysis of *Araucaria heterophylla* extract via an UPLC-MS/MS method and its formulation as an antibacterial phytonanoemulsion gel. *Sci. Rep.* **2022**, *12*, 12557. https://doi.org/10.1038/s41598-022-16188-1.

110. Gonfa, Y.H.; Tessema, F.B.; Bachheti, A.; Rai, N.; Tadesse, M.G.; Singab, A.N.; Chaubey, K.K.; Bachheti, R.K. Anti-inflammatory activity of phytochemicals from medicinal plants and their nanoparticles: A review. *Curr. Res. Biotechnol.* **2023**, *6*, 100152.

111. Baymiev, A.K.; Chuvatkina, A.K.; Vladimirova, A.A.; Matniyazov, R.T.; Mavzyutov, A.R.; Baymiev, A.K. Analysis of Antibacterial Action Mechanisms of Medicinal Plant Extracts Using Dual Reporter System Dualrep2. *Antibiot Khimioter = Antibiot. Chemother.* **2023**, *68*, 11–16. https://doi.org/10.37489/0235-2990-2023-68-7-8-11-16. (In Russian)

112. Nesterovich, V.M.; Belykh, D.A.; Gorokhovets, N.V.; Kurbatov, L.K.; Zamyatnin, A.A.; Ikryannikova, L.N. Secondary metabolites of plants and their possible role in the “age of superbugs”. *Biomeditsinskaya Khimiya* **2023**, *69*, 371–382.

113. Keerthana, S.; Charumathy, M.; Gangadhar, L.; E.S., A. Comparision of in vitro and ex vivo experimental analysis of aloe vera extracts on the antioxidant and antimicrobial activity. *Int. J. Manag. Technol. Eng.* **2019**, *IX*, 3531–3540.

114. Amanzholkyzy A, Zhumagaliyeva S, Sultanova N, Abilov Z, Ongalbek D, Donbayeva E, Niyazbekova A, Mukazhanova Z. Hydrogel Delivery Systems for Biological Active Substances: Properties and the Role of HPMC as a Carrier. *Molecules*. 2025; 30(6):1354. https://doi.org/10.3390/molecules30061354

115. Sultanova N. A., et. al. Antioxidant and antimicrobial activities of Tamarix ramosissima // Journal of Ethnopharmacology. 2001. № 2–3 (78). С. 201–205.

116. Sultanova N. A., et. al. Biologically Active Terpenoids from Tamarix Species // Eurasian Chemico-Technological Journal. 2013. № 3 (15). С. 219.

117. Sultanova N. A., Makhmoor T., Yasin A., Abilov Zh.A., Omurkamzinova V.B, Atta-ur-Rahman, Choudhary M.I. Isotamarixen - A New Antioxidant and Propyl Endopeptidase-Inhibiting Triterpenoid from *Tamarix hispida* //Planta Medica. 2004. Vol. 70. N 1. Pp. 65-67. DOI: 0009-3130/04/4002-0192.

118. Belokurov S.S., Narkevich I.A., Flisyuk E.V., Kaukhova I.E., Aroyan M.V. Modern methods of extracting medicinal plant raw materials (review) // Pharmaceutical Chemistry Journal. 2019. T. 56. № 6. p. 45–50. DOI: 10.30906/0023-1134-2019-53-6-48-53.

119. Belokurov S.S., Flisyuk E.V., Narkevich I.A., Luzhanin V.G., Shilov S.V., Novikova K.O. Comparative Analysis of Perspective Extragation Methods for Receiving Extractions from Fenugreek Seeds. *Drug development & registration*. 2019;8(3):49-55. (In Russ.)https://doi.org/10.33380/2305-2066-2019-8-3-49-55

120. Vilkhu K., Mawson R., Simons L., Bates D. Applications and opportunities for ultrasound assisted extraction in the food industry - A review //Innovative Food Science and Emerging Technologies. 2008. Vol. 9. N 2. Pp. 161-169. DOI: 10.1016/j.ifset.2007.04.014.

121. Patist A., Bates D. Ultrasonic innovations in the food industry: From the laboratory to commercial production //Innovative Food Science and Emerging Technologies. 2008. Vol.9. N2. Pp. 147-154. DOI: 10.1016/j.ifset.2007.07.004.

122. Hua-Feng Zhang, Tian-Shun Yang, Zuo-Zhou Li, Ying Wang. Simultaneous extraction of epimedin A, B, C and icariin from Herba Epimedii by ultrasonic technique // Ult. Sonochemistry. 2008. Vol. 15. N 4. Pp. 376-385. DOI: 10.1016/j.ultsonch.2007.09.002.

123. Valeeva A.R., Makarova N.V., Valiulina D.F. Optimisation of conditions for extracting bioactive compounds exhibiting antioxidant properties from hawthorn fruit (*Crataegus*) //Izvestiya Vuzov. Prikladnaya Khimiya i Biotekhnologiya. 2019. Vol. 9. N. 2. Pp. 239–249. DOI: 10.21285/2227-2925-2019-9-2-239-249.

124. Апаева А.В., Ямансарова Э.Т., Куковинец О.С., Зворыгина О.Б. Влияние ультразвукового облучения на извлечение флавоноидов из зеленой массы гречихи //Вестник Башкирского университета. 2016. Т.21. №1. С. 69-72.

125. Wang S., Shi Y., Zhang G., Meng Y., Zhang N., Madhujith T. Optimization of Extraction of Flavonoids and Triterpenoids from Loquat Leaves Using Response Surface Methodology // Food Industry. 2018. Vol. 3. N 3. Pp. 33–45. DOI: 10.29141/2500- 1922-2018-3-3-6.

126. Ameer K., Shahbaz H.M., Kwon J.H. Green Extraction Methods for Polyphenols from Plant Matrices and Their Byproducts: A Review // Comprehensive Reviews in Food Science and Food Safety. 2017. Vol. 16. N 2. Pp. 295–315. DOI: 10.1111/1541-4337.12253.

127. Попова О.С., Скрыпник Л.Н. Сравнительная характеристика эффективности различных методов экстракции полифенолов из растений семейства Яснотковые //Успехи современного естествознания. 2017. № 6. С. 34-38.

128. Tang-Bin Zou, Min Wang, Ren-You Gan, Wen-Hua Ling. Optimization of ultrasound-assisted extraction of anthocyanins from mulberry, using response surface methodology //International journal of molecular sciences. 2011. Vol. 12. N 5. Pp. 3006–3017. DOI: 10.3390/ijms1205300.

129. Sharma A., Gupta M.N. Ultrasonic pre-irradiation effect upon aqueous enzymatic oil extraction from almond and apricot seeds //Ult. Sonochemistry. 2006. N 6. Pp. 529-534. DOI: 10.1016/j.ultsonch.2005.09.008.

130. Patel DK, Prasad SK, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property //Asian Pacific Journal of Tropical Biomedicine. 2012. Vol 2. N4. Pp. 320-330. DOI: 10.1016/S2221-1691(12)60032-X.

131. Timmins P.,.Pygall S.R, Melia C.D. Hydrophilic Matrix Tablets for Oral Controlled Release,Springer-Verlag, New York, 2014. – P. 500.

132. Mahmoudi, M.; Lynch, I.; Ejtehadi, M. R.; Monopoli, M. P.; Bombelli, F. B.; Laurent, S. Protein-Nanoparticle Interactions: Opportunities and Challenges. Chem. Rev. - 2011. – Vol. 111, Is. 5. – P. 5610−5637.

133. Archivio D., Filesi M.C., Di Benedetto R., Gargiulo R., Giovannini C., Masella R. Polyphenols // Dietary Sources and Bioavailability. Annali dell'Istituto Superiore Di Sanità. – 2007. – Vol. 43, Is. 4. – P. 348.

134. Yuan G., Zhang P., Shao M.-a., Luo Y., Zhu X., Energy and water exchanges overa riparian Tamarix spp. stand in the lower Tarim River basin under a HyPerrid climate // Agric. Forest Meteorol.- 2014. – Vol. 194, Is. 6. – P. 144–154.

135. Sharma, S.K., Parmar, V.S. Novel constitutes of Tamarix species // Journal of Scientific and Industrial Research. - 1998. - Vol. 57, Is. 2. - P. 873–890.

136. Lamaison, J.L., Petitjean-Freytet, C., Duband, F., Carnat, A.P. Rosmarinic acid content and antioxidant activity in French Lamiaceae // Fitoterapia 62. – 1990. - Vol. 7, Is. 2. - P.166–171.

137. Massaro M., Colletti C.G., Lazzara G., Riela S. The Use of Some Clay Minerals as Natural Resources for Drug Carrier Applications. *Journal of Functional Biomaterials,* **2018**, 9, No 4, 58. https://doi.org/10.3390/jfb9040058

138. Gylymkhan N.T., Zhumagalieva Sh.N., Abilov Zh.A. Possibilities of using bentonite clays in medicine. Reports of the National Academy of Sciences of the Republic of Kazakhstan, **2016**, № 4, 24-33.

139. Park Ju-H., Shin H-J., Kim M. H., Kim Ji-S., Kang N., Lee J-Y., Kim Ki-T. and ets Application of montmorillonite in bentonite as a pharmaceutical excipient in drug delivery systems. *J Pharm Investig.,* **2016**, 46, No 4, 363-375. https://doi.org/10.1007/s40005-016-0258-8

140. Nazarova F.Sh., Matkarimova G.M., Nazarova G.Kh. Lechebnye svoistva bentonita. Achievements of Science and Educatio, **2020**, №5, 59.

141. Гарасько Е.В., Родионова А.Н., Алексеева О.В., Багровская Н.А., Агафонов А.В. Структура и биологическая активность композитов гидроксиэтилцеллюлозы/бентонит. *Успехи современного естествознания,* **2015**, № 11-1, 20-25.

142. Devi N., Dutta J. Preparation and characterization of chitosan-bentonite nanocomposite films for wound healing application, *International Journal of Biological Macromolecules,* **2017**, 104 (B), 1897-1904. https://doi.org/10.1016/j.ijbiomac.2017.02.080

143. Жумагалиева Ш.Н. Глинистые композиции натриевой соли карбоксиметилцеллюлозы в качестве носителя рихлокаина. *Известия НАН РК. Серия химическая,* **2008**, №5, 63-66

144. Notarbartolo M., Massaro M., Barbosa R., Emili C., Liotta L., Poma P., Raymo F., ets Exploring the cellular uptake of hectorite clay mineral and its drug carrier capabilities. *Colloids and Surfaces B: Biointerfaces,* **2022**, 220, 112931. https://doi.org/10.1016/j.colsurfb.2022.112931

145. Wang Q., Wang M., C henghong L., Yan L., Wu X., Li L. Functionalizing graphene with clay nanosheets as a protein carrier. *Colloid and Interface Science Communications*, **2022**, 48, 100618. https://doi.org/10.1016/j.colcom.2022.100618

146. Phongphut A., Chayasombat B., Cass E.G. A., Sirisuk A., Phisalaphong M., Prichanont S., Thanachayanont Ch. Clay/au nanoparticle composites as acetylcholinesterase carriers and modified-electrode materials: A comparative study. *Applied Clay Science,* **2020**, 194, 105704. https://doi.org/10.1016/j.clay.2020.105704

147. Cacciotti I., Lombardelli C., Benucci I., Esti M. Clay/chitosan biocomposite systems as novel green carriers for covalent immobilization of food enzymes. *Journal of Materials Research and Technology,* **2019**, 8, No 4, 3644-3652. https://doi.org/10.1016/j.jmrt.2019.06.002

148. Jain Sh., Dutta A., Kumar J., Shakil N.A. Preparation and characterization of dicarboxylic acid modified starch-clay composites as carriers for pesticide delivery. *Arabian Journal of Chemistry,* **2020**, 13, No 11, 7990-8002. http://dx.doi.org/10.1016/j.arabjc.2020.09.028

149. El-Hamshary H., El-Newehy H. M., Abdulhameed M. M., El-Faham A., Elsherbiny A. S. Evaluation of clay-ionene nanocomposite carriers for controlled drug delivery: Synthesis, in vitro drug release, and kinetics. *Materials Chemistry and Physics,* **2019**, 225, 122-132. https://doi.org/10.3390/molecules28155895

150. Cheikh D., García-Villén F., Majdoub H., Zayani M. B., Viseras C. Complex of chitosan pectin and clay as diclofenac carrier. *Applied Clay Science,* **2019**, 172, 155-164. http://dx.doi.org/10.1016/j.clay.2019.03.004

151. Сапаргалиев Е.М., Батурина Г.М., Шатобин А.А., Раисова М.К. Способ получения основы для медицинских, косметических и ветеренарных препаратов // Патент Республики Казахстан № 940211.1. Бюл. №4. 1997.

152. Алексеева И. В. Разработка лекарственных форм для лечения ран. «Фармация», 2003.- С.43-45.

153. *Gosudarstvennaya farmakopeya Respubliki Kazakhstan.* [The State Pharmacopoeia of Kazakhstan]. Almaty, 2008, vol. 1, 592 p. (in Russ.).

154. Eskalieva B.K. Fitopreparaty i khimiya prirodnykh biologicheski aktivnykh veshchestv – A.: Kazakh universiteti., 2013. – 22-50 б.

155. Frolova L.N., Kovaleva E.L., Sakanyan E.I., Kuteinikov V.Yu., Shelestova V.V., Cheremisina M.A. "Sravnitel'nyy analiz nomenklatury lekarstvennogo rastitel'nogo syr'ya, ispol'zuemogo v otechestvennoy i mirovoy farmakopeynoy praktike." Vedomosti Nauchnogo tsentra ekspertizy sredstv meditsinskogo primeneniya. – 2020. – T. 10, № 1 – C. 29-40.

156. Tokul Ölmez Ö, Şahin B, Çakır C, Öztürk M. (2020). Rapid and easy method for simultaneous measurement of widespread 27 compounds in natural products and foods. *Journal of Chemical Metrology*. https://doi.org/10.25135/jcm.38.20.03.1589

157. Parvin, M.S., Das, N., Jahan, N. *et al.* Evaluation of in vitro anti-inflammatory and antibacterial potential of *Crescentia cujete* leaves and stem bark. *BMC Res Notes* **8**, 412 (2015). https://doi.org/10.1186/s13104-015-1384-5

158. Shinde, U.A.; Phadke, A.S.; Nair, A.M.; Mungantiwar, A.A.; Dikshit, V.J.; Saraf, M.N. Membrane stabilizing activity—A possible mechanism of action

for the anti-inflammatory activity of *Cedrus deodara* wood oil. *Fitoterapia* **1999**, *70*, 251–257.

159. Alves, T.M.A., Silva, A.F., Brandao, M., Grandi, T.S.M., Smania, E.F.A., Smania, Jr. A., Zani, C.L. **(2000).** Biological screening of brazilian medicinal plants. *Mem Inst Oswaldo Cruz*, 95: 367-373

160. Kivack, B., Mert, T., and Tansel, H. (**2001**). Antimicrobial and Cytotoxic activities of *Ceratonia siliqua* L. extracts. *Turk. J. Biol*., 26:197-200

161. Carballo. L. J., Hernandez-inda. L. Z., Perez. P., and Gravalos, M.D. (**2002**). A comparison between two brine shrimp assays to detect in vitro cytotoxicity in marine natural products. *Bio Med Central Biotechnology*, 2, 1-10

162. Mayer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nicholas P. E., and McLaughin, J. L. (**1982**). Brine Shrimp: A convenient general bioassay for active plant constituents. *Planta Medica*, 45: 31-34

163. D.J. Finny, Probit Analysis. 3rd edition, Cambridge University Press, Cambridge, p 333 (1971)

164. Kojima Y., Usuki A., Kawasumi M., Okada A., Kurauchi T., Kamigaito O. Synthesis of nylon 6 – clay hybrid by montmorillonite intercalated with e – caprolactam//J. Polym. Sci., Part A, -1993.-Vol.31.-P. 983-986.

165. Kojima Y., Usuki A., Kawasumi M., Okada A., Kurauchi T., Kamigaito O. One-pot synthesis of nylon 6 – clay hybrid//J. Polym. Sci., PartA, -1993.-Vol.31.-P. 1755-1758.

166. Герасин В.А., Зубова Т.А., Бахов Ф.Н., Баранников А.А., Мерекалова Н.Д., Королев Ю.М., Антипов Е.М. Структура нанокомпозитов полимер/Na^+-монтмориллонит, полученных смешением в расплаве //Российские нанотехнологии. -2007. -Т.2. -№1-2. -С. 90-105.

167. G. BishwaBiditaVaradwaj, Surjyakanta R.,K. M. Parida.A stable amine functionalized montmorillonite supported Cu, Ni catalyst showing synergistic and co-operative effectiveness towards C–S coupling reactions //**RSC Advances,**-2013. -№**3.**–P. 7570-7578.

168. Jaideep B. Bharate, Rajni Sharma, SubrayashastryAravinda, Vivek K. Gupta, Baldev Singh, Sandip B. Bharate and Ram A. Vishwakarma. Montmorillonite clay catalyzed synthesis of functionalized pyrroles through domino four-component coupling of amines, aldehydes, 1,3-dicarbonyl compounds and nitroalkanes //RSC Advances,-2013. -№**3. –P.** 21736-21742.

169. Yu peng, Zhongying Li,RuirenTang.Epoxidation of cyclohexene with O_2 over the composite catalysts of Mn-montmorillonite coordinated with novel Schiff-base ligands //RSC Advances, -2013. -№ **3. –P.** 19965-19970.

170. N.B. Sakhibov, M.Yu. Yunusov, A.R. Kariev, M.D. Isobaev. Adsorbtsionnye svoystva bentonitovykh i paligorskitovykh glin Tadzhikistana // Izvestiya akademii nauk Respubliki Tadzhikistan, otdelenie fiziko-matematicheskikh, khimicheskikh, geologicheskikh i tekhnicheskikh nauk, 2011, №4 (145), p. 110–115.

171. A.I. Tuchkova, E.A. Tyupina. Poluchenie modifitsirovannogo bentonita dlya ochistki vakuumnykh masel ot Cs-137 // Perspektivnye materialy, -2011. -p. 307-311.

172. Mostafa Hossein Baki, FarzanehShemirani, RouhollahKhani and MehrnooshBayat. Applicability of diclofenac–montmorillonite as a selective sorbent for adsorption of palladium(II); kinetic and thermodynamic studies //Analytical Methods**,** -2014. -№6. –P. 1875-1883.

173. A.I. Vezentsev, S.V. Korolkova, V.D. Bukhanov. Teksturnye kharakteristiki i sorbtsionnye svoystva prirodnoy i magniy-zameshchennoy montmorillonit-soderzhashchey gliny // Nauchnye vedomosti, seriya estestvennye nauki, 2010, T.9, №11, p. 119–123.

174. N.G. Taran, N.F. Dityar, I.N. Ponomarev. Izuchenie vliyaniya obrabotki vinomaterialov aktivirovannym bentonitom na ikh mineral'nyy i fenol'nyy sostav // Nauchnye trudy GNU SKZNIISiV, 2013, T.4, p. 116–121

175. Bortnikov S.V., Gorenkova G.A. Modifikatsiya shchelochnozemel'nogo bentonita oleatom natriya // Al'manakh sovremennoy nauki i obrazovaniya, 2013, № 8 (75), s. 32–34.

176. Mezhidov V.Kh., Viskhanov S.S., Daudova A.L. Khimicheskiy sostav bentonita (mestorozhdeniya Chechenskoy Respubliki) // Izvestiya vysshikh uchebnykh zavedeniy. Severokavkazskiy region. Seriya: Tekhnicheskie nauki, 2013, № 4 (173), s. 67–70.

177. Vezentsev A.I., Trubitsyn M.A. Montmorillonitovye gliny kak potentsial'nyy sorbent patogennykh veshchestv i mikroorganizmov // Sorbtsionnye i khromatograficheskie protsessy, 2012, T.12, vyp. 6, s. 998–1004

178. S.S.Guptad, G. Bhattacharya. Adsorption of heavy metals on kaolinite and montmorillonite: a review //Physical Chemistry Chemical Physics -2012. -№**14.**–P. 6698-6723.

179. P. K. Verma,P. N. Pathak,P. K. Mohapatra,S. V. Godbole,R. M. Kadam,A. A. Veligzhanin,Y. V. ZubavichusS. N. Kalmykov. Influences of different environmental parameters on the sorption of trivalent metal ions on bentonite: batch sorption, fluorescence, EXAFS and EPR studies //Environmental Science: Processes &Impacts .-2014. -№2. –P. 6789-6794.

180. Архиреев В.П., Ибрагимов М.А., Трофимова Ф.Ф., Демидова М.И. Влияние состава слоистых силикатов типа бентонитов на термостойкость резин из силоксанового каучука //Вестник Казанского технологического университета,-2009. -№2. -С. 60-63.

181. Qilong Tai, Xueying Shan, Lei Song, Siuming Lo, Richard K.K, Yuan HuA. Polymeric flame retardant and surfactant-free montmorillonite nanocomposites: Preparation and exfoliation mechanism discussion //Polymer Composites, -2014.-Vol. 35(1), -P. 167–173.

182. Terent'ev A.N. Bentonit i vozmozhnoe ego primenenie v meditsine. – Ashkhabad, 1994. – 274 s.

183. Khalifaev D.R. Perspektivnoe ispol’zovanie bentonitov dlya proizvodstva farmatsevticheskikh preparatov // Materialy kongressa rabotnikov zdravookhraneniya Respubliki Tadzhikistana, 1997, T.11, s. 284–286.

184. Murav’ev I.A., Zhakova N.A. Issledovanie bentonitovykh glin dlya prigotovleniya mazey–suspensiy // Aptechnoe delo, 1964, №5, s. 23–25.

185. Fel’dshteyn M.M., Zezin A.B. Priroda vzaimodeystviya detergentov s polipeptidami i polielektrolitami // Mol. Biol., 1974, T.8, №1, s. 142–153.

186. Musabekov K.B., Legkunets R.E., Zhubanov B.A., Abilov Zh.A. Vzaimodeystvie polielektrolitov s poverkhnostno-aktivnymi veshchestvami // Trudy Instituta khim. nauk AN KazSSR: Khimiya monomerov i polimerov. – Alma-Ata, 1980, Ch.13, s. 104–121.

187. Issledovanie i ispol’zovanie glin i glinistykh mineralov. Pod red. Tazhibaevoi P.T. – Alma-Ata: Nauka, 1970. – 307 s.

188. Tarasevich Yu.T., Ovcharenko F.D. Adsorbtsiya na glinistykh mineralakh. – Kiev: Naukova dumka, 1975. – 351 s.

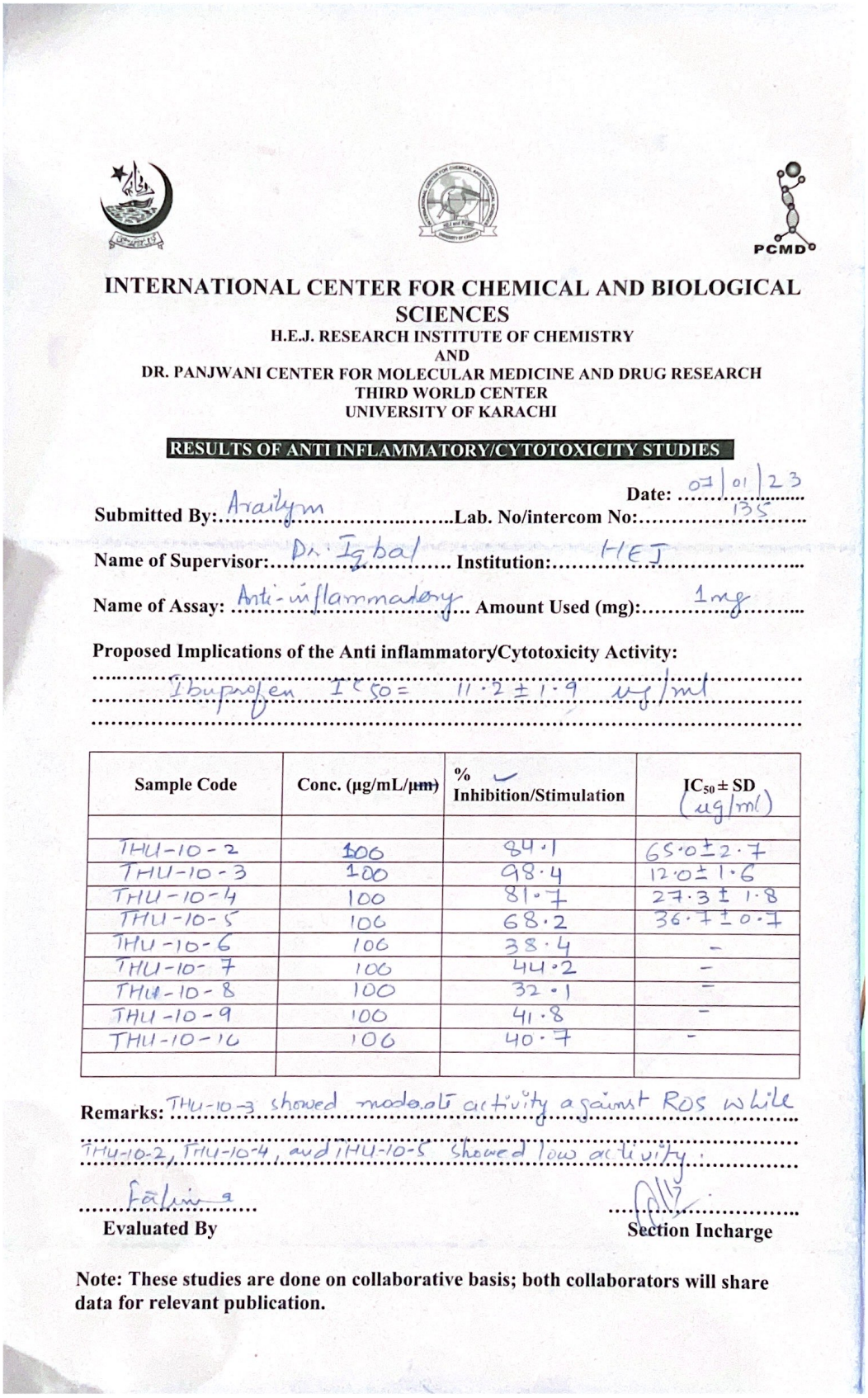
189. Khil’ko S.L., Titov E.V. Osobennosti techeniya vodnykh suspenziy paligorskitovoy i bentonitovoy gliny // Kolloid. zhurn., 2002, T.64, №5, s. 697–703.

190. Pettit, R.K., Weber, C.A., Kean, M.J., Hoffmann, H., Pettit, G.R., Tan, R., Franks K.S., and Horton, M.L. Microplate alamar blue assay for Staphylococcusepidermidis Biofilm susceptibility testing. // Antimicrob Agents Chemother, 2005,49(7), 2612–2617.

191. Jan Hilhorst, Vera Meester, Esther Groeneveld, Jan K. G. Dhont, and Henk N. W. Lekkerkerker, Structure and Rheology of Mixed Suspensions of Montmorillonite and Silica Nanoparticles // J. Phys. Chem. B, 2014, 118 (40), pp 11816–11825

192. G. Schramm. Osnovy prakticheskoy reologii i reometrii. – Moskva: “KolosS”, 2003, s. 215–289.

193. 193. Kartalov M.G., Dmitruk S.E., Dmitruk V.S., Romanenko T.V. Issledovanie strukturno-mekhanicheskikh svoystv mazi “Kartalin” // Byulleten’ sibirskoy meditsiny, 2009, T.8, №3, s. 48–52



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RESULTS OF ANTI INFLAMMATORY/CYTOTOXICITY STUDIES

Date: 07/01/23

Submitted By: Arailym Lab. No/intercom No: 135

Name of Supervisor: Dr. Iqbal Institution: HEJ

Name of Assay: Anti-inflammatory Amount Used (mg): 1mg

Proposed Implications of the Anti inflammatory/Cytotoxicity Activity:

Ibuprofen IC_{50} = 11.2 ± 1.9 µg/ml

Sample Code	Conc. (µg/mL/µm)	% Inhibition/Stimulation	IC_{50} ± SD (ug/ml)
THU-10-2	100	84.1	65.0 ± 2.7
THU-10-3	100	98.4	12.0 ± 1.6
THU-10-4	100	81.7	27.3 ± 1.8
THU-10-5	100	68.2	36.7 ± 0.7
THU-10-6	100	38.4	-
THU-10-7	100	44.2	-
THU-10-8	100	32.1	-
THU-10-9	100	41.8	-
THU-10-10	100	40.7	-

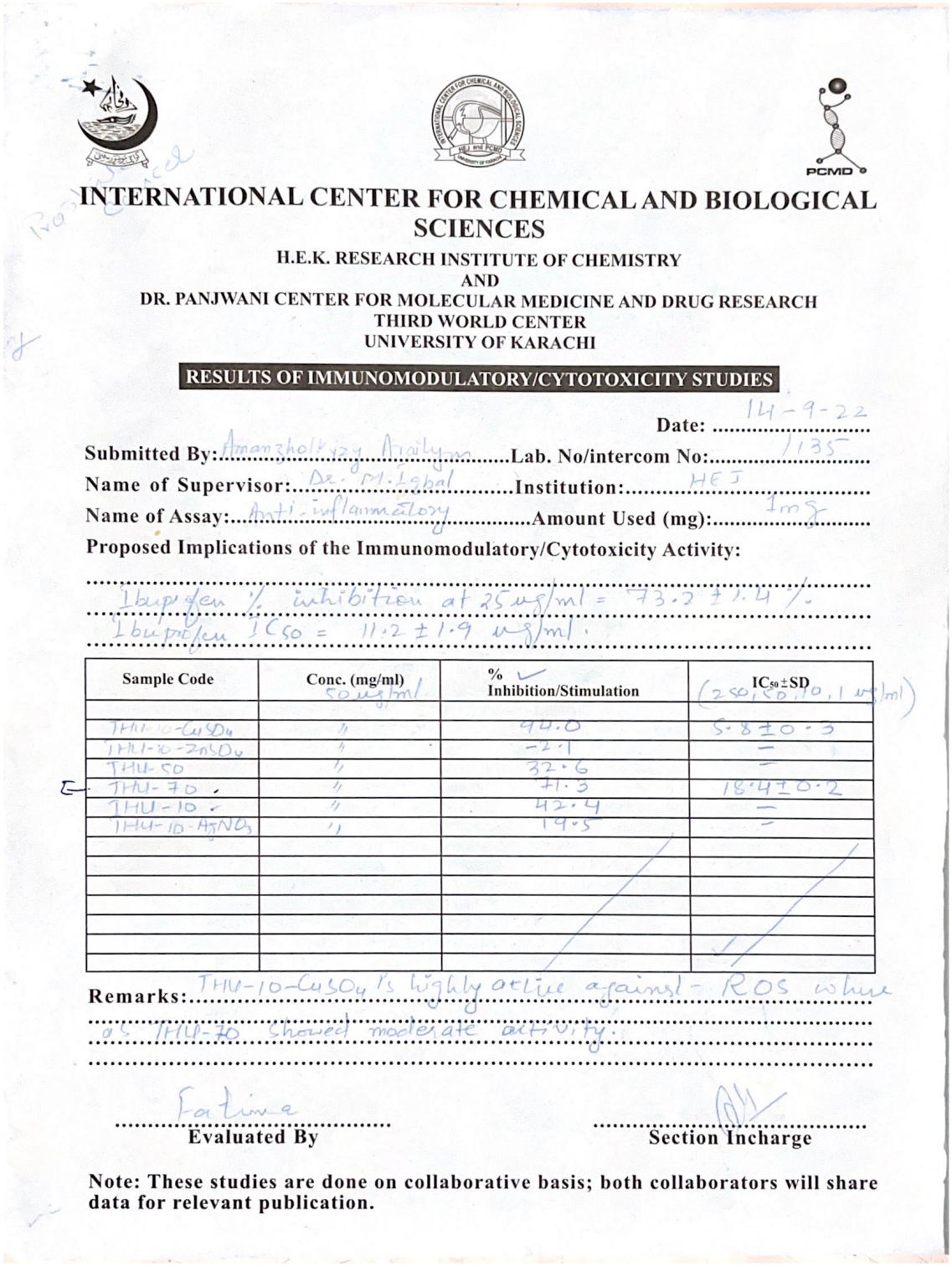
Remarks: THU-10-3 showed moderate activity against ROS while THU-10-2, THU-10-4, and THU-10-5 showed low activity.

Fatima
Evaluated By

Section Incharge

Note: These studies are done on collaborative basis; both collaborators will share data for relevant publication.

Figure A.1 – Results of anti-inflammatory/cytotoxicity studies



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RESULTS OF IMMUNOMODULATORY/CYTOTOXICITY STUDIES

Date: 14-9-22

Submitted By: Amanzholkyzy Ainilyam ..... Lab. No/intercom No: 1135

Name of Supervisor: Dr. M. Iqbal ..... Institution: HEJ

Name of Assay: Anti-inflammatory ..... Amount Used (mg): 1mg

Proposed Implications of the Immunomodulatory/Cytotoxicity Activity:

Ibuprofen % inhibition at 25 µg/ml = 73.2 ± 1.4 %.
Ibuprofen IC_{50} = 11.2 ± 1.9 µg/ml.

Sample Code	Conc. (mg/ml) 50 µg/ml	% Inhibition/Stimulation	IC_{50} ± SD (250, 50, 10, 1 µg/ml)
THU-10-$CuSO_4$	//	40.0	5.8 ± 0.3
THU-10-$ZnSO_4$	//	-2.1	—
THU-50	//	32.6	—
THU-70	//	±1.3	18.4 ± 0.2
THU-10	//	42.4	—
THU-10-$AgNO_3$	//	19.5	—

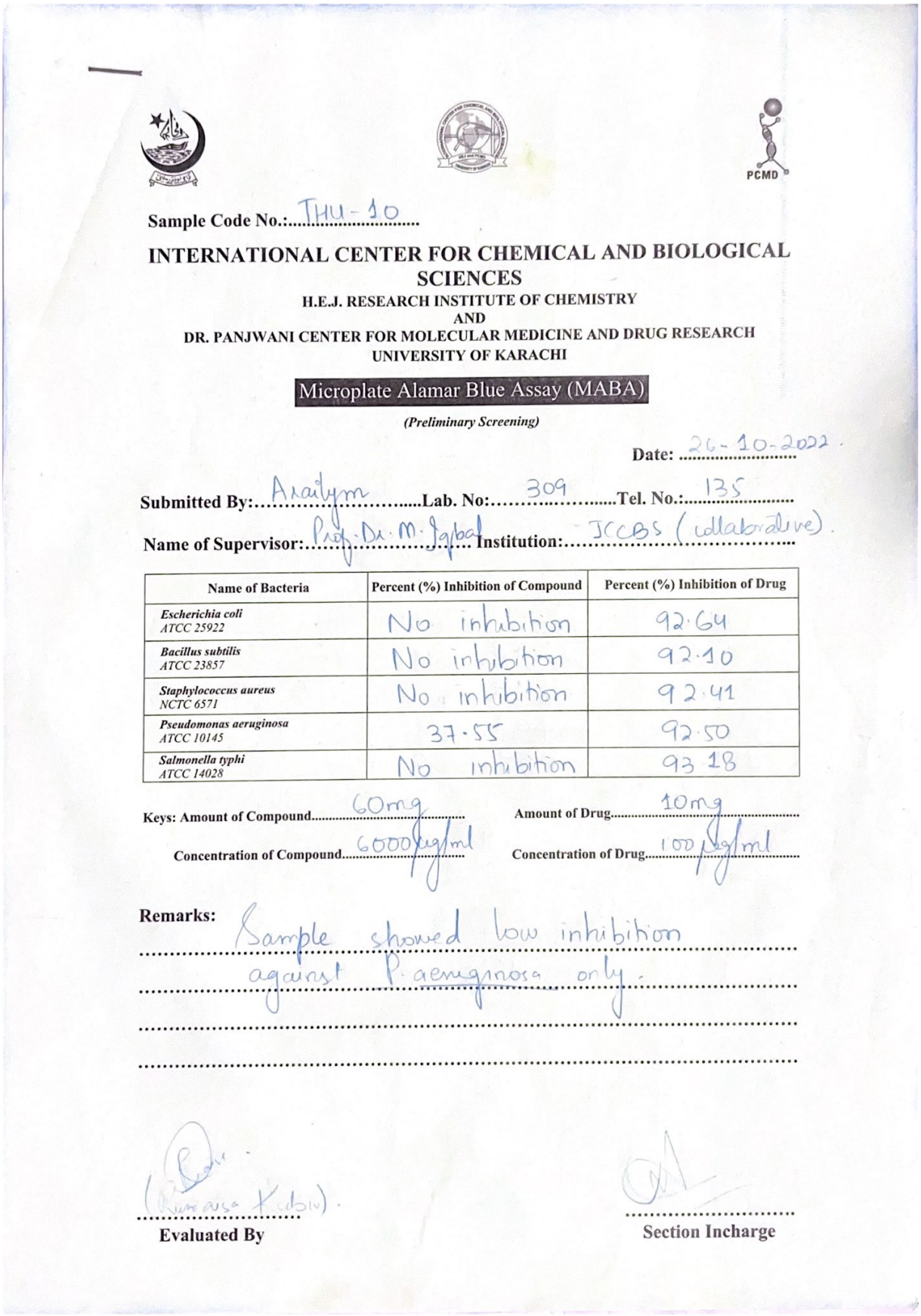
Remarks: THU-10-$CuSO_4$ is highly active against ROS while ROS THU-70 showed moderate activity.

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Note: These studies are done on collaborative basis; both collaborators will share data for relevant publication.

Figure A.2 – Results of anti-inflammatory/cytotoxicity studies



Sample Code No.: THU-10

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Microplate Alamar Blue Assay (MABA)

(Preliminary Screening)

Date: 26-10-2022

Submitted By: Arailym Lab. No: 309 Tel. No.: 135

Name of Supervisor: Prof. Dr. M. Iqbal Institution: ICCBS (collaborative)

Name of Bacteria	Percent (%) Inhibition of Compound	Percent (%) Inhibition of Drug
Escherichia coli ATCC 25922	No inhibition	92.64
Bacillus subtilis ATCC 23857	No inhibition	92.10
Staphylococcus aureus NCTC 6571	No inhibition	92.41
Pseudomonas aeruginosa ATCC 10145	37.55	92.50
Salmonella typhi ATCC 14028	No inhibition	93.18

Keys: Amount of Compound: 60mg Amount of Drug: 10mg

Concentration of Compound: 6000µg/ml Concentration of Drug: 100µg/ml

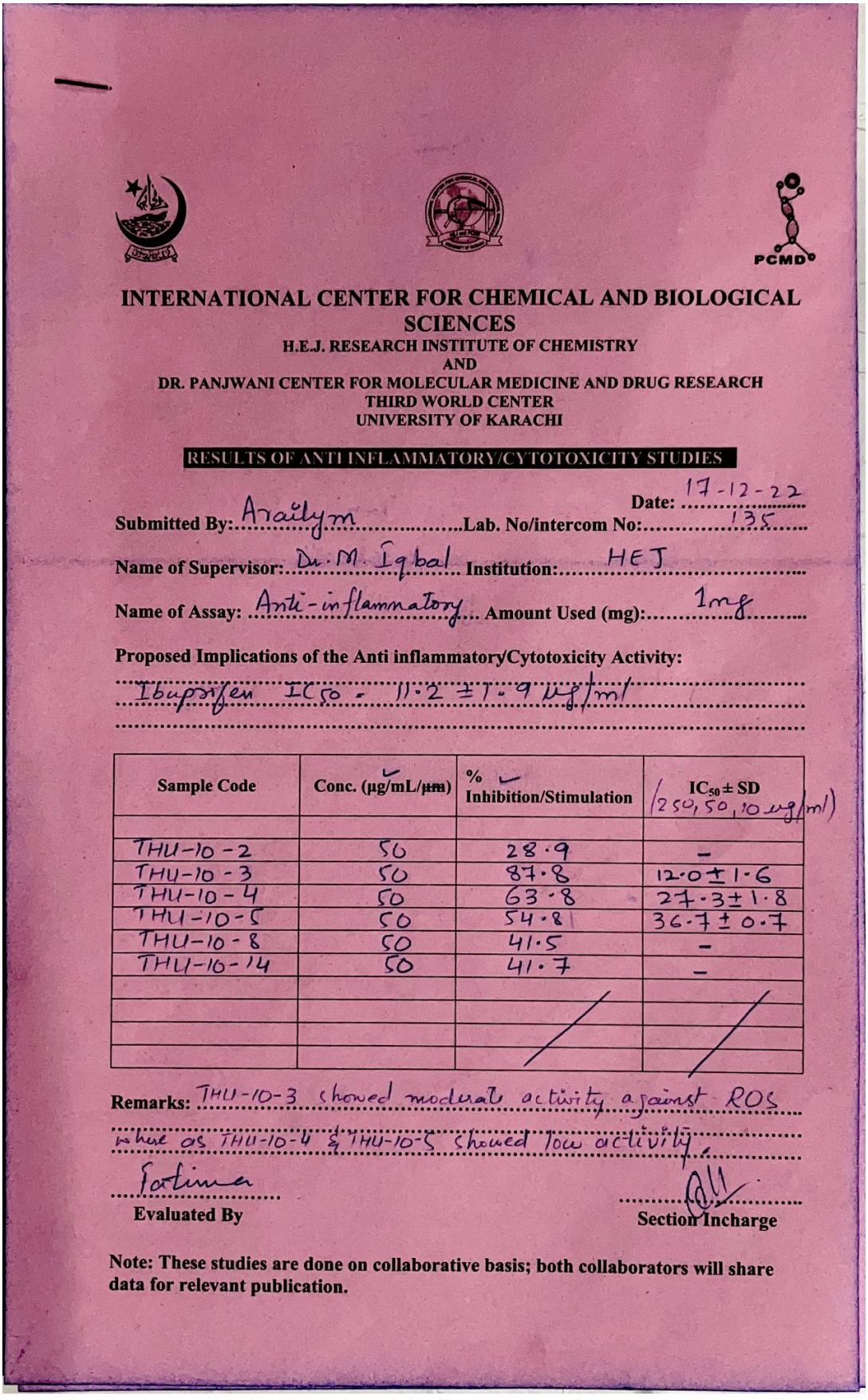
Remarks: Sample showed low inhibition against P. aeruginosa only.

(Sumaira Kabir)

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Figure A.3 – Results of anti-inflammatory/cytotoxicity studies



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RESULTS OF ANTI INFLAMMATORY/CYTOTOXICITY STUDIES

Date: 17-12-22

Submitted By: Arailym ..... Lab. No/intercom No: 135

Name of Supervisor: Dr. M. Iqbal Institution: HEJ

Name of Assay: Anti-inflammatory Amount Used (mg): 1mg

Proposed Implications of the Anti inflammatory/Cytotoxicity Activity:

Ibuprofen IC50 = 11.2 ± 1.9 µg/ml

Sample Code	Conc. (µg/mL/~~µm~~)	% Inhibition/Stimulation	IC_{50} ± SD (250, 50, 10 ug/ml)
THU-10-2	50	28.9	-
THU-10-3	50	87.8	12.0 ± 1.6
THU-10-4	50	63.8	27.3 ± 1.8
THU-10-5	50	54.8	36.7 ± 0.7
THU-10-8	50	41.5	-
THU-10-14	50	41.7	-

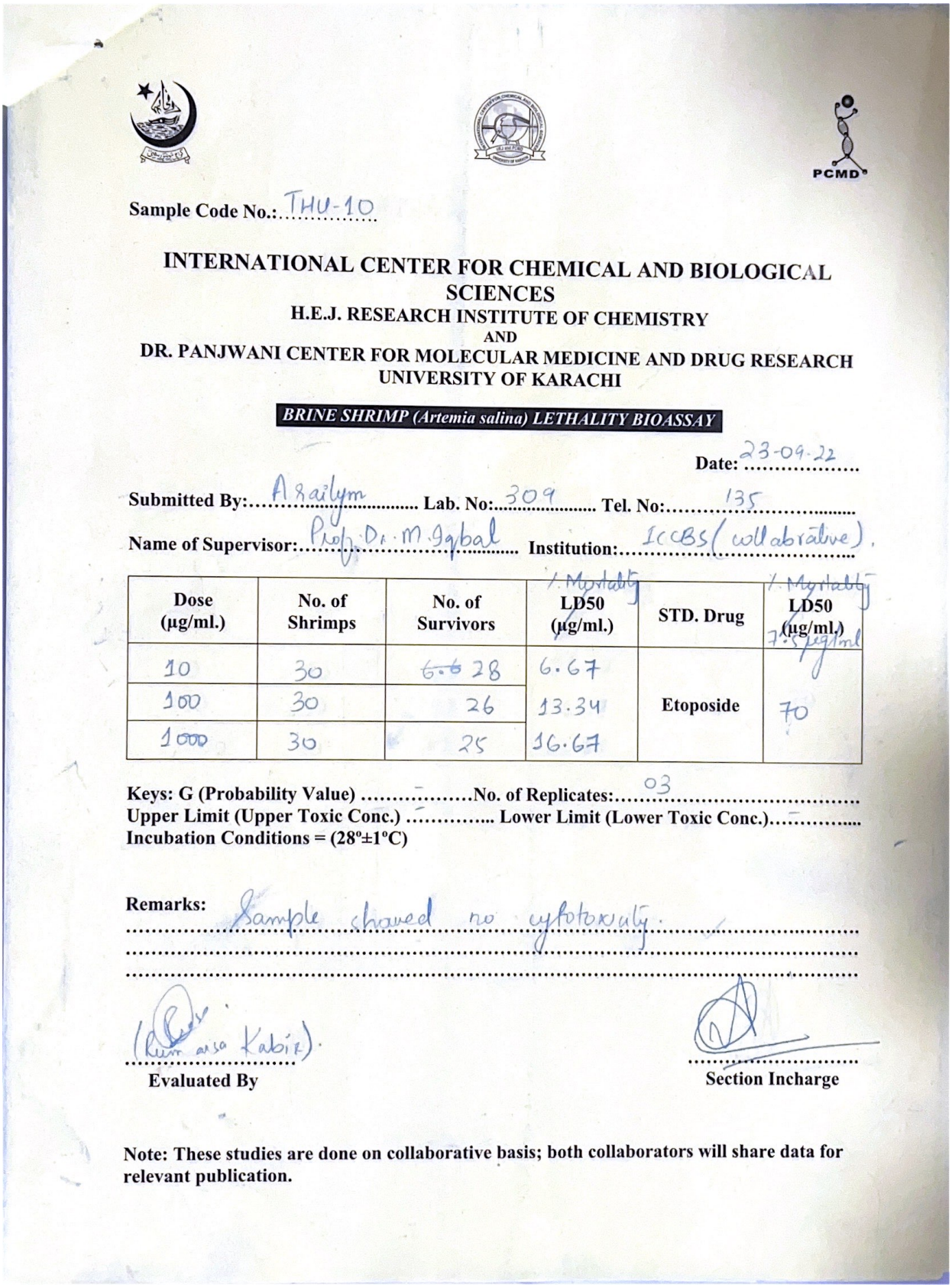
Remarks: THU-10-3 showed moderate activity against ROS whereas THU-10-4 & THU-10-5 showed low activity.

Fatima
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Section Incharge

Note: These studies are done on collaborative basis; both collaborators will share data for relevant publication.

Figure A.4 – Results of anti-inflammatory/cytotoxicity studies



Sample Code No.: THU-10

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BRINE SHRIMP (Artemia salina) LETHALITY BIOASSAY

Date: 23-09-22

Submitted By: Arailym Lab. No: 309 Tel. No: 135

Name of Supervisor: Prof. Dr. M. Iqbal Institution: ICCBS (collabrative).

Dose (µg/ml.)	No. of Shrimps	No. of Survivors	% Mortality LD50 (µg/ml.)	STD. Drug	% Mortality LD50 (µg/ml.)
10	30	~~6.6~~ 28	6.67	Etoposide	7.5 µg/ml
100	30	26	13.34		70
1000	30	25	16.67		

Keys: G (Probability Value) ……No. of Replicates: 03
Upper Limit (Upper Toxic Conc.) …….. Lower Limit (Lower Toxic Conc.)……..
Incubation Conditions = (28°±1°C)

Remarks: Sample showed no cytotoxicity.

(Rumaisa Kabir).
Evaluated By

Section Incharge

Note: These studies are done on collaborative basis; both collaborators will share data for relevant publication.

Figure A.5 – Results of anti-inflammatory/cytotoxicity studies

APPENDIX B

Sample № Content, %	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Bentonite clay	20	25	30	20	25	30	20	25	30	20	25	30	20	25	30
Glycerine	5	5	5	5	5	5	1	1	1	2	2	2	1	1	1
Vaseline	5	5	5	-	-	-	-	-	-	2	2	2	5	5	5
Twin-80	1	1	1	-	-	-	-	-	-	1	1	1	1	1	1
Potassium sorbate	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2
Water	68,8	63,8	58,8	74,8	69,8	64,8	78,8	73,8	68,8	74,8	69,8	64,8	72,8	67,8	62,8
Sample № Content, %	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Bentonite clay	20	25	30	20	25	30	20	25	30	20	25	30	20	25	30
Glycerine	5	5	5	5	5	5	5	5	5	1	1	1	2	2	2
Vaseline	3	3	3	-	-	-	-	-	-	2	2	2	2	2	2
Twin-80	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2
Potassium sorbate	70,8	65,8	60,8	72,8	67,8	62,8	71,8	66,8	61,8	75,8	70,8	65,8	74,8	69,8	64,8
THU-10	1	1	1	2	2	2	3	3	3	1	1	1	1	1	1
Sample № Content, %	***31***	***32***	***33***	**34**	**35**	**36**	**37**	**38**	**39**	**40**		**41**		**42**	
Бентонит	***25***	***25***	***25***	20	20	20	25	25	25	30		30		30	
Глицерин	***1***	***1***	***1***	2	2	2	1	1	1	1		1		1	
Сорбат калия	***0,2***	***0,2***	***0,2***	0,2	0,2	0,2	0,2	0,2	0,2	0,2		0,2		0,2	
Вода	***72,8***	***71,8***	***70,8***	76,8	75,8	74,8	72,8	71,8	70,8	67,8		66,8		65,8	
ТН-10	***1***	***2***	***3***	1	2	3	1	2	3	1		2		3	

Figure B1 - Samples of hydrogels based on bentonite clay