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Effect of pH and water irradiation with the electromagnetic field on the gelation of gelatin solutions

The rate of gelation was determined from the curve slope of the dependence of the gelatin solution relative viscosity on time at different pH. It was found that slow gelation of 2 % gelatin solution occurs at T = 293 K, and the degree of fluidity significantly depends on pH. The most stable jellies are formed at the isoelectric point (pH = 4.7). There are significant differences in the physical state of jellies prepared on irradiated water and control samples. Prepared on unirradiated water jellies are more mobile and retain fluidity at T = 293 K regardless of pH. There is a partial or full melting of the jellies with the increase in temperature at 297 K; however solid state remains for irradiated systems at pH = 4 and 4.7. It was shown that the viscosity of the irradiated solution and the rate of its increase are higher in comparison with the control samples at all pH values except pH = 2. The observed phenomenon can be caused by the weakening of the hydration of polymer macromolecules in the activated water, which facilitates their association and the formation of a structured system.

Keywords: gelatin, gelation rate, relative viscosity, electromagnetic field, frequency, medium acidity, irradiated water.

Introduction

Currently a large amount of experimental material has been accumulated, indicating significant changes in the physical and chemical properties of water [1–3] and aqueous solutions of polymers as a result of exposure to magnetic and electromagnetic fields. Thus, the effect of the electromagnetic field on glutamic acid solutions was studied by A. Ninno and A.K. Castellano using IR-spectroscopy [4].

It was shown that the solutions with a pH less than the isoelectric point (IEP) demonstrated a shift towards deprotonation of a carboxyl group as a result of field exposure. The deprotonation of residual amino groups was observed for solutions with pH above the IEP. The same authors studied the effect of weak electromagnetic fields on the structure of L-glutamine and L-phenylalanine in aqueous solution. It was assumed that the magnetic field changes the structure of water around hydrophobic molecules and their interaction, which allows the aggregation of amino acids molecules [5]. The influence of low-intensity extrahigh-frequency (EHF) radiation (27–120 GHz) on the processes of structuring of water and aqueous solutions of amino acids has been studied [6]. The strengthening of the structure of water and upgrading of hydrophobic interactions near the macromolecules of the polymer as a result of field exposure have been demonstrated. The effect of microwave electromagnetic radiation on the formation of supermolecular particles in aqueous solutions of non-hydrolyzed polyacrylamide has been studied in [7]. It was shown that the heating of the sample contributes to the emergence of large supermolecular particles. The possibility of pulse-discharge technologies using in the food industry with the aim to develop the hydration of biopolymers and improve the physical and chemical properties of the products has been discussed in [8].

The idea of relationship between the structure of water and the biological molecules dissolved in it is based on the methods of high frequency therapy used in the treatment of respiratory and blood circulation. Structural properties of water, solutions of electrolytes and non-electrolytes and their changes in the electromagnetic field have been analyzed in [9, 10]. The boundary of the first structural zone where the order is based on the original tetrahedral structure of water has been determined. It was shown that the appropriate concentrations are important for tissue cells, since they control the characteristic changes in the physical and chemical properties of aqueous systems and the possibility of jel-like structures formation. The effect of a magnetic field on the sol-gel transition of methylcellulose in water was studied by Wang K. et al. They found that the influence of the magnetic field reduced the sol-gel transition temperature by 3 °C, showing an effect

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similar to that of salt additives. There is a slight increase in jelly hardness [11]. The influence of a pulsed magnetic field on the rate of change in the mechanical strength of fibers was shown using examples of a number of fibrous polymeric materials (viscose, polyacrylonitrile, polyamide, cotton, cellulose wool) [12]. The effect of electromagnetic water treatment on the dyeing of woolen fabrics has been determined [13]. The action of an external magnetic field on the cis-trans isomerization of polybutadiene rubber has been demonstrated [14].

Previous studies have shown significant changes in physical and chemical properties of water, caused by irradiation of water with a low-intensity high-frequency (30–240 MHz) electromagnetic field (EMF) and subsequent shift in intermolecular interaction [15–17]. It was shown that the magnitude of the observed changes in the water surface tension, rate of evaporation and parameters of wetting depends on the EMF frequency and exposure time. It was also found that the degree of expansion of biopolymers such as gelatin, carboxymethylcellulose (CMC) and its sodium salt change in irradiated water, increasing the viscosity of these high molecular mass compound solutions [18–20]. The change in the energy of solvatation processes in activated water can be the reason of the observed phenomena as a result of the strengthening of its supramolecular organization, which, in turn, determine the viscosity of polymer solutions, the rate and degree of polymer expansion.

The aim of this work was to study the effect of pH and preliminary irradiation of water with an electromagnetic field on the rate of gelation of gelatin solutions.

Gelatin is a polydisperse mixture of polypeptides. It is formed from collagen with prolonged alkaline treatment of cartilage, tendons, bones, dermis with subsequent extraction with water at 50–100 °C [21]. Gelatin is a very promising matrix material for in vitro cell culture and tissue engineering. When forming a medical implant of soft tissue gelatin improves the mechanical properties of hydrogels. In the pharmaceutical industry gelatine is used to manufacture hard and soft capsules, in the production of artificial plasma extenders, in the production of hemostatic agents, the hemostatic dressings.

Experimental

The high frequency generator G3–19A with an output power of 1 W and a range of variation of the frequency from 30 to 200 MHz has been used as the source of the electromagnetic field. A 200 ml capacitive cell was used to expose water with EM field.

We used deionized water purified by reverse osmosis, specific conductivity of which was 1.2 μs/m. Water samples were exposed with the field frequency of 130 MHz for 3 hours. The choice of EMFs frequency was due to previously conducted experiments, in which the exposure fields of the given frequency leads to significant changes in water properties (electrical conductivity, surface tension, heat and rate of evaporation) [15–16]. Time of exposure has been also selected on the basis of previous studies. In this case, the exposure efficiency is controlled by the value of the water specific conductivity, comparing its initial and finite value. As a result of field exposure, the electrical conductivity increased by 2.7±0.2 times. To prepare gelatin solutions, we used water kept in hermetically sealed plastic vessels for a week after irradiation. During this time, no relaxation of the electrical conductivity of water to the initial value was observed.

2 % solutions of powdered food gelatin (GOST 11293–89) have been used for studies. The choice of the concentration is due to the fact that solutions of this concentration flow, gel slowly (over days) at room temperature and when the temperature drops to 15 °C gelation proceeds within 15–40 min, that allows to study the kinetics of this process. Solutions of gelatin were prepared in 2 stages. First a portion of gelatin was placed in 50.0 ml flasks. Then 20 ml of activated or non-activated water at room temperature was added and left to swell for 30–40 minutes. After that, the mixture was placed in a water bath (60–70 °C) and gelatin was dissolved with stirring until a clear solution. The resulting solutions were adjusted to the mark with water and cooled to room temperature. The required pH values were obtained by adding 0.1 M HCl or NaOH to the water used for the preparation of solutions. The acidity of the medium was controlled using the pH-meter "Anion 4100" with an accuracy of ± 0.05 pH units. Three series of parallel experiments were carried out.

Determination of kinematic viscosity was carried out using flow-through capillary viscometer VPI-2 with a capillary diameter d = 1,31 mm. The relative viscosity of solutions was calculated by the relative expiration time out of the viscosimeter of the solution of gelatin and water. The rate of gelation was determined from the slope of the curve of the dependence of the gelatin solution relative viscosity on time at different pH. The gelation time was determined from the time the solution stopped flowing out of the viscometer. The study of the kinetics of the solutions gelation process was carried out at a temperature of 288 K (15 °C). The

required temperature was maintained with a TJ-TB-01 thermostat (temperature measurement accuracy ±0.1 °C).

Results and Discussion

As a result of the studies, it was found that slowly (within a day) gelation of 2 % gelatin solution proceeds at T = 293 K, and the degree of their fluidity depends significantly on pH. Flowage or non-flowage of the obtained systems was estimated by the presence or absence of their outflow from the tube when it was turned over. The most solid gels are formed in the IEP when pH = 4.7. Noticeable differences in the physical state of the jellies have been observed in those prepared from the activated water (f = 130 MHz), and control samples (f = 0). Jellies, prepared from the non-activated water were turbid and fluent. They kept the fluidity regardless pH. The only jelle, obtained at pH = 10 was in non-flowing state. Jellies, prepared from activated water at all pH values except pH = 2 showed solid properties (Table 1) and were transparent.

Table 1

State of the gelatin jellies at T = 293 K and 1 day after preparation

pH	2.0	4.0	4.7	6.0	8.0	10
State of jelly (f = 0)	flowing	slow-flowing	slow-flowing	slow-flowing	flowing	non-flowing
State of jelly (f = 130 MHz)	flowing	non-flowing	non-flowing	non-flowing	non-flowing	non-flowing

It is known that the structure is stabilized in jellies, which is appeared in the decrease of their flowage. After 4 days the non-activated systems became less fluid, and the activated kept a solid state and did not leak out of the vessels in which they were stored (Table 2).

Table 2

State of gelatin jellies at T = 293 K and 4 day after preparation

pH	2.0	4.0	4.7	6.0	8.0	10
State of jelly (f = 0)	slow-flowing	slow-flowing	slow-flowing	slow-flowing	slow-flowing	non-flowing
State of jelly (f = 130 MHz)	flowing	non-flowing	non-flowing	non-flowing	non-flowing	non-flowing

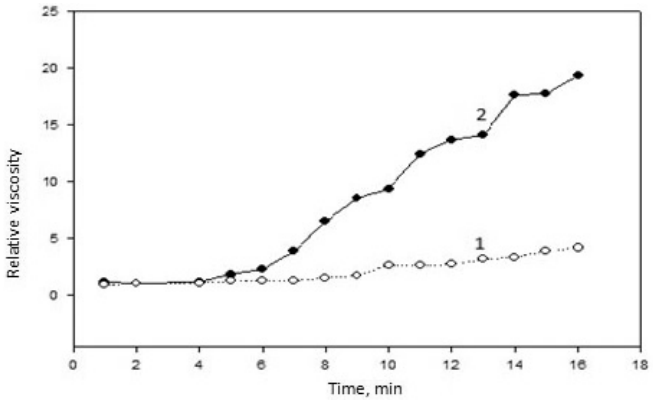
Partial or full melting of the jellies occurred with the increase in temperature to 297 K, however, solid state remained for activated systems at pH = 4 and 4.7 (Table 3).

Table 3

State of gelatin jellies at T =297 K

pH	2.0	4.0	4.7	6.0	8.0	10
State of jelly (f = 0)	flowing	flowing	slow-flowing	flowing	flowing	flowing
State of jelly (f = 130 MHz)	flowing	non-flowing	flowing	slow-flowing	slow-flowing	slow-flowing

The kinetics of a 2 % solution of gelatin gelation at T = 288 K has been studied. The obtained jellies were melted in water bath when heated and then quickly cooled in a thermostat to specified temperature. Cooling of the gelatin solutions was carried out in the viscometer. The viscosity of solutions was measured with an interval of 1 min when reaching T = 288 K. It was shown that the viscosity of the activated solutions and their rate of increase is much higher compared with control samples at all pH values. Only at pH = 2 the viscosity of the studied and control samples increased with the same rate, and along with this no gelation was observed within 1 hour. Figure 1 shows the kinetic curves of increase in the relative viscosity of the activated and non-activated gelatin solutions at pH = 10.



1 — non-activated water; *2* — activated water (130 MHz)

Figure 1. Dependence of relative viscosity of 2 % solutions of gelatin on the time at T = 288 K and pH = 10

It is known that gelation process runs faster in solutions of proteins of the same concentration, provided that the protein molecules have no electrical charge and they are less hydrated, i.e. they are in isoelectric state. Therefore, the rate of gelation depends on the acidity of the medium and best of all occurs at a pH corresponding to the isoelectric point (IEP) of the protein [21], which was confirmed by our experiments. Rates and time of gelation of polymer solutions at different pH values are presented in Table 4.

Table 4

Rate (- d/ηdt) and time (t) of gelation of 2 % gelatin solutions at T =288 K

pH	2.0	4.0	4.7	6.0	8.0	10
$(d\eta/dt)\cdot 10$, min^{-1} (f = 0)	1.1±0.2	1.3±0.3	1.6±0.1	1.4±0.2	1.3±0.3	1.2±0.2
$(d\eta/dt)\cdot 10$, min^{-1} (f = 130 MHz)	1.2±0.1	1.8±0.4	3.9±0.3	2.5±0.4	2.2±0.2	2.2±0.2
t, min (f=0)	-	42±4	19±2	24±3	36±2	48±5
t, min (f = 130 MHz)	58±3	18±1	11±1	13±3	18±2	16±2

The fluidity of the jelly is determined by the strength of the bond that occurs between the polymer macromolecules during the gelation process. The grid nodes can be caused by the hydrogen bonds, the interaction of electric charges or dipoles and chemical bonds. The strength of the bonds in the jell is small in the case if this bond is hydrogen or electrostatic (dipole), and its ability to melting and decomposition increases. Gelatin jellies are good examples of such systems [21]. The macromolecules of proteins comprising gelatin are positively charged in the acidic medium due to the suppression of dissociation of carboxylic groups and protonation of amino groups; the force of electrostatic repulsion is high, which prevents the association of molecules and the formation of a polymer three-dimensional grid in an aqueous medium. Gelling does not occur in such case. The charge of macromolecules is small (or zero in the IEP at pH = 4.7) in weak acid and weak-alkaline medium, which leads to weakening of the electrostatic repulsive forces. As a result, the process of formation of intermolecular bonds (dipole-dipole and hydrogen) is facilitated and the gelling of gelatin solutions occurs. The intensification of the gelling processes in water exposed to EMF may be due to the weakening of the hydration of ionogenic groups of the polymer due to increase of dipole-dipole interaction between water molecules. Strengthening the cohesive interaction in the aquatic medium was confirmed in [16]. The result is a reduction in the degree of dissociation of carboxylic groups and decrease in the total charge of the macromolecules, which contributes to their association and the formation of a structured system. The well-known fact that the addition of salts containing well-hydrated ions (sulfates, citrates) to gelatin solutions accelerates this process, indicates the influence of the hydration degree of the polymers polar groups during gelling. The greater the ability of the ion to hydrate, the more active is the dehydration of the polymer macromolecules in its presence, what facilitates their connection to each other and the formation of a structured system. Thus, it can be assumed that the effect of EMF on water is similar to the effect of elec-

trolyte additives. An increase in the viscosity of electromagnetic field solutions in activated water can also be a consequence of the enhancement of the macromolecules association due to the adhesion of their dehydrated sections.

Conclusions

The dependence of gelation of 2 % gelatin solution on pH at 293 and 297 K has been determined. It has been shown that solid systems are formed at pH = 4–6 and 10. Gelling does not occur at pH=2.

Strengthening of the gelatine structuring in water, exposed to the electromagnetic field of 130 MHz frequency was shown, that appears in the formation of solid systems at all pH values except pH=2.

The kinetics of gelation of gelatin solutions according to the changes in relative viscosity during the time has been studied. The processes acceleration in activated water has been demonstrated.

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Желатин ерітінділерінің қатаюына pH пен суды электромагниттік өріспен өңдеудің әсері

Гельдің түзілу жылдамдығы ортаның pH-ның әр түрлі мәндерінде желатин ерітіндісінің салыстырмалы тұтқырлығының уақытқа тәуелділік қисығы көлбеуімен анықталды. T = 293 K кезінде 2 % желатин ерітінділерінің қатуы баяулайды, ал олардың аққыштық дәрежесі pH-қа байланысты. Ең берік желе pH = 4,7 изоэлектрлік нүктеде түзіледі. Сәулелендірілген суда дайындалған желе мен бақылау үлгілерінің физикалық жағдайында айтарлықтай айырмашылықтар байқалады. Сәулеленбеген суда дайындалған желе неғұрлым жылжымалы және pH-қа қарамастан T = 293 K кезінде аққыштықты сақтайды. Температура 297 K дейін көтерілгенде желе ішінара немесе толық балқиды, бірақ сәулелендірілген жүйелер үшін pH = 4 және 4,7 кезінде қатты күй сақталады. Бақылау үлгілерімен салыстырғанда сәулеленген ерітінділердің тұтқырлығы және оның өсу жылдамдығы pH = 2-ден басқа pH барлық мәндерінде жоғары екендігі көрсетілген. Байқалған құбылыстар сәулелендірілген судағы полимерлі макромолекулалардың гидратациясының әлсіреуіне байланысты болуы мүмкін, бұл олардың ассоциациясын және құрылымдық жүйенің қалыптасуын жеңілдетеді.

Кілт сөздер: гель, желатин, гельдің түзілу жылдамдығы, салыстырмалы тұтқырлық, электромагниттік өріс, жиілік, ортаның қышқылдығы, сәулеленген су.

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Влияние pH и обработки воды электромагнитным полем на застудневание растворов желатина

Скорость гелеобразования определена по наклону кривой зависимости относительной вязкости раствора желатина от времени при различных значениях pH среды. Установлено, что при T = 293 K происходит медленное застудневание 2 % растворов желатина, а степень их текучести существенно зависит от pH. Наиболее прочные студни образуются в изоэлектрической точке при pH = 4,7. Наблюдаются заметные различия в физическом состоянии студней, приготовленных на облученной воде, и контрольных образцов. Студни, приготовленные на необлученной воде, более подвижны и сохраняют текучесть при T = 293 K независимо от pH. При повышении температуры до 297 K происходит частичное или полное плавление студней, однако для облученных систем при pH = 4 и 4,7 твердообразное состояние сохраняется. Показано, что вязкость облученных растворов и скорость ее нарастания выше по сравнению с контрольными образцами при всех значениях pH, кроме pH = 2. Наблюдаемые явления могут быть обусловлены ослаблением гидратации макромолекул полимера в облученной воде, что облегчает их ассоциацию и образование структурированной системы.

Ключевые слова: гель, желатин, скорость гелеобразования, относительная вязкость, электромагнитное поле, частота, кислотность среды, облученная вода.

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