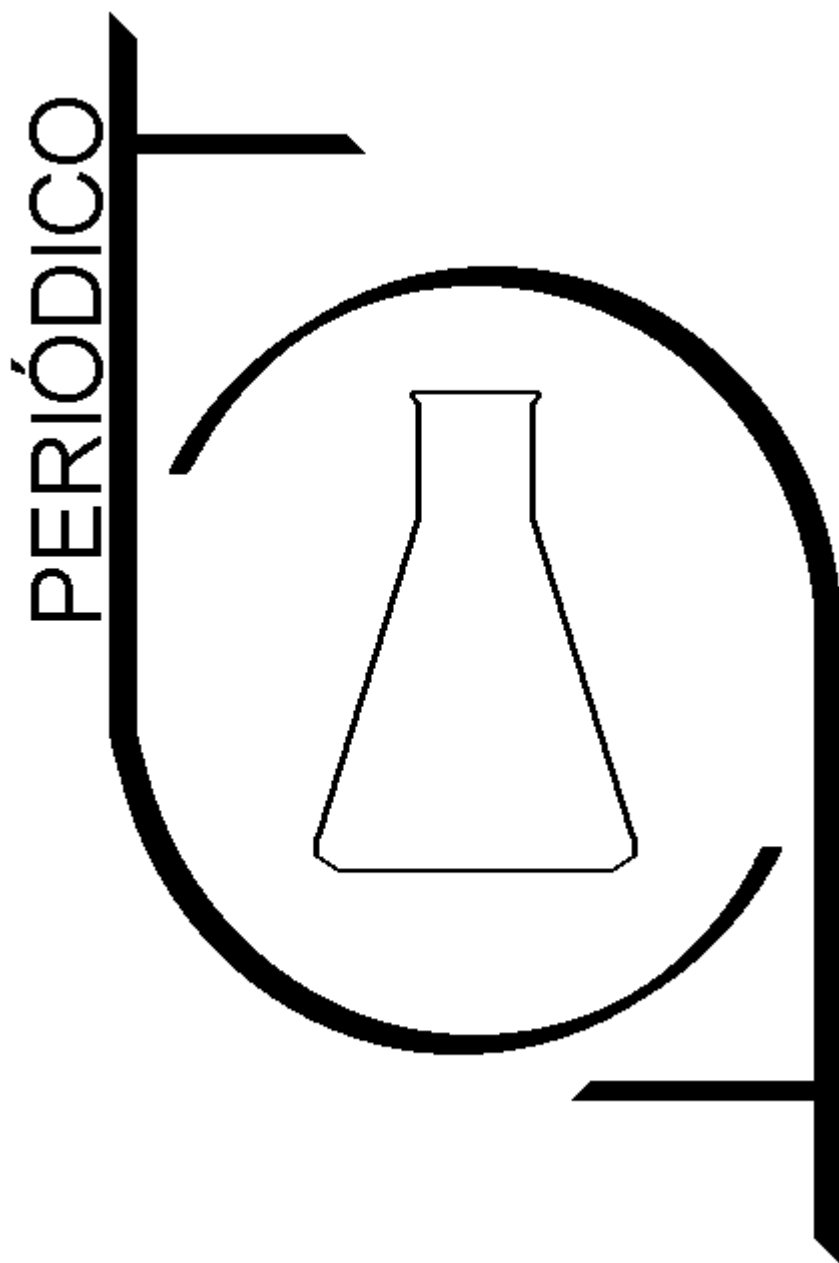


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OBTENÇÃO DE HIDROGELS MISTOS BASEADOS EM BIOPOLÍMEROS E ESTUDO DE SUAS PROPRIEDADES REOLÓGICAS**PREPARATION OF MIXED HYDROGELS BASED ON BIOPOLYMERS AND THE STUDY OF THEIR RHEOLOGICAL PROPERTIES****ПОЛУЧЕНИЕ СМЕШАННЫХ ГИДРОГЕЛЕЙ НА ОСНОВЕ БИОПОЛИМЕРОВ И ИЗУЧЕНИЕ ИХ РЕОЛОГИЧЕСКИХ СВОЙСТВ**

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RESUMO

Um método bem conhecido, acessível e barato de modificação de biopolímeros é simplesmente sua mistura com polímeros sintéticos e naturais. Como resultado, semelhante às ligas metálicas, é possível obter complexo interpolímeros com propriedades predeterminadas. Apesar do fato de que os complexos interpolímeros terem sido estudados e aplicados há muito tempo, na prática doméstica o estudo de biopolímeros naturais é limitado. O objetivo deste estudo é apresentar e estudar o problema de estruturação em soluções de biopolímeros e polímeros sintéticos, uma vez que, com baixo teor de matéria seca, essas estruturas têm muitas propriedades do corpo sólido. Polímeros anfóliticos naturais são polieletrólitos, o que também é de grande importância para estudos futuros. Ao analisar os resultados de estudos de complexos formados por um polieletrólito linear sintético com o parceiro carregado – a proteína, torna-se possível criar novos complexos de polieletrólitos “inteligentes” que podem sofrer alterações de fase em faixas estreitas e controladas de pH. Foi estabelecido o efeito positivo da carboximetilcelulose de sódio na gelificação de hidrogéis de gelatina a 5%. Foi estabelecido que a presença de aditivos de sais inorgânicos leva a um aumento na expansão de géis de gelatina. Foi também estabelecido que os géis mais duráveis são menos propensas ao envelhecimento. A importância prática desses estudos é determinada pelo fato de que em várias indústrias de alimentos, sabão, tinta e verniz e outras, é necessário obter estruturas com as propriedades desejadas. A formação dos géis também pode ser um fenômeno indesejável que deve ser evitado, por exemplo, na produção de fibras químicas, colas e soluções de bronzeamento. Além disso, na maioria dos casos, os próprios objetos biológicos são os géis de proteínas com certas propriedades mecânicas.

Palavras-chave: *estrutura e dinâmica de gel de gelatina, gelificação de gelatina, colapso de redes heterogêneas, expansão de gel de gelatina, adsorção de gelatina.*

ABSTRACT

A well-known, affordable, and cheap method of modifying biopolymers is their simple mixing with synthetic and natural polymers. As a result, similar to metal alloys, polymer-polymer complexes with predetermined properties can be obtained. Although interpolymers complexes became a subject of study and application a long time ago, in domestic practice, the study of natural biopolymers is limited. The purpose of this study was to present and study the issue of structuring in solutions of bio- and synthetic polymers, as with a low dry matter content such structures have many properties of a solid. Natural ampholytic polymers are polyelectrolytes, which is also of great importance for their further study. By analyzing the results of studies of complexes formed by a synthetic linear polyelectrolyte with a charged partner – protein, it becomes possible to create new “smart” polyelectrolyte complexes that can undergo phase changes in narrow, controlled pH ranges. The positive effect of sodium carboxymethyl cellulose on the gelation of 5% gelatine hydrogels was established. It was determined that the presence of additives of inorganic salts leads to an increase in the swelling of gelatine gels. It was established

that more durable gels are less prone to aging. The practical significance of these studies is determined by the fact that in many industries (food, soap, paint, and varnish), it is necessary to obtain structures with tailor-made properties. Gel formation may also be an undesirable phenomenon that must be prevented, for example, in the production of chemical fibers, glues, and tanning solutions. Furthermore, biological objects themselves in most cases, by their nature, are protein gels with specific mechanical properties.

Keywords: *structure and dynamics of gelatine mixed hydrogels, adsorption on solid surfaces, collapse of heterogeneous networks, swelling and aging of mixed gelatine hydrogels.*

АННОТАЦИЯ

Хорошо известным, доступным и дешевым методом модификации биополимеров является их простое смешение с синтетическими и природными полимерами. В результате, подобно сплавам металлам, можно получить полимер-полимерные комплексы с заранее заданными свойствами. Несмотря на то, что интерполимерные комплексы начали изучаться и применяться давно, в отечественной практике изучение природных биополимеров ограничено. Цель данного исследования – представить и изучить проблему структурирования в растворах био- и синтетических полимеров, так как при незначительном содержании сухого вещества такие структуры обладают многими свойствами твердого тела. Природные амфолитные полимеры являются полиэлектролитами, что также имеет огромное значение для их дальнейшего изучения. Анализируя результаты исследований комплексов, образованных синтетическим линейным полиэлектролитом с заряженным партнером – белком, становится возможным создание новых «умных» полиэлектролитных комплексов, способных претерпевать фазовые изменения в узких, контролируемых диапазонах pH. Установлено положительное влияние натрийкарбоксиметилцеллюлозы на гелеобразование 5% гидрогелей желатина. Установлено, что присутствие добавок неорганических солей приводит к увеличению набухания студней желатина. Установлено, что более прочные студни менее подвержены старению. Практическое значение этих исследований определяется, тем, что в ряде отраслей промышленности пищевой, мыловаренной, лакокрасочной и других надо получать структуры с заданными свойствами. Образование студней может оказаться и нежелательным явлением, которое надо предотвращать, например, в производстве химических волокон, клеев, растворов дубителей и т.д. Кроме того, сами биологические объекты в большинстве случаев по своей природе представляют собой студни белков с определенными механическими свойствами.

Ключевые слова: *структура и динамика смешанных желатиновых гидрогелей, адсорбция на твердых поверхностях, коллапс гетерогенных сеток, набухание и старение смешанных желатиновых гидрогелей.*

1. INTRODUCTION:

The possibility of a directed change in the properties of biopolymers due to external influences, such as the formation of stable association products with macromolecules and low molecular weight compounds (alcohols, carbohydrates, salts, etc.), makes them one of the most promising materials. In addition, another relevant study is of the effect of the interaction of the mixture components on the rheological and conformational properties of macromolecules due to non-covalent polymer interactions. The study of interpolymer complexes based on natural biopolymers is necessary for the further development of the technology for the production of environmentally friendly products that are promising for agriculture, medicine, food industry, cosmetology, etc. (Lanik, 1986; Tsereteli and Smirnova, 1991; Irshak and Varyukhin, 1995; Shandryuk *et al.*, 2002).

Feature of the formed biopolymer systems

is the mechanical properties of the resulting interpolymer complexes, allowing their use in technical industries. For example, mixed systems of polysaccharide-based biopolymers are used in the oil industry as reagents as drilling agents and flushing fluids. It is proved that the inclusion of small amounts of mixed systems based on gelatine and polyvinyl alcohol, sodium caseinate and polyvinyl alcohol in the feed composition increases the biological value of feed and increases the productivity of animal and poultry product, which makes it promising to apply mixed systems based on natural polymers in animal husbandry (Izmailova *et al.*, 1964; Pchelin, 1972; Vasiliev, 1981).

The mechanism and laws of formation, deformation, and destruction of dispersed structures of various types from liquid-like to high-strength materials of modern technology are the subject of the physicochemical mechanics of dispersed systems. From literature data (Gurevich, 1975; Busk, 1984; Deryagin, 1990; Katsuyoshi, 1993) it is known that an important

condition for the formation of a gel is the lack of the ability of the polymer to dissolve in a solvent unlimitedly. This condition, reduction of the interaction of the polymer and the solvent, can be achieved by changing the temperature, the nature of the solvent, the introduction of precipitators, specific additives. The fact that the loss of solubility does not lead to demixing is connected with the fact that the polymer chain molecules bind in solution during gelation into a single spatial structure that covers the entire volume.

An essential condition for gelation is the achievement of a specific, so-called critical concentration of the solution. Gelation can also occur when certain additives are introduced into polymer solutions, which lead to the formation of intermolecular or chemical bonds between different polymer macromolecules (Karásek and Meissner, 1992). The gelation process is also affected by the structure of the macromolecule itself. Gel formation also depends on the flexibility of the polymer chain and its conformation in solution (Yiebke *et al.*, 1994). Some ordering in the arrangement of polymer molecules is necessary for the emergence of secondary structures. However, with a large ordering of polymer macromolecules relative to each other, a precipitate forms. It should be noted that the gel state is the usual state of natural substances. Gels of various nature arouse the interest of researchers due to the emergence of the frame, which determines the mechanical properties of gels in the structure (Irshak and Varyukhin, 1995).

By definition of S.P. Papkov (1971), the gel phase is a matrix in which the second, low-concentrated polymer equilibrium phase is located. Such structures are characterized by the practical absence of irreversible deformation of the gel as a whole. Studying the rheological properties of jellies and their dependence on the type of stresses, temperature, duration of various influences, including the composition of the components allows to indirectly judge on the structure of gels, in particular, on their affiliation to a certain type (Valevsky, 1983; Hidekazu and Yoshihito, 1993; Kuleznev, 1993).

The purpose of this study was to present and study the issue of structuring in solutions of bio- and synthetic polymers, as with a low dry matter content such structures have many properties of a solid.

2. LITERATURE REVIEW:

In recent years, the principle of molecular recognition, which is based on steric effects,

electrostatic interaction, and hydrogen bonding, has been widely used in organic and polymer chemistry as a way to create new supramolecular materials. In the work of Shandryuk (Shandryuk *et al.*, 2002), a directed modification of systems is performed by introducing additives capable of hydrogen bonding with a polymer matrix. Rheological studies demonstrated that liquid crystal hydrogen-bonded networks are capable of large reversible deformations (up to 4-5-fold degrees of extension). Relatively many works are devoted to gelation of aqueous gelatine solutions. Most of these works are collected in Veys' monograph (Veys, 1971). It follows that the minimum concentration of gelatine gel formation is 0.5% and does not depend on pH in the region of 4-8 at temperatures below 18°C; at temperatures above 25°C, the gelation concentration increases and becomes dependent on the pH of the medium: in the region $\text{pH} = 4.6 \div 4.8$ and at a temperature of 0°C 0.6% gelatine solution does not gelate for 20 hours.

Gelatine gels were repeatedly subjected to structural and mechanical studies due to having solid-like properties. However, there are very conflicting ideas in the matter of the structure of gelatine gels. In particular, the fact that gelatine gels belong to two- or single-phase gels causes controversy and doubts among researchers. Two possible assumptions are made regarding the cause of gelation of gelatine solutions after the conformational transition of its molecules from a coil to a spiral. The first is that there is a mechanism of local crystallization of spiralized macromolecules upon the formation of gel of the first type. According to the second hypothesis, due to the transition of gelatine to a rigid conformation, the polymer loses its solubility and forms a gel. In his work, A. Veys points out the unlikelihood of the gelation mechanism based on the renaturation of the structure of tropocollagen. Research conducted by G.I. Tsereteli and O.I. Smirnova (Tsereteli and Smirnova, 1991) points at the formation of metastable collagen-like structures in the process of gelation.

In his work, V.G. Vasiliev, having studied the effect of chemical crosslinking on the elastic modulus, concluded that a substantial increase does not occur in the latter. Crosslinking fixes the glomerular conformation of macromolecules, which prevents the formation of intermolecular bonds and, consequently, structure formation (Vasiliev, 1981). A series of works is devoted to the study of the rheological properties of gelatine solutions and jellies of various concentrations in a narrow temperature range and the replacement of

water with organic solvents (Rogovina *et al.*, 1971; Grigoryeva, 1975; Lanik, 1986). The properties of gelatine-water gels were experimentally studied at various temperatures, gelatine concentrations and frequencies of mechanical stresses. The relationship between the properties of gels and the shape and structure of polymer molecules was evaluated (Ross-Murphy, 1992; Hsu and Jamilson, 1993; Golfen and Borhard, 1994; Bocchard, 1998). An increase in high elasticity is also observed due to a possible change in both the nature of the bond in the spatial network and the conformations of macromolecules and a significant dependence of the elastic modulus of gelatine on the nature of the solvent. Authors believe that the Ferry-Eldridge equation is applicable to the researched systems with sufficient accuracy.

The study of the structure and dynamics of gelatine gels during temperature jumps by method of holographic relaxation spectroscopy was considered by Chi and Wolfgang (Chi and Wolfgang, 1990). The authors determined that in the process of normal cooling at a speed of 2 K/min two types of grids are formed. One type refers to a coarse structure formed by aggregation of collagen-like triplet standard helices, and the other – to a fine structure formed by links between gelatine molecules. Some works are devoted to the study of gelatine gels near the gelation threshold and in the immediate vicinity of the sol-gel transition point (Takahiro and Masayuki, 1990).

The published data on the thermal effect of gelation of gelatine are contradictory. Thus, having examined the sol-gel similarly to glass-liquid, absence of phase transition upon gelation is indicated (Nikolaev and Neumann, 1957). In contrast, other authors demonstrated that gelation of gelatine solutions is similar to crystallization and is accompanied by a thermal effect (Bobrova *et al.*, 1970; Gordovsky, 1971).

Many works cover the research of swelling and collapse of heterogeneous networks, including gelatine gels in an aqueous medium, as one of the characteristics of the void ratio of the formed structures. Patlazhan's (2002) work displayed that when polymer networks swell with an inhomogeneous crosslinking distribution, local internal stresses arise, the magnitude of which depends on the degree of swelling, the type and structure of the network. The effective swelling coefficient of such networks is less than the corresponding average value. Its relative change, including the statistical properties of internal stresses, are determined by the structural features

of the network, expressed through the correlation function of fluctuations of macromolecular chains connecting the neighboring nodes. The dependence of light scattering on the degree of swelling is investigated. It is concluded: with increasing degree of swelling, the scattering intensity passes through a maximum.

In the work of (Xion and Zheng Pei, 1997), research on the change in the volume of gelatine gels depending on the pH and ionic strength of aqueous solutions was conducted. The dependence of the swelling gel force (the force exerting pressure on the polymer network when it is enclosed in a cell of constant volume) is noted on these factors. The influence of changes in the volume of gels and swelling forces on chemomechanical energy, including on changes in rheological properties, is pointed out. V.V. Klepko and co-workers studied the kinetics and equilibrium of swelling of gelatine gels in a good solvent (Klepko and Melnichenko, 1994). It was demonstrated that in the studied range of ripening times (1÷5) hours, sol-gel transition temperatures (4÷22)°C and gelatine concentration in the initial solution (0.02÷0.13), the equilibrium degree of swelling depends on the initial concentration gel as $F_0 - 0.4$, which corresponds to Flory's theory.

Proceeding from the consideration of swelling as a diffusion-controlled process, it was concluded that the use of first-order kinetics is valid only at the initial and middle stages of the swelling process. At the stage of extensive swelling, first-order kinetics is not applicable. The explanation for this observation was made on the basis of the assumptions that the swelling rate is directly proportional to the relative swelling in time τ and the specific area S_{int} , which limits the places of the polymer network that have not yet reacted with water in time τ , but will be hydrated (Hans, 1992). The use of a new interferometric sensor and a special interferometer allowed the authors of the work (Chi and Chui-Sing, 1994) to study the kinetics of swelling of thin gelatine films. In situ measurements were performed both at times of several minutes and several days. It is noted that the data on swelling are well-suited for the kinetics of the first-order reaction, which contradicts the previous work. The discrepancies were explained by the significant difference in the processes in the solvent and in air, including the specificity of gelatine gels. The presence of a correlation between the shear modulus, the longitudinal modulus of elasticity, and the cooperative diffusion coefficient is noted.

The subject of the study is also the nature

of the bonds responsible for gelatine gelation. Some data give preference to nonpolar groups; according to other data, hydrogen and hydrophobic bonds play an important part in gelatine gelation (Izmailova *et al.*, 1964; Pchelin, 1972). The study of the interaction between water-soluble polymers, in particular proteins and surfactants, is necessary in connection with the expanding use of such joint systems in a number of biological and industrial processes. The work (Fruhner and Kzetzschmar, 1989; Roe *et al.*, 1998) researches the interaction between gelatine and ionic surfactant-alkyl sulfates and alkyl trimethylammonium ions by the direct method of surface-active selective electrolysis with a liquid membrane of o-nitrotoluene containing a complex of cetyltrimethylammonium dodecyl. The interaction of polyphenols with gelatine amino acids was studied in (Shi *et al.*, 1994). It was demonstrated that such an interaction is largely determined by hydrophobic forces.

In connection with the widespread use of gelatine in the pharmaceutical and food industries, adsorption of this fibrillar protein on solid adsorbents is being studied. The general regularities of the kinetics of gelatine adsorption at the solid-liquid border are discussed in (Bajpai, 1994). In (Bajpai, 1995), kinematic measurements of gelatine adsorption on alumina were performed. To analyse the adsorption process and its kinetic parameters, the magnetic formalism technique was used. The effect of gelatine pH, salt concentration in solution, and anion valency on the adsorption process is noted. The study of gelatine adsorption on small particles of magnetic iron oxide is the subject of work of (Hirotooshi *et al.*, 1997). The authors investigated the adsorption of gelatine depending on various parameters (temperature, pH, salt concentration). It indicates an increase in the adsorption and molecular weight of gelatine with an increase in the initial concentration of gelatine.

Some works are devoted to the study of polymer complexes containing gelatine as one of its components. P.N. Gilsenan, R.V. Richardson investigated the gelatine-pectin cogels (Gilsenan and Richardson, 1998). The effect of the structure of gelatine micellar solutions in the isooctane-water (bis-2-ethylhexyl) sodium sulfosuccinate system on gelation was considered in (Krasovskii and Andreeva, 1996). A number of works covers aqueous systems of gelatine-sodium alginate. The authors (Mukhin and Tolstoguzov, 1980), upon studying the compatibility of proteins and polysaccharides, found that aqueous mixtures of gelatine and sodium alginate at single ionic

strength and pH above the isoelectric point of gelatine are single-phase. An assumption was put forward that the compatibility of gelatine with anionic polysaccharides is conditioned by the formation of complexes of different nature between them. A study of mixed gels of gelatine and calcium alginate (Tolstoguzov, 1980) indicates the existence of two independent spatial networks.

The authors I.V. Fedusenko, T.E. Eryshkanova, V.I. Klenin (Fedusenko *et al.*, 2001) studied the gelation process in the gelatine-sodium alginate-water system with a total polymer concentration of 3 wt. %. It was demonstrated that gelation in the system is conditioned by local crystallization of polymers. When the gelatine content in the polymer mixture is 90%, a complex is formed. Gels of this composition have a higher melting (gelation) point and a greater value of the elastic modulus. Works of I.V. Fedusenko, L.I. Khomutov deal with the rheological behaviour of gelatine + sodium alginate + water system depending on the ratio of gelatine and sodium alginate at a total polymer concentration of (2-4) %. The dependence of the tensile strength of the forming structural formations depending on the content of sodium alginate in the mixture and temperature is shown. The appearance of spatially cross-linked thermoreversible structures in such ternary polymer systems is also discussed (Fedusenko and Khomutov, 1996).

In the work of E.A. Reshetnyak (Reshetnyak *et al.*, 2005), properties of gelatine are compared to modified gelatine (degree of modification of 10, 30 and 85%). Using the method of capillary viscometry, it was demonstrated that upon hydrophobization of gelatine, its macromolecular tangles become more compact and symmetrical. At the maximum degree of hydrophobization of gelatine, its adsorption activity increases by approximately 4 times. Methods were developed for the preparation of a polymer complex of phthaloyl gelatine with proxanol and other additives (Pobedimsky *et al.*, 2000; Elin *et al.*, 2002), characterized by the presence of a significant number of functional groups, large size, and the possibility of being used as a sorbent for wastewater treatment in such industries as petrochemical, pharmaceutical, etc.

A large role is given to the introduction of small additives of gelatine in composite materials, which enables the increase in the physicomechanical properties of composites and reduces their cost. In this regard, the work (Kudaibergenova *et al.*, 2008) studied the possibility of obtaining homogeneous and

interconnected composites on the basis of gelatine and bentonite clay with insignificant amounts of clay (0.2-2%). The degree of swelling of various compositions was determined using a scanning microscope, the morphology and structure of composite gels were determined using a scanning electron microscope, and the rheological properties of the compositions were studied using a Brookfield DV-II+PRO device (USA). An assumption on the formation of complexes through hydrogen bonds is put forward. It is shown that the compositions obtained in terms of homogeneity, stability, interoperability and structure-forming properties correspond to the requirements for carriers of medicinal and biologically active compounds.

The rheological properties of gelatine-calcium and sodium alginates system are also considered in detail in the dissertation of Evtushenko (2008). The work of A.V. Varlamov, A.N. Zuev, E.E. Latinsky (Varlamov *et al.*, 2008) displays that upon hydrophobizing gelatine with succinylauril, the macromolecular tangles become more compact and symmetrical. At the maximum degree of hydrophobization of gelatine, its adsorption activity increases by approximately 4 times. Chemical modification of gelatine with lauric acid succinimide ester changes the rheological properties, greatly changes the hydrodynamic parameters of protein macromolecules.

The structure formation of solutions of various protein molecules was studied in Kazakhstan by A. Zholbolsynova (Kozlov *et al.*, 1984; Zholbolsynova *et al.*, 1992). The process of structure formation in aqueous solutions of edible gelatine in the presence of organic additives (carbohydrates) was studied, the bonds determining the strength of the structure and the conformational transformations of macromolecules were determined. The conformation of gelatine macromolecules was studied by measuring the specific optical rotation. It is shown that the addition of carbohydrates increases the strength of the resulting structure, reduces the induction period. A conformational change in macromolecules accompanies the process of gel formation. It is assumed that the stabilization of the resulting structure is conditioned by hydrophobic bonds and an increase in the number of intermolecular bonds.

Further studies examined the effect of synthetic and biopolymers on the structuring and rheological properties of gelatine (polyvinyl alcohol, polyvinylpyrrolidone, sodium humate and nitrohumate, salts of inorganic acids, carbohydrates, organic alcohols, surfactants)

(Salikova *et al.*, 1999; Salikova and Zholbolsynova, 1999; Salikova and Zholbolsynova, 2000; Salikova *et al.*, 2000; Salikova *et al.*, 2001; Zholbolsynova and Salikova, 2001; Salikova *et al.*, 2002; Bektemisova *et al.*, 2009a; Bektemisova *et al.*, 2009b; Zhakina, *et al.*, 2009; Bektemisova *et al.*, 2009c; Salikova *et al.*, 2014).

3. MATERIALS AND METHODS:

Here we consider the effect of sodium carboxymethyl cellulose on the processes of structure formation, the rheological properties of mixed gels and the adsorption processes of gelatine. To clarify the gelation mechanism, we used the kinetic approach, since this allows us to consider the processes of nucleation and formation of spatial structures.

The object of the research in this part of the study was gelatine, on the basis of which experiments were carried out to determine the structure formation in its mixed systems with sodium carboxymethyl cellulose. We used edible gelatine, purified and reduced to an isoelectric state. The moisture content of the preparation is 15%, the ash content is 12%, and the molecular weight is 70.000. Proceeding from the analysis, all previous studies, a working gelatine concentration of 5% was selected. The process of structure formation was studied through the following parameters: structuring time, melting point, ultimate shear stress at different pH.

As in most cases, the polymer-polymer-low molecular weight liquid systems are incompatible with a wide range of component composition changes, the effect of the polymer volume ratio on the structuring process was studied. It was established that mixed gelatine gels are formed at certain optimal ratios. The compatibility criterion was the shape of the viscosity – composition curves. A linear relationship was observed, which signifies the formation of a homogeneous system when the selected components are mixed up to 50% NaCMC in the mixture; when a share of NaCMC in the mixture is above 50% vol., system demixing is observed. As a result of determining the conditions for the formation of mixed solutions, the range of variation of the NaCMC fraction in the mixture of 0÷50 (vol%) was chosen. It was suggested that during the formation of crystalline nuclei, the process will occur at different rates depending on the content of NaCMC. Table 1 presents data on the effect of the NaCMC content in a mixture with gelatine on the structuring time of mixed systems as an indicator of the rate of

formation of three-dimensional structures.

The identification of the structuring time demonstrated that, appropriately, the pH of the medium affects the structure formation of mixed gels. pH = 5, as the isoelectric state of gelatine, due to the lack of resistance to structuring from the side of its charged chains, leads to the shortest time for structuring mixed gels. Because of the change in the properties of gelatine aqueous solutions due to modification of properties of the solvent by the presence of NaCMC, an increase in the content of the modifier in the mixture (up to the previously established limit of 50% vol.) leads to an acceleration of the structure formation process. Kinetic studies of the ultimate shear stress of 5% mixed gelatine gels and NaCMC allowed to identify the time interval necessary to achieve the equilibrium strength of systems equal to 4 days (Figure 1).

To identify the effect of pH of the medium, the composition of the mixture on the melting temperature, measurements were performed for 5% concentrations formed in gel in the course of four days (Table 2).

4. RESULTS AND DISCUSSION:

Since collagen folding is a cooperative phenomenon characterized by high activation energy; therefore, the melting points should be the same regardless of the gel composition. Our results show an increase in T_{mel} . With an increase in the content of NaCMC, which proves the participation of NaCMC in the stabilization of the network structure. It is necessary to note the distinct melting temperature during the measurement, which is evidence of the network structure of the gel stabilized by secondary forces.

This is because the value of T_{mel} depends on the relative number of hydrogen and hydrophobic bonds involved in each series of interacting chain segments; the melting temperature rises with an increase in the number of bonds per segment and becomes more defined. The distinctness of T_{mel} is directly related to the fact that the rupture of interacting pairs of chain segments involves the simultaneous rupture of several bonds. Adding NaCMC to the system does not change the effect of pH. Gels have the highest melting point at pH corresponding to the isoelectric state of gelatine. In parallel with measurements of the melting temperature in the same gels, their tensile strength was identified (Table 3).

It was concluded that the presence of a charge in gelatine macromolecules at a pH other

than the isoelectric state impairs the rheological properties of mixed gelatine gels and sodium carboxymethyl cellulose. Studying the adsorption of biopolymers at the liquid-solid interface is of great practical and theoretical importance. The extremely important role of proteins as biological objects is well known. Proteins provide cell adhesion and tissue formation. For the functional properties of biological systems, the surface activity of proteins is of no small importance. The part of surface phenomena in protein systems is very great in natural and technological processes.

Thus, the study of structure formation in mixed 5% gelatine and sodium carboxymethyl cellulose gels suggests that the synthetic polymer has a positive effect on gelation of gelatine. An increase in the content of NaCMC leads to an acceleration of the gelation process and the formation of stronger spatial structures.

Concentration of proteins at the phase boundaries, technological stages of emulsification and stabilization are widespread in the food, chemical, pharmaceutical industries. In animal husbandry, the use of biopolymers as fillers is known. In addition, surface phenomena involving proteins are often accompanied by structure formation or phase transitions; therefore, the study of adsorption provides additional information on the structuring of aqueous solutions. With that, the adsorption of macromolecular compounds from solutions on a solid surface is most important, since polymer adhesion, structural and mechanical properties of the high molecular weight compound boundary layers, the structure and the most important characteristics of monolayers and various composite materials are associated with it.

An analysis of the literature data shows that most of the experimental work on the study of polymer adsorption was performed using dilute polymer solutions, when intermolecular interaction in solutions can be neglected. However, even in this case it is impossible to draw unambiguous conclusions about the influence of various factors on the adsorption of polymers. Adsorption of biopolymers, in particular gelatine, is devoted to a small amount, especially for the adsorption of mixed solutions of gelatine with artificial polymers. Therefore, we studied the kinetics of adsorption of a mixture of gelatine with sodium carboxymethyl cellulose (NaCMC), the dependence of the adsorption on the amount and nature of the adsorbent, the specific surface of the adsorbent, and the concentration of the mixture. Preliminary studies of the rheological properties established the optimal ratio of gelatine and NaCMC in the

mixture – 1:1. Based on initial experiments, the optimum adsorption temperature was determined – 50°C; lowering the temperature below the optimum value leads to undesirable gelation of the system.

The study of the kinetics of adsorption is important from the standpoint of identifying factors determining the duration of the formation of adsorption layers. A study of the kinetics of gelatine adsorption with NaCMC displayed that the maximum contact time of the studied mixed aqueous solutions is 8-10 hours, the adsorption equilibrium is achieved within 2-3 hours and is practically independent of the mixture concentration; with an increase in the mixture concentration, the adsorption equilibrium is reached faster (Figure 2). The contour of the curves indicates that the experimental points fit well on the Langmuir adsorption equilibrium curve. Achieving equilibrium within a few hours, unlike pure gelatine, when equilibrium is reached faster (30 min-1 hour) (Zholbolsynova *et al.*, 1986), proves structural changes in NaCMC-modified gelatine. Gelatine macromolecules lose their flexibility, the diffusion rate to the surface of a solid decreases.

The established range of variation in the concentration of the adsorbent (0.5-3) g/dl of the solution is optimal for the adsorption of gelatine. The study of adsorption at such amounts of adsorbent (Table 4) allowed to establish the working concentration of the adsorbent equal to 0.5 g/dl of the solution. The decrease in adsorption with increasing adsorbent content can be explained by a decrease in their effective specific surface area due to adsorbent aggregation. The effect of the mixture concentration on the adsorption value of the gelatine and NaCMC mixture is provided in Table 5.

The data indicates that an increase in the concentration of the mixture leads to a larger adsorption value. An increase in the concentration of the mixture above 1% causes system structuring. At a given concentration of the mixture (1%) in the solution, the process of formation of large aggregates begins, including up to several hundred macromolecules of gelatine bonded with NaCMC. The effect of the specific surface area on the adsorption of a mixture of gelatine with NaCMC is presented in Table 6.

With an increase in the specific surface area of the adsorbent, which facilitates adsorption of large aggregates, the specific adsorption of gelatine with NaCMC increases. To identify the effect of the nature of the adsorbent on the specific

adsorption value, a mixed solution of gelatine with NaCMC was adsorbed on alumina oxide, activated carbon, ground brick, bentonite and silica gel (Table 7).

The results indicate that the adsorption of the test mixture depends on the chemical nature of the substrate, which is explained by the influence of the formed adsorption layer, which completely shields the surfaces of any solids. The results show that the best adsorption properties for mixed gelatine systems with NaCMC are hydrophilic surfaces with a well-developed macro- and microstructure of pores, such as silica gel and ground brick (significant intermolecular interactions in the adsorption layer are observed). Non-polar adsorbents with an activated surface, such as activated carbon, are also good adsorbents for gelatine aggregates with NaCMC. Adsorption on charged surfaces of alumina oxide is characterized by a significant energy barrier. The specific adsorption on such surfaces is significantly smaller.

A study of the adsorption of gelatine with NaCMC proves rheological studies, the results display structural changes in NaCMC-modified gelatine. When gelatine is mixed with NaCMC, larger structures and aggregates are formed in the solution, which lead to an increase in the time for the establishment of adsorption equilibrium, which is explained by spatial difficulties in the formation of adsorption layers. It should be noted that the specific adsorption value depends on the quantity and nature of the adsorbent. It was established that hydrophilic and activated surfaces are good adsorbents for gelatine with NaCMC.

Gels of various nature arouse the interest of researchers because of the emergence of the framework in the structure, determining their mechanical properties. Furthermore, a gelatinous state is a common state of natural polymers. The study of the formation of spatial structures of various types in disperse systems, their properties and the management of this process opens up unlimited possibilities for obtaining materials with tailor-made properties.

One of the essential conditions for gelation is to achieve a specific concentration of the solution, the so-called critical concentration, including the limited solubility of the polymer in this solvent. Reducing the interaction of the polymer and the solvent can be achieved by changing the temperature, the nature of the solvent, including the introduction of specific additives into the solvent. Thus, gelation can be enhanced when some additives (alcohols, various salts, heavy

metal oxides, etc.) are introduced into the polymer solution, which lead to the formation of intermolecular or chemical bonds between different polymer macromolecules (Asaubekov *et al.*, 1996). Indirect evidence of the hardening gel structure is its ability to swell. The stabilization of the gel structure can be analysed by its resistance to aging.

The object of research, in this case, was also gelatine, based on which experiments were carried out to determine the swelling and aging of its gels using inorganic salts, such as sodium chloride, calcium chloride, aluminum chloride. In this work, edible gelatine is used, purified, and reduced to isoelectric state. The moisture content of the preparation is 15%, the ash content is 12%, and the molecular weight is 70.000. Based on the analysis of literature data (Zholbolsynova, 1973), the working concentration of gelatine and the amount of sodium, calcium, and aluminum chloride additives were selected. In the research, selective nature of the swelling process (gelatine swells in water and in aqueous solutions and does not swell in liquid organic substances) was considered; therefore, water was used as a solvent for swelling gelatine gels.

The obtained experimental data are presented in Tables 8-10. From the provided data, it follows that the presence of additives of inorganic salts leads to an increase in the swelling of gelatine gels. Thus, the degree of swelling increased by 100% with the addition of sodium chloride, by 73% with the addition of calcium chloride, and by 60% with the addition of aluminum chloride. Investigation of the effect of the pH of the medium on the degree of gelatine swelling in the presence of inorganic salts suggests that a deviation of the pH of the medium from the isoelectric state also leads to a change in swelling degree. At pH = 9 and pH = 3, an increase in the degree of swelling is observed compared to the isoelectric point. With that, at pH = 3, the degree of swelling is somewhat greater than at pH = 9. This is due to the influence of a charge that is distributed throughout the gelatine gel chain at pH = 3 and is localized in certain regions at pH = 9. A similar dependence is observed for gelatine gels both in the presence of sodium chloride and of calcium and aluminum chlorides.

The results also display that aluminum chloride has the greatest effect on increasing the degree of swelling. It turned out that the maximum degree of gelatine gel swelling is observed with the introduction of NaCl, average – with CaCl₂, minimum – with AlCl₃. The results on the effect of the anion charge on the gelatine gel swelling

correlate with the results of studying their influence on the ultimate shear stress. With an increase in the anion charge, an increase in the ultimate shear stress (gel strength) occurs, which manifests itself, in this case, in a decrease in the gel swelling, which may be due to an increase in the osmotic pressure inside the network because of the different amounts of low molecular weight ions (Bekturov *et al.*, 1999). Measurement of the degree of swelling of 5% gelatine gel with the introduction of sodium, calcium, and aluminum chlorides allowed to graphically consider the dependence of the kinetics of its swelling on the cation charge at pH = 5 (Figure 3).

Figure 4 demonstrates a characteristic curve of gelatine gel swelling in the presence of NaCl additive depending on pH. From Figure 4 it follows that the degree of swelling depends on the pH of the medium and increases with a shift in pH to the acidic and alkaline regions. At the isoelectric point swelling is minimum.

Gels of macromolecular compounds are not subject to the process of their aging, and a change in some of their properties during prolonged standing is conditioned by the slow action of foreign substances capable of oxidation present in them. It was suggested that the presence of low molecular weight additives in the polymer hydrogel, contributing to the structure formation and hardening of gels, will destabilize the spatial system and contribute to its aging. In this case, we determined the aging of 5% gelatine gel with the introduction of 0.5M sodium, calcium, aluminum chlorides at different pH. The study of aging was carried out through studying changes in the size and mass of the initial sample (gel height, Δh, mm), changes in mass Δm, g.

Measurements were also taken over time. The results are presented in Tables 11-13. The procured data indicate that the presence of salts accelerates the aging process with a content of sodium, calcium, and aluminum chlorides at a concentration of 0.5 mol/L; the procured data correlate with the results of a study of the swelling process. The results of a study of the aging processes in gelatine in the presence of inorganic salts suggests the greater the charge of the anion the greater the presence of salts affects the strength of the gel. The aging process of gelatine hydrogels in the presence of inorganic salts depends on the pH of the medium. Stronger gels are less prone to aging.

5. CONCLUSIONS:

The positive effect of sodium

carboxymethyl cellulose on the gelation of 5% gelatine hydrogels was established. An increase in the content of NaCMC in a mixture with gelatine leads to an acceleration of the gelation process and the formation of stronger spatial structures. The participation of NaCMC in stabilizing the network structure was indirectly noted by the distinct melting temperature upon measurement.

The study of surface phenomena at the phase division boundary "mixed gelatine hydrogels – solid medium" indicated that quantitatively the adsorption of the test mixture depends on the chemical nature of the supporting medium, which is explained by the influence of the formed adsorption layer. The best adsorption properties for mixed gelatine systems with NaCMC was established – hydrophilic surfaces with well-developed macro- and microstructure of pores, such as silica gel and ground brick. Non-polar adsorbents with an activated surface, such as activated carbon, are also good adsorbents for gelatine aggregates with NaCMC. Adsorption on charged surfaces of alumina oxide is characterized by a significant energy barrier. The value of specific adsorption on such surfaces is set to be much smaller. The effect of pH on the structuring processes, the adsorption of mixed hydrogels of gelatine as a polyampholytic polymer is confirmed.

The gelation process of gelatine-based hydrogels was also studied from the standpoint of its stability over time in the presence of inorganic salts. It was established that the presence of additives of inorganic salts leads to an increase in the swelling of gelatine gels. The presence of inorganic salts also accelerates the aging process of gelatine hydrogels with a content of sodium, calcium, and aluminum chlorides at a concentration of 0.5 mol/l. The higher the charge of the salt anion, the greater the modification of gelatine hydrogels with inorganic salts affects the strength of the gel. It is established that more durable gels are less prone to aging. The process of swelling and aging of gelatine hydrogels in the presence of inorganic salts also depends on the pH of the medium.

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Table 1. The effect of the composition and pH of 5% mixed gelatine-based aqueous systems on sodium carboxymethyl cellulose on the structuring time

Content of NaCMC, %	Structuring time, min. at pH:		
	3	5	9
0	53	32	35
5	52	31	34
15	50	31	33
25	48	30	32
35	47	29	32
50	45	28	31

Table 2. Effect of the composition and pH of 5% of mixed aqueous systems based on gelatine and NaCMC on the melting temperature

Content of NaCMC, %	Melting temperature, t_{mel} , °C, at pH:		
	3	5	9
0	30.1	34.5	32.5
15	31.6	35.6	33.0
25	32.5	36.0	34.1
35	33.1	36.5	35.2
50	34.2	37.8	36.6

Table 3. Dependence of the maximum strength of 5% mixed gelatine gels and NaCMC on the composition at different pH

Content of NaCMC, %	Critical shear stress, P_m , kg/m ² , at pH:		
	3	5	9
0	150.0	250.1	250.2
5	153.3	254.3	253.6
15	158.1	260.4	258.7
25	163.8	268.6	263.8
35	169.1	276.1	270.2
50	177.2	287.2	277.5

Table 4. Dependence of the specific adsorption of a mixture of gelatine and NaCMC on the mass of silica gel, the concentration of the mixture = 1 %; $S_{sp} = 14 \text{ m}^2/\text{g}$; $t = 50^\circ\text{C}$

Adsorbent mass, m, g	0.5	1.0	1.5	2.0	2.5	3.0
Specific adsorption, G, mg/g	423	385	340	290	255	210

Table 5. Dependence of the adsorption value of a mixture of gelatine and NaCMC on silica gel on the concentration of the mixture, m (adsorbent) = 0.5 g; $S_{sp} = 14 \text{ m}^2/\text{g}$; $t = 50^\circ\text{C}$

Mixture concentration, C, %	0.2	0.3	0.5	0.7	0.9	1.0
Specific adsorption, G, mg/g	78	140	223	318	405	423

Table 6. Dependence of the specific adsorption of a mixture of gelatine and NaCMC on the specific surface of silica gel, m (adsorbent) = 0.5 g; $C_{mix} = 1\%$; $t = 50^\circ\text{C}$

Silica gel specific surface, S_{sp} , m ² /g	12	14	18	25
Specific adsorption, G, mg/g	363	423	544	755

Table 7. Dependence of the specific adsorption of a mixture of gelatine and NaCMC on the nature of the adsorbent, m (adsorbent) = 0.5 g; $S_{sp} = 14 \text{ m}^2/\text{g}$; $C_{mix} = 1 \%$; $t = 50^\circ\text{C}$

Adsorbent	Specific adsorption, G, mg/g
Alumina oxide	366
Bentonite	377
Silica gel	423
Activated carbon	646
Ground brick	753

Table 8. Kinetics of the degree of swelling of 5% gelatine gels ($\text{pH} = 5$) in the presence of salts

Concentration of salts, mol/l	Time, h	Swelling degree, α , %		
		NaCl	CaCl_2	AlCl_3
0	0	0	0	0
	1	18	18	18
	3	25.6	25.6	25.6
	5	31.2	31.2	31.2
	7	37.8	37.8	37.8
	9	38	38	38
	0	0	0	0
0.5	1	36	31.2	28.8
	3	51.2	44.4	40.9
	5	62.4	54.2	49.9
	7	75.6	65.6	60.5
	9	76	65.9	60.8

Table 9. Kinetics of the degree of swelling of 5% gelatine gels ($\text{pH}=3$) in the presence of salts

Concentration of salts, mol/l	Time, h	Swelling degree, α , %		
		NaCl	CaCl_2	AlCl_3
0	0	0	0	0
	1	22	22	22
	3	46.6	46.6	46.6
	5	58	58	58
	7	60.4	60.4	60.4
	9	60.8	60.8	60.8
	0	0	0	0
0.5	1	44	38	36.3
	3	93.2	80.9	76.8
	5	116	100.6	95.7
	7	120.8	104.8	99.6
	9	121.8	105.5	100.3

Table 10. Kinetics of the degree of swelling of 5% gelatine gels (pH=9) in the presence of salts

Concentration of salts, mol/l	Time, h	Swelling degree, α , %		
		NaCl	CaCl ₂	AlCl ₃
0	0	0	0	0
	1	20	20	20
	3	30	30	30
	5	37	37	37
	7	41.6	41.6	41.6
	9	41.8	41.8	41.8
0.5	0	0	0	0
	1	40	34.7	32
	3	60	52.1	48
	5	74	64.2	59.2
	7	83.2	72.2	66.6
	9	83.6	72.5	66.8

Table 11. The kinetics of aging of 5% gelatine gels (pH=5) in the presence of salts

Concentration of chlorides, mol/l	Time, h	NaCl		CaCl ₂		AlCl ₃	
		Δm , g	Δh , mm	Δm , g	Δh , mm	Δm , g	Δh , mm
0	0	0	0	0	0	0	0
	24	0.9	1.3	0.9	1.3	0.9	1.3
	48	1.58	2.3	1.58	2.3	1.58	2.3
	72	1.98	2.9	1.98	2.9	1.98	2.9
	96	2.23	3.2	2.23	3.2	2.23	3.2
	120	2.47	3.5	2.47	3.5	2.47	3.5
0.5	0	0	0	0	0	0	0
	24	1.86	2.6	1.56	2.3	1.08	2.03
	48	3.16	4.6	2.74	4	1.85	2.66
	72	3.96	5.8	3.43	5.03	2.3	3.3
	96	4.46	6.4	3.87	6	2.5	3.6
	120	4.94	7	4.2	6.1	2.7	4

Table 12. The kinetics of aging of 5% gelatine gels (pH=3) in the presence of salts

Concentration of chlorides, mol/l	Time, h	NaCl		CaCl ₂		AlCl ₃	
		Δm , g	Δh , mm	Δm , g	Δh , mm	Δm , g	Δh , mm
0	0	0	0	0	0	0	0
	24	1.35	1.93	1.35	1.93	1.35	1.93
	48	2.37	3.39	2.37	3.39	2.37	3.39
	72	2.97	4.25	2.97	4.25	2.97	4.25
	96	3.34	4.77	3.34	4.77	3.34	4.77
	120	3.69	5.27	3.69	5.27	3.69	5.27
0.5	0	0	0	0	0	0	0
	24	2.7	3.86	2.35	3.35	2.16	3.1
	48	4.74	6.78	4.1	5.88	3.8	5.42
	72	5.94	8.15	5.15	7.37	4.75	6.8
	96	6.68	10	5.8	8.7	5.3	8
	120	7.38	10.5	6.4	9.1	5.6	8.4

Table 13. The kinetics of aging of 5% gelatine gels (pH=9) in the presence of salts

Concentration of chlorides, mol/l	Time, h	NaCl		CaCl ₂		AlCl ₃	
		Δm , g	Δh , mm	Δm , g	Δh , mm	Δm , g	Δh , mm
0	0	0	0	0	0	0	0
	24	0.99	1.42	0.99	1.42	0.99	1.42
	48	1.74	2.49	1.74	2.49	1.74	2.49
	72	2.19	3.13	2.19	3.13	2.19	3.13
	96	2.46	3.61	2.46	3.61	2.46	3.61
	120	2.72	3.89	2.72	3.89	2.72	3.89
0.5	0	0	0	0	0	0	0
	24	1.98	2.84	1.71	2.46	1.58	2.27
	48	3.48	2.98	3.02	4.3	2.8	3.9
	72	4.38	6.26	3.8	5.4	3.5	5
	96	4.92	7.22	4.27	6.3	3.9	5.7
	120	5.4	7.5	4.5	6.6	4	6

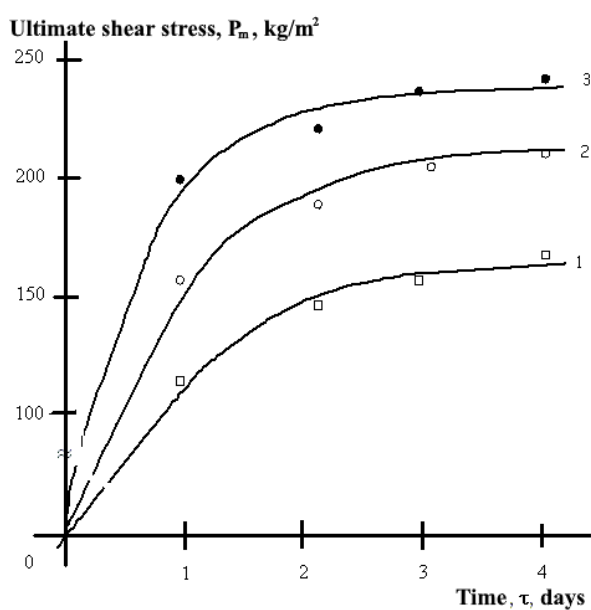


Figure 1. Kinetics of changes in the ultimate shear stress of 5% mixed systems of gelatine and NaCMC in a ratio of 85:15 of pH. Designation of curves, pH: 1– 3; 2 – 9; 3 – 5

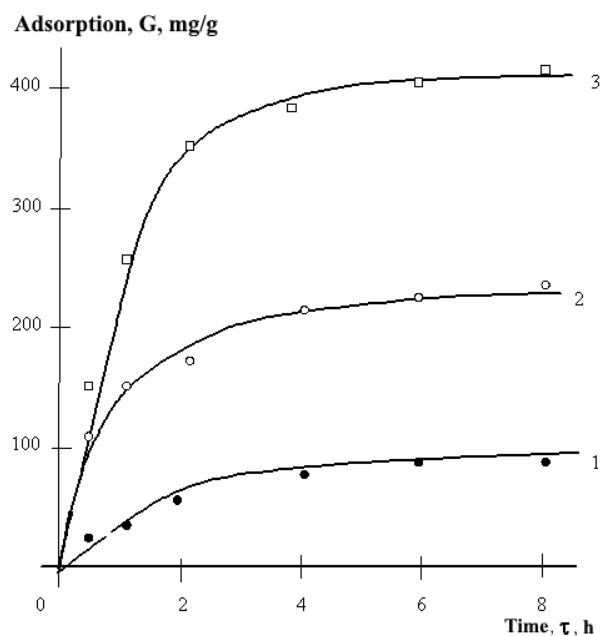


Figure 2. Kinetics of changes in the adsorption of a mixture of gelatine and NaCMC on silica gel, the adsorbent mass is 0.5 g; $S_{sp} = 14 \text{ m}^2/\text{g}$. The designation of the curves, the concentration of the mixture, %: 1 – 0.2; 2 – 0.5; 3 – 1.0

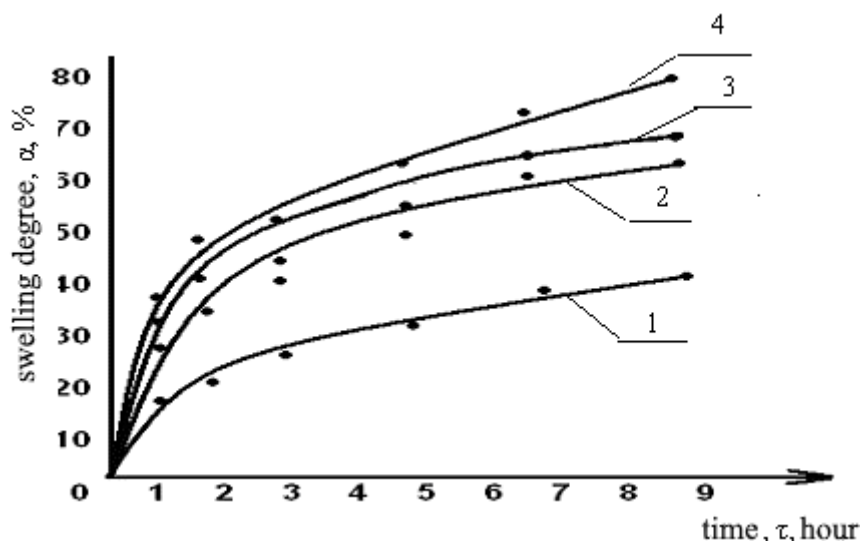


Figure 3. The dependence of the kinetics of swelling of 5% gelatine gel (pH=5) on the cation charge. Designation of curves: additive: 1 – absent; 2 – AlCl_3 ; 3 – CaCl_2 ; 4 – NaCl

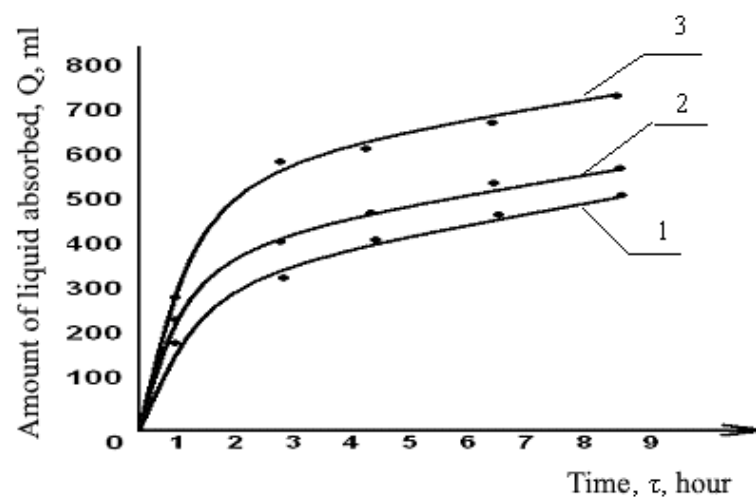


Figure 4. Swelling kinetics of 5% gelatine gel in the presence of sodium chloride depending on pH.
 Note: Curve designations, pH: 1 – 5; 2 – 9; 3 – 3