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Short Communication

BIOPHARMACEUTICAL STUDY OF BINARY POLOXAMER SYSTEMS AS *IN SITU* DRUG DELIVERY SYSTEMS POLOXAMER POLYCOMPLEXES: THE STUDY

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ABSTRACT

Objective: The article presents the results of studying the parameters of *in situ* systems based on poloxamers.

Methods: Natural, synthetic and semi-synthetic polymers were considered as additional gel-forming agents. The above-mentioned agents were cellulose derivatives, as well as rare-cross-linked acrylic polymers, alginic acid salts, and xanthan gum. The gelation temperature was visually recorded during the gradual heating. Mucoadhesion was determined by measuring the separation force of the sample from the mucosal model using pig stomach mucin. The concentration of gel formation was determined using an Ostwald capillary viscometer.

Results: During the experiment, 20 samples based on poloxamer 407 18% were examined with the addition of excipients in concentrations of 0.5-2.0%. It was found that the introduction of additional gel-forming agents has a positive effect on the mucoadhesive properties of poloxamer 407. To increase the temperature of gelation of the binary mixture, it is necessary to take into account the own concentration of gelation of the introduced additional polymer, which was confirmed by capillary viscometry. Also, additional viscometric studies revealed the reasons for the absence of a thermoreversible effect in the composition of a poloxamer with xanthan gum and confirmed its use as an excipient in the thermoreversible matrix of poloxamer 407 is impractical. Composition with the best-investigated biopharmaceutical properties-the composition with HEC in a concentration of 0.5%

Conclusion: The obtained results can be explained by the fact that when the concentration of additional polymer was exceeded, binary mixtures showed a decrease in the gel formation temperature up to a complete loss of the ability to temperature-dependent sol-gel transition. The choice of an additional gel-forming agent to improve the properties of the system should be based on some specific properties that affect the thermoreversible properties of the system.

Keywords: *In situ* system, Polymer, Poloxamer, Mucoadhesion, Gelation temperature

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To date, poloxamers are the most promising polymers used to create reversible thermosensitive bases used as *in situ* delivery systems. Unlike other excipients that can change the viscosity of their solutions depending on temperature, poloxamers do not change their rheological and technological characteristics, passing from the sol state to the gel and back [1].

Even though the technological properties of pharmaceutical compositions based on poloxamer 407 are already well studied, it is of interest to develop and study the characteristics of compositions with other gel-forming agents to create thermoreversible systems with improved biopharmaceutical properties [1-5].

Monocomponent gels based on poloxamers have several disadvantages: high occlusion, relatively low gelation temperature and yield strength, medium osmotic activity, low mucoadhesive ability, and very low adsorption activity [1-3]. In dry form, the excipients do not have a pronounced taste and smell, but gel compositions with a high polymer content often require correction of organoleptic parameters since they are applied topically in the oral cavity, intranasally, or orally. Due to the narrow range of concentrations used to obtain thermoreversible systems, it is proposed to vary the technological characteristics of the dosage form by introducing an additional gel-forming agent into the composition. The resulting systems based on poloxamers can have thermoreversible properties or, unfortunately, lose them [4].

According to the analysis of scientific publications, carbomers, other poloxamers that do not have thermoreversible properties (poloxamer 188, poloxamer 338, etc.), cellulose esters, chitosan, alginate salts, gums, hyaluronic acid, polyethylene glycols are most often used as additional components for creating improved thermoreversible *in situ* systems [6-15]. All these excipients having different technological (osmotic activity, mucoadhesion, viscosity) and their own pharmacological properties (chitosan, hyaluronic acid) make a significant contribution to the properties of the finished *in situ* system. Thus, the biopharmaceutical and technological properties of a thermoreversible system consisting of two or more polymers directly depend on the type and concentration of the additional excipient being injected. To optimize the process of pharmaceutical development of *in situ* systems, it is important to create ready-made combinations of polymers with certain design and properties (gelation temperature, mucoadhesion value, gel viscosity, gelation time, etc.). To solve this problem, it is necessary to determine the influence of specific polymers-components of systems on the properties of the thermoreversible complex and to justify the range of possible concentrations of their introduction, which does not allow the loss of a thermosensitive phase transition.

This work aims to provide the scientific base for the choice of additional gel-forming agents and their concentrations for creating binary thermoreversible systems based on poloxamer 407.

The poloxamer Kolliphor® P 407 (BASF) was used as a thermoreversible component. Additional gel-forming agents for creating combined systems were: hydroxyethylcellulose (HEC) Natrosol® 250 HHX (Ashland), hydroxypropylmethylcellulose (HPMC) Benecel® K100M PHARM (Ashland), carbomer Carbopol® 981 NF Polymer (Lubrizol), sodium alginate Protanal® CR 8133 (FMC), xanthan gum Grinsted® Xantan 80 (Dupont Nutrition and Health). All the described components met the requirements of the United States Pharmacopoeia and Pharmacopoeia Europe. The gelling agents were chosen in such a way as to differ in nature, molecular weight, molecular structure, as well as biopharmaceutical properties (viscosity, mucoadhesion, etc.).

The concentration of the thermoreversible component of the systems, according to the recommendations of the manufacturer,

was 18.00%. Additional gel-forming agents were selected in such a way as to represent polymers of different chemical structures, with different densities, compositions, and configurations of gel structures at the molecular level. The concentration range of the mentioned agents was from 0.5 to 2.0%. Thus, it was supposed to study the effect of a group of polymers on a thermoreversible matrix rather than a specific additional substance.

The poloxamer gel was obtained by the cold method [16]. The suspension of poloxamer 407 was dispersed in purified water on a magnetic stirrer IKA Topolino Mobil (Germany). The experimental sample was left for structure at a temperature of 5-8 °C for 24 h.

Solutions of gel-forming agents were prepared by the recommendations of the manufacturer.

Systems based on poloxamer were obtained by introducing an additional gel-forming agent into the poloxamer dispersion with constant stirring. The experimental samples were left for structure at a temperature of 5-8 °C for 24 h.

The obtained systems were stored at a temperature of 5-8 °C. The gelation temperature, as well as the concentration of gel formation and the characteristics of mucoadhesion, were studied.

The gelation temperature was measured during the gradual heating of the sample from a temperature of 20 °C to 50 °C in a water bath IKA® HBR4 digital (Germany) in a glass beaker using a thermal sensor. The temperature of the sample at which the sol-gel transition was visually recorded was taken as the gel formation temperature [2, 3]. The average value of the gelation temperature was determined based on five measurements.

The separation force of the sample from the mucosal model using pig stomach mucin-type II (Sigma Aldrich) determined the mucoadhesion of the experimental samples. The determination of mucoadhesion was carried out at a temperature of 20±0.5 °C using a lever mechanism. The mucoadhesion value was calculated as the product of the mass of the load and the acceleration of gravity (g = 9.81 m/s^2) and expressed in Newtons. Based on the average values for five dimensions, the separation force was calculated [17].

The concentration of gel formation was determined using an Ostwald capillary viscometer. The measurements were carried out at a temperature of 20±1 °C, the flow time was determined as the average after five tests.

The dynamic viscosity of the samples was calculated by the formula:

$$\eta=\rho Kt$$

Where η is the dynamic viscosity (mPa·s)

ρ is the density (g/ml)

K is the constant of the viscometer (K=3.203 mm^2·s^{-2})

t is the average time for which the sample passes the distance from the start mark to the finish mark (s).

The concentration value preceding a sharp (more than twice) increase in the viscosity was taken as the concentration of gel formation.

During the experiment, 20 models of thermoreversible multicomponent placebo samples were obtained. The results of determining the gelation temperature are shown in table 1.

In parallel, a control experiment was conducted with a reference composition containing 18.0% poloxamer 407 without the introduction of additional gel-forming agents. The average value of the gelation temperature was 26±0.5 °C.

Table 1: The gelation temperature of the systems, measured on the seventh day after manufacture

Gel-forming agent	Concentration of gel-forming agent, %			
	0.50	1.0	1.5	2.0
	Gelation temperature, °C			
HEC	27.0±0.5	26.0±0.5	<22.0	<22.0
HPMC	28.0±0.5	24.0±0.5	<22.0	<22.0
Carbomer	23.5±0.5	>50.0	<22.0	<22.0
Sodium alginate	28.0±0.5	24.5±0.5	24.0±0.5	<22.0
Xanthan gum	*	*	*	*

*No thermal reversal effect; the data are given as mean±SD (n=5)

For samples containing poloxamer 407 and xanthan gum in a concentration of 0.5 to 2.0%, the absence of a thermoreversible effect was shown–they were gels or dispersions of different viscosities both at the storage temperature (5-8 °C) and during the experiment (23-25 °C).

The systems containing HEC, HPMC, and carbomer as additional gel-forming agents in a concentration exceeding 1.0% were sols at a storage temperature (5-8 °C). However, they demonstrated a sol-gel transition when the temperature was increased to room temperature (<22.0 °C). Thus, these systems did not meet the purpose of the experiment, suggesting that the sol-gel transition should be carried out at the injection site at a temperature of about 30 °C.

The sample containing the carbomer in the concentration of 1.0% did not demonstrate a reliably determined sol-gel transition within the conditions of the experiment. It was a sol, both at storage and room temperature.

According to the data, the formulations containing a minimum amount of additional gelation agent (0.5%) showed the best thermoreversible properties: the gelation temperature was increased by 1.0-2.0 °C compared to the reference sample.

The mucoadhesive properties were determined for these experimental formulations to reveal the complex effect of the excipient on the biopharmaceutical properties. The results of the experiment are shown in fig. 1.

In parallel with the tests of experimental samples, a control experiment was conducted with a reference sample containing poloxamer 407 at a concentration of 18.0%. The average value of mucoadhesion was 17.73 N (fig. 1)

It was shown that the introduction of additional excipients has a positive effect on mucoadhesion, while the HEC has the greatest effect on the mucoadhesive properties (an increase of 31.13 N).

It was shown that the addition of excipients to thermoreversible formulations based on poloxamer 407 is advisable to increase the main biopharmaceutical characteristics of *in situ* delivery systems: gelation temperature and mucoadhesion. However, the introduction of the polymers at a concentration above 0.5% reduced the gelation temperature and, ultimately, led to the loss of thermoreversible properties.

This effect could be caused by the gel-forming agent, which formed a three-dimensional structure and so prevented the thermoreversible effect of the poloxamer 407. A similar phenomenon is described by many authors, including Sanjeevani S. Deshkar *et al.* [11]. A decrease in the gelation temperature (up to the loss of thermoreversible properties) is often associated with the high hydrophilicity of additional gelating agents included in the complex. In turn, an increase in the hydrophilicity of the complex as a whole leads to rapid gelation at lower temperatures [7-12].

To confirm this, solutions of the polymers were obtained and the range of gelation concentrations was determined using an Ostwald capillary viscometer (table 2).

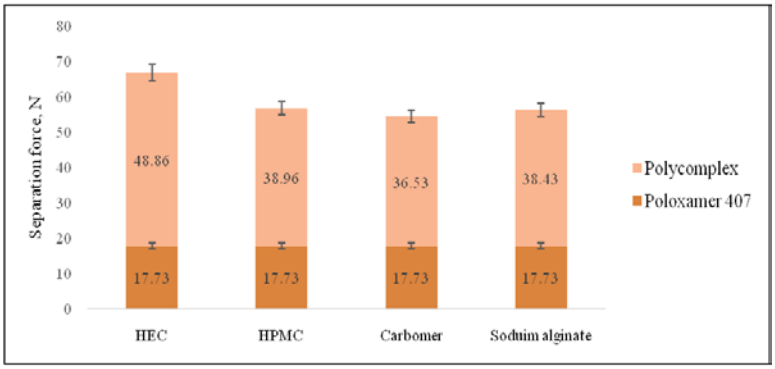

Fig. 1: The dependence of the values of mucoadhesion on the composition of the systems based on poloxamer 407; the data are given as mean±SD (n=5)

Table 2: Viscosity of polymer solutions used as additional gel-forming agents

	Concentration of polymer, %			
	0.25	**0.50**	**1.0**	**1.5**
	Viscosity, Pa·s			
HEC	33.210±2.490	36.310±2.723	2043.120±20.430	8523.490±42.617
HPMC	89.760±4.488	120.200±6.010	1176.180±11.761	1978.610±19.786
Carbomer	0.0120±0.001	0.089±0.006	0.367±0.027	0.470±0.035
Sodium alginate	11.530±0.864	13.760±1.032	38.990±2.924	46.510±3.488
Xanthan gum	8.130±0.609	30.215±2.266	-*	-*

*It cannot be measured on a capillary viscometer due to the high viscosity of the solution; the data are given as mean±SD (n=5)

According to the data, the critical gelation concentration of HEC, HPMC, carbomer, and sodium alginate lies in the range from 0.5 to 1.0 %. Thus, the optimal concentration of the additional polymer (0.5%), determined in the studies of the gelation temperature and mucoadhesion, is below the critical gelation concentration. Making a significant contribution to improving the biopharmaceutical properties of binary poloxamer systems, the additional gel-forming agents do not violate the molecular structure of the gel and do not interfere with the thermoreversible effect.

It should also be noted that the experimental data obtained are consistent with the results of other studies. Thus, in the work of Sanjeevani S. Deshkar *et al.* [11], the composition with an HEC of 0.4% is also recognized as the most successful of all the studied formulations with various gel-forming agents as a vaginal *in situ* gel with fluconazole. Besides, additional rheology studies allowed to explain the absence of a thermoreversible effect in the compositions containing the xanthan gum in a concentration of more than 0.5%. The change in viscosity by more than four times was demonstrated by a solution of xanthan gum in a concentration of 0.5% compared to 0.25%. Thus, the three-dimensional structure formed by xanthan gum, even when the polymer is introduced at a low concentration, prevents the movement of poloxamer molecules and prevents the occurrence of the thermoreversible effect [6].

According to the experiment, the polymers selected for the creation of binary poloxamer systems belong to different groups and have a different structure of intermolecular bonds in the process of gelation. However, according to previous studies [7], each of the introduced gel-forming agents has a high degree of mucoadhesion in a one-component solution or dispersion. During the experiment, it was shown that despite the good mucoadhesive properties of each polymer separately, the degree of increased mucoadhesion could vary in the composition of the systems with poloxamer 407. The most successful was the introduction of the HEC in a concentration of 0.5%-the resulting system showed an increase in the gelation temperature compared to the control and a significant increase in mucoadhesion, which is an important quality indicator for *in situ* delivery systems.

It is known that the mechanism of interaction between mucose and adhesive polymers is based on bonding, mainly due to the emerging hydrogen and Van der Waals bonds [18]. The mucoadhesive properties of polymers are influenced by such characteristics as molecular weight, the flexibility of polymer chains, the nature of the three-dimensional structure, and polymer concentration. In preliminary studies of the mucoadhesive properties of individual commercially available, the average (n=5) values of mucoadhesion determined in the separation force experiment conducted under conditions similar to this study were determined polymers [17, 18]. For 2.0% (by weight) in polymer solutions, the separation forces from the mucin layer were: HEC-24.59 N, HPMC-21.84 N, carbomer-23.38 N, sodium alginate-20.43 N. It should be noted that the studied polymers have similar values of mucoadhesion. However, for a polymer of the Natrosol ® 250 HHX brand in a thermo-reversible complex with poloxamer 407, the value of mucoadhesion for separation is 1.2-1.3 times higher than other binary complexes studied. To find out the cause of this phenomenon and to study the contribution of the properties of individual polymers to the amount of mucoadhesion of the complex, additional research is necessary.

The creation of thermoreversible matrices based on poloxamers is an urgent problem in the technology of *in situ* drug delivery systems. The choice of an additional gel-forming agent to improve the properties of the matrix should be based on some specific properties that affect the thermoreversible properties of the composition. One of these characteristics is the critical gelation concentration and the mucoadhesive properties in polymer solutions at concentrations preceding the gel-forming process.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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