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Optimisation of the composition of safe dental gel with IL-1β antagonist for the treatment of inflammatory periodontal diseases

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Aim. The study aims to develop the composition of the oromucosal gel with the IL-1β interleukin antagonist for complex treatment of inflammatory periodontal diseases.

Materials and methods. Experimental studies were conducted based on the Department of Medicines Technology of Zaporizhzhia State Medical and Pharmaceutical University and the Training Medical and Laboratory Center of Zaporizhzhia State Medical and Pharmaceutical University of the Ministry of Health of Ukraine.

As an active component, in the recipe of oromucosal gel, used antagonist of the interleukin IL-1B. Excipients: D-panthenol (the plasticizer), carboxymethylcellulose sodium salt (the viscosity modifier and mucoadhesive component), Tween-80 (the enhancer absorption), benzalkonium chloride (the preservative), sodium hydrophosphate + citric acid (the phosphate buffer solution), purified water. The experiments used existing and auxiliary ingredients of pharmaceutical purity, which were obtained from Sinbias LLC, Istok-Plus LLC, LLC "Mobile Medical". For the design of the experiment, the methodology of the response surface (Box-Behnken Design) is used: Na CMC, Tween-80, D-panthenol; and four answers: pH, viscosity test, system type, mucoadhesive properties.

Research methods: rheological research (viscosity test, amplitude test, frequency test, mucoadhesive test, thixotropy test) performed in oscillation mode on the modular compact Rheometer Anton Paar MCR 302 (CP50-1 SN71317), which, compared to cylindrical devices, requires a much smaller amount of gel sample and allows the planned tests in the oscillation mode, the temperature in the experiments was provided with a built-in thermostat (Peltier Temperature Control, C-PTD 200).

Pharmaco-toxicological studies were conducted on 46 white outbred rats of both sexes, weighing 160–180 grams, which were received from Vivarium by the Institute of Pharmacology and Toxicology. The studies were performed on enough animals, all manipulations were carried out by the provisions on the use of animals in biomedical experiments. The results of the study were calculated using the standard statistical package of the Statistica for Windows 13 (StatSoft Inc., No. JPZ804I382130ARCN10-J), as well as SPSS 16.0, Microsoft Office Excel 2003.

Results. According to the design of the experiment, it is established that the components of the system do not change the value of pH in experimental oromucosal gels (6.5500 ± 0.0334). The Anova for Quadratic Model statistical analysis data certify the significant impact of Na CMC, and Tween-80 factors on the viscous characteristics of oromucosal gels (F-value > p-value). Comparison of mucoadhesive characteristics of the studied samples of oromucosal gels was made using dynamic mechanical analysis and the results of statistical analysis of ANOVA for Quadratic Model highlight the significant influence of Na CMC, D-panthenol, Tween-80. Optimized oromucosal gel test data is obtained that its structure is restored after the applied effort, namely the restoration of the structure after 10 seconds occurs by 69.5 %, after 30 s by 76.1 %, after 180 s by 85.4 %, after 180 s which allows to predict the stability of the dosage form, both after manufacture and after use.

Conclusions. The composition of the oromucosal gel of anti-inflammatory action with the IL-1β interleukin antagonist for the complex treatment of inflammatory diseases of periodontal is developed. The optimized composition of the oromucosal gel obtained has satisfactory performance of kinetic stability and thixotropic properties. The developed gel for dentistry meets all the requirements for harmlessness and safety for dosage forms of this group toxicity, lack of local irritant and allergic action.

Ключові слова:
IL-1β, оромукозний гель, фармацевтична розробка, фармакологічні дослідження, гостра токсичність.

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Розроблення складу безпечного оромукозного гелю з антагоністом IL-1β для лікування запальних захворювань пародонта

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Мета роботи – розроблення складу оромукозного гелю з антагоністом інтерлейкіну IL-1β для комплексного лікування запальних захворювань пародонта.

Матеріали і методи. Експериментальні дослідження здійснили на базі кафедри технології ліків Запорізького державного медико-фармацевтичного університету та Навчально-наукового медико-лабораторного центру Запорізького державного медико-фармацевтичного університету МОЗ України.

Як компонент з активною дією в рецептурі оромукозного гелю використали антагоніст інтерлейкіну IL-1b. Допоміжні речовини: D-пантенол (пластифікатор), карбоксиметилцелюлози натрієва сіль (модифікатор в'язкості, мукоадгезивний компонент), твін-80 (енхасер адсорбції), бензалконію хлорид (консервант), динатрію гідрофосфат + кислота лимонна (фосфатний буферний розчин), вода очищена. В експериментах використані активні й допоміжні інгредієнти фармацевтичної чистоти, які отримано від ТОВ НВФ «СІНБІАС», ТОВ «Исток-Плюс», ТОВ «МОБІЛЬ МЕДІКАЛ». Для дизайну експерименту використано методологію поверхні відповіді (Box-Behnken Design) з трьома рівнями факторів (Na CMC, Tween-80, D-panthenol) та чотирма відповідями (pH, тест в'язкості (Viscosity), тип системи (Type system), мукоадгезивні властивості (Mucoadhesive)).

Методи дослідження: реологічні дослідження (тест в'язкості досліджених гелів, амплітудний, частотний, мукоадгезивний тести, тест тиксотропії) виконано в осциляційному режимі на модульному компактному реометрі Anton Paar MCR 302 (CP50-1 SN71317), що порівняно з циліндричними пристроями потребує значно меншої кількості зразка гелю, а також дає змогу в осциляційному режимі проводити заплановані тести. Температуру в дослідах забезпечували вбудованим термостатом (Peltier temperature control for concentric cylinder systems, C-PTD 200). Фармако-токсикологічні дослідження здійснили на 46 білих безпородних щурах обох статей із масою тіла 160–180 г, що одержані з віварію Інституту фармакології та токсикології. Дослідження виконали на достатній кількості тварин, всі маніпуляції здійснили відповідно до Положення про використання тварин у біомедичних дослідах. Результати дослідження опрацювали, використавши стандартний статистичний пакет ліцензійної програми Statistica for Windows 13 (StatSoft Inc., № JPZ804I382130ARCN10-J), а також SPSS 16.0, Microsoft Office Excel 2003.

Результати. Відповідно до дизайну експерименту, встановлено, що компоненти системи не змінюють значення pH дослідних оромукозних гелів (6,5500 ± 0,0334). Результати статистичного аналізу (ANOVA for Quadratic model) показали достовірний вплив факторів Na CMC, Tween-80 на в'язкісні характеристики виготовлених зразків оромукозних гелів (F-value > p-value). Мукоадгезивні характеристики досліджених зразків оромукозних гелів порівняли, виконавши динамічний механічний аналіз. У результаті статистичного аналізу з використанням ANOVA for Quadratic model встановили вірогідний вплив факторів Na CMC, D-panthenol, Tween-80 на мукоадгезивні характеристики (F-value > p-value). Дані тесту тиксотропії оптимізованого оромукозного гелю свідчать, що його структура відновлюється після прикладеного зусилля. Так, відновлення структури через 10 секунд відбувається на 69,5 %, через 30 с – на 76,1 %, через 180 с – на 85,4 %. Це дає підстави прогнозувати стабільність лікарської форми після виготовлення та застосування.

Висновки. Розробили склад оромукозного гелю протизапальної дії з антагоністом інтерлейкіну IL-1β для профілактики та комплексного лікування запальних захворювань пародонта. Цей склад оромукозного гелю забезпечує задовільні показники кінетичної стійкості та тиксотропні властивості. Розроблений оромукозний гель відповідає всім вимогам щодо нешкідливості та безпечності, що ставлять до лікарських форм цієї групи (мають низьку токсичність, не чинять місцевоподразнювальної та алергізуючої дії).

Inflammatory periodontal diseases exhibit extensive distribution, progressive nature, and chronic course of the ailment, thereby acquiring a prominent position in the spectrum of oral cavity diseases. Moreover, chronic inflammatory processes, diminished resistance to microorganisms, and premature tooth loss can lead to an uptick in atypical periodontitis among younger individuals. WHO experts have noted that periodontal disease affects approximately 80 % of school-aged children across various nations, with prevalence reaching 100 % among adults [1,2].

The causes of such pathological processes are exogenous and endogenous factors: accumulation of tartar due to irregular brushing of teeth or metabolic disorders; bacterial infections that lead to inflammation of the gums or deeper tissues, which leads to serious forms of periodontitis, periodontal disease, gingivitis; genetic predisposition to the occurrence of diseases of the oral cavity – susceptibility to bacterial infections, increased sensitivity of the gums; the presence of bad habits, such as tobacco smoking, which leads to poor blood circulation to the gums or a violation of the diet, which contributes to the deterioration of the supply of nutrients and vitamins; hormonal state of the body – hormonal changes such as pregnancy, menstruation, menopause, and others can also contribute to inflammation; the presence of chronic diseases of the body – diabetes, immunodeficiency states, chronic inflammatory processes also affect the occurrence of periodontal inflammation [3,4,5].

Currently, there is a considerable arsenal of pharmaco-therapeutic agents that are used for the prevention and treatment of inflammatory processes in periodontal tissues, the use of which is based on influencing the link of the relevant pathological process: antiseptics, antibacterial drugs, anti-inflammatory drugs, enzyme preparations, desensitizers, immunomodulators, sorbents, antioxidants and their various combinations to increase therapeutic effectiveness [6,7].

Given the complex nature of the disease, its chronic progression, the emergence of antibiotic-resistant microorganisms, and the potential for further complications, there is a clear need for a new oromucosal treatment that can deliver active pharmaceutical ingredients (APIs) directly to the affected area without significant systemic absorption. To achieve this, a local treatment with polymeric gelling agents and mucoadhesive properties is proposed, which will help to prolong the concentration of APIs and enhance their efficacy.

Modern views on the pathogenesis of inflammatory diseases of the oral cavity characterize the prospects for the use of metabolic, endothelial, antioxidative and anti-inflammatory agents. Thus, an important link in the pathogenesis of inflammatory processes in the oral mucosa is the expression of pro-inflammatory cytokines – IL-1b, TNF-a, an increase in the activity of iNOS and the activation of nitrosating stress, accompanied by an increase in cytotoxic forms of NO.

There is a selective IL-1b antagonist, the substance of which is obtained biotechnologically from *E.coli* TG1 (pTAC-hIL-1ra), in its structure containing 153 amino acid fragments. This agent interrupts IL-b, the dependent cascade mechanisms of ischemic neurodestruction. This antagonist normalizes glutathione (GSH)-dependent mechanisms of HSP70 expression in mitochondria and brain cytosol in acute ischemia. High neuroprotective, anti-ischemic, antioxidative, anti-apoptotic activity and harmlessness of this agent have been shown. The interleukin antagonist has been successfully used for the treatment of rheumatoid arthritis, cerebrovascular disease, and pericarditis [8,9].

All of the above determines the relevance and prospects of creating a dosage form (oromucous gel) based on it for the treatment of chronic generalized periodontitis.

The selection of excipients in pharmaceutical development requires careful consideration and experimentation to create a stable dosage form with high therapeutic efficacy throughout its shelf life. To streamline this process and ensure quality control, researchers can employ mathematical and *in silico* methods to predict outcomes and conserve resources.

Aim

This research aims to enhance the structure of oromucosal gel containing interleukin antagonist IL-1β to effectively prevent and treat complex inflammatory periodontal diseases.

Materials and methods

Experimental studies were conducted based on the Department of Medicines Technology of Zaporizhzhia State Medical and Pharmaceutical University and the Training Medical and Laboratory Center of Zaporizhzhia State Medical and Pharmaceutical University of the Ministry of Health of Ukraine.

As an active component, in the recipe of oromucosal gel, used antagonist of the interleukin IL-1B. Excipients: D-panthenol (the plasticizer), carboxymethylcellulose sodium salt (the viscosity modifier and mucoadhesive component), Tween-80 (the enhancer absorption), benzalkonium chloride (the preservative), sodium hydrophosphate + citric acid (the phosphate buffer solution), purified water. For the experiments, only pharmaceutical-grade active and auxiliary ingredients were utilized, which were sourced from Ukraine suppliers such as SINBIAS LLC, Istok-Plus LLC, and MOBIL MEDICAL LLC.

Design of experiments. To develop a robust experiment design, we utilized the response surface methodology, specifically the Box-Behnken Design. These statistical techniques are commonly employed to create a model and analyze responses that are influenced by optimization factors [10,11]. The development optimization process was conducted based on three factors (x), each at three levels (low, medium, high), and four distinct answers (y), as detailed in *Table 1*.

The laboratory technology for producing prototypes of oromucosal gels involves several steps. Firstly, half the amount of prescribed water is measured and purified. Disodium hydrogen phosphate and citric acid are then added to this water, and the resulting mixture is heated to 80 °C while being constantly stirred with a magnetic stirrer. Na CMC is added, and the polymer is left to swell without heating. This mixture is called mixture A. Secondly, the other half of the prescribed water is measured and purified, and it is mixed with benzalkonium chloride, Tween-80, D-panthenol, and IL-1β at a temperature of 25 °C while being constantly stirred with a magnetic stirrer. This mixture is called mixture B. Once mixture A has cooled down to 25 °C, mixture B is added to it and mixed without heating. The resulting gel is then left in a cool place (5 °C) for one day.

Characteristics of the obtained samples of oromucosal gels

pH test. A pH test was conducted by weighing 10.0 g of gel and placing it in a measuring cylinder. This was then mixed with 100 ml of purified water using a magnetic stirrer. Finally, the pH was measured with a 15 OM pH meter device equipped with a glass electrode.

Table 1. Factors and their levels used in the design of the oromucosal gel experiment with IL-1β

Factor	Parameter	Levels		
		Low (-)	Medium (0)	Hight (+)
x1	Na CMC, %	1	1.5	3
x2	D-panthenol, %	1	3	5
x3	Tween-80, %	0.5	0.75	1.5

Kinetic stability of oromucosal gel. The gel sample was centrifuged in a centrifuge at 6000 rpm for a duration of 15 minutes. The coefficient of kinetic stability was calculated using the formula:

$H_k = H_1 / H_{all}$,

where H_1 – the height of the layer of fluid that can be released from the oromucosal gel;

H_{all} – the total height of the gel;

A system is considered kinetically stable if $H_k = 0$.

Rheological studies. The experimental gels underwent rheological studies using the oscillation mode of the Anton Paar modular compact rheometer MCR 302. Measuring devices employed in the study included the CP50-1 cone-plate system with a 50 mm diameter and 1-degree cone angle SN71317. Temperature control was achieved through a built-in thermostat, specifically the Peltier temperature control for concentric cylinder systems (C-PTD 200).

Viscosity test of test gels. A quantity of 5 g of gel was precisely weighed and delicately placed onto the plate. The RheoCompass software was utilized to position the cone a mere 0.1 mm away from the plate, enabling accurate measurements. At a shear rate (γ) of 50 1/s, viscosity values (measured in mPa × s) were recorded. The temperature during the experiment was strictly maintained at 25 °C.

Amplitude test. Weighing 5 g of gel, it was placed on a plate and the cone was positioned 0.1 mm away using the RheoCompass software. An amplitude test was performed with the following parameters: angular frequency of oscillations (ω) – 10 [Rad/s], deformation (%) – logarithm 0.01 – 100, measurement point time (s) – automatically. Throughout the experiment, the RheoCompass software tracked the accumulation module G' and the loss module G'' from deformation (γ), allowing for the determination of the structural transition (yield strength of the sample (τ0)) at the point of transition to the graph curves G' and G''. Additionally, the software established the boundary of the linear viscoelastic region (LVER) where the gel sample retained its structure. The amplitude test was carried out under a temperature of 25 °C.

Frequency test. A quantity of 5 g of gel was precisely weighed and delicately placed onto the plate. Using the advanced RheoCompass software, we placed a cone 0.1 mm away from the plate and conducted a frequency test ranging from 100 to 0.1 rad/s, with a strain amplitude of 0.5 % and at a temperature of 25 °C. Throughout the experiment, the software accurately calculated the accumulation modulus G', loss modulus G'', and loss factor tg δ (also known as the "loss factor") by dividing G'' by G'.

Mucoadhesive test. The experiment was conducted using a Tack test, which involved the Anton Paar Model MCR 302 device. A solution of 2 % pig's stomach mucin (M2378, Sigma Aldrich) was prepared in water and used to coat the cone and platinum working surface. The surface was then incubated at 37 °C for 10 minutes and dried until a film was formed [12]. Next, 0.1 g of gel was weighed onto the mucin-coated plate. Using the RheoCompass firmware, the cone was placed 0.1 mm away from the hob and the normal force (N) required to detach the cone from the plate at 25 °C was measured.

Thixotropy test. We weighed 0.5 g of gel and placed it onto a plate. Using the RheoCompass software, we positioned the cone 0.1 mm away from the plate and

Table 2. Received the formulation design, along with the factors and responses

Run	Factor 1 A: CMC, %	Factor 2 B: D-panthenol, %	Factor 3 C: Tween-80, %	Response 1 pH	Response 2 Viscosity, mPa × s	Response 3 Type system, 1/0	Response 4 Mucoadhesive, N
1	2	5	0.5	6.5	1429.4	0	6.9
2	2	1	1.5	6.5	1184.5	0	6.6
3	3	5	1.0	6.5	5242.9	1	10.4
4	1	3	1.5	6.5	362.12	0	5.8
5	3	3	0.5	6.5	3864.3	1	9.0
6	2	3	1.0	6.4	1163.7	0	6.8
7	1	1	1.0	6.5	338.71	0	5.4
8	3	1	1.0	6.5	4591	1	9.5
9	3	3	1.5	6.6	8475.1	1	11.5
10	2	3	1.0	6.5	1214.9	0	6.4
11	1	5	1.0	6.5	390.17	0	5.9
12	2	5	1.5	6.6	1005.8	0	6.9
13	2	3	1.0	6.6	1533.3	0	7.2
14	2	1	0.5	6.5	1139.1	0	6.7
15	1	3	0.5	6.6	323.32	0	5.5

studied the restoration rate of a oromucosal gel prototype with three intervals (3ITT) through direct examination. The experiment was conducted in three stages. First, we measured at a low shear rate (0.1 s^{-1}) to observe the sample's behavior at rest. Next, we measured at a shear velocity of 100 s^{-1}, which characterizes the behavior of the sample during application. Finally, we measured at a low shear rate of -0.1 s^{-1} to determine how quickly the sample regained its structure.

Pharmaco-toxicological research methods. The experiment involved 46 outbred white rats of both sexes, weighing between 160 and 180 grams, sourced from the Institute of Pharmacology and Toxicology's vivarium. All procedures were carried out in compliance with the biomedical experiment animal usage regulations (Strasbourg, 1986, amended in 1998), as well as the "General Ethical Principles of Animal Experiments" [13,14,15]. The acute toxicity studies followed the recommendations of the SFC of the Ministry of Health of Ukraine and other guidelines. Each group had 6 animals, and an oral dose of the test gel was administered using a dosing syringe in an optimal volume of 1.0 ml/100 g of body weight [16]. Over 14 days, changes in the cardiovascular system, respiratory system, central nervous system, and motor activity were monitored, along with the mortality rate of the animals.

The gel's local irritating effect was studied following the recommendations of the SFC of the Ministry of Health of Ukraine. A dispenser was used to apply 0.01 ml of gel to the conjunctiva of both eyes of the animals in the experimental group, while the control group was given purified water. The animals were observed for three days.

To study the gel's active cutaneous anaphylaxis, hair was removed from a 4 × 4 cm area on the lateral surface of the animals' bodies. Then, 0.5 g of gel was applied to the area, and the animals were placed in separate cages for four hours. Sensitization was detected five days after the last application of the drug by applying 0.3 g of gel to the skin of the ear. The intensity of anaphylactic shock was evaluated at 6, 12, and 24 hours, according to the Weigle index. These tests were also conducted following the SFC.

Statistical research methods. The results of the study were calculated using the standard statistical package of the Statistica for Windows 13 (StatSoft Inc., No. JPZ804I382130ARCN10-J), as well as SPSS 16.0, Design Expert, Microsoft Office Excel 2003.

Results

Planning of the optimal composition of the oromucosal gel with IL-1β was carried out using Box-Behnken Design, with three levels of factors: Na CMC, Tween-80, D-panthenol; and four responses: pH, viscosity test, Type system, Mucoadhesive *(Table 2)*.

Organoleptic characteristics. As per the experimental design, oromucosal gel samples were collected, which varied in color from transparent to faint white-yellow, and had different densities, consistency, and no discernible odor. The intensity of coloration was dependent on the amount of tween-80 used in the formulation.

Hydrogen index (pH). The pH index is critical in maintaining the chemical stability of a formulation. It is influenced by several factors and can impact the mucous membranes in the oral cavity, leading to potential health issues. To ensure consistency in pH levels, disodium hydrogen phosphate and citric acid are added to the formulation as phosphate buffer components.

Statistical analysis of the obtained results of the effect of variable factors on the pH of the obtained oromucosal gels is given in *Table 3*.

As expected, the results obtained for determining the pH of oromucosal gel samples do not change in oromucosal gel formulations, due to the presence of a phosphate buffer solution in the prescription composition.

Rheological characteristics. Viscosity at a given shear stress is one of the rheological characteristics that allows you to compare the consistency properties of experimental gels and identify the possible effect of excipients of the formulation on it. The results of determining the consistency characteristics of oromucosal gels are given in *Table 4*.

The obtained data of the statistical analysis of the ANOVA for Quadratic model indicate the significant influence of factors A – Na CMC, C – Tween-80 on the viscosity characteristics of the manufactured samples of oromucosal gels (F-value > p-value). The relationship between the viscosity value of the gel preparation and the factors is shown in *Fig. 1–3* and is highlighted in the equation:

Table 3. Influence of variable factors on the pH value of oromucosal gels

Source	Sequential p-value	Lack of Fit p-value	Adjusted RBI	Predicted RBI	–
Mean	<0.0001	–	–	–	Suggested
Linear	0.8799	0.9481	-0.2004	-0.5412	
2FI	0.3867	0.9701	-0.1534	-0.4972	
Quadratic	0.7094	0.9630	-0.4318	-0.9318	
Cubic	0.9630	–	-2.1818	–	Aliased

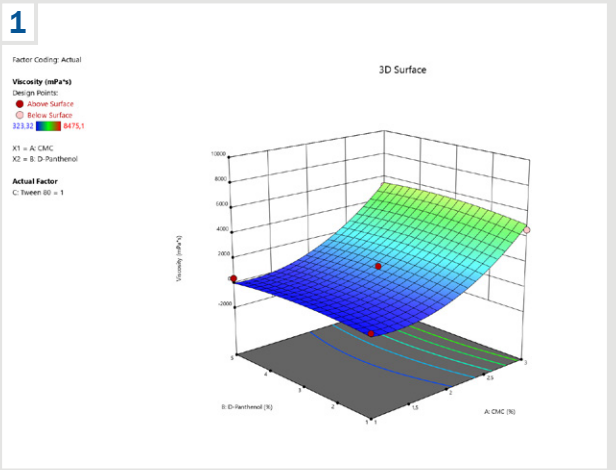
Table 4. Influence of variable factors on the value of viscosity characteristics of oromucosal gels

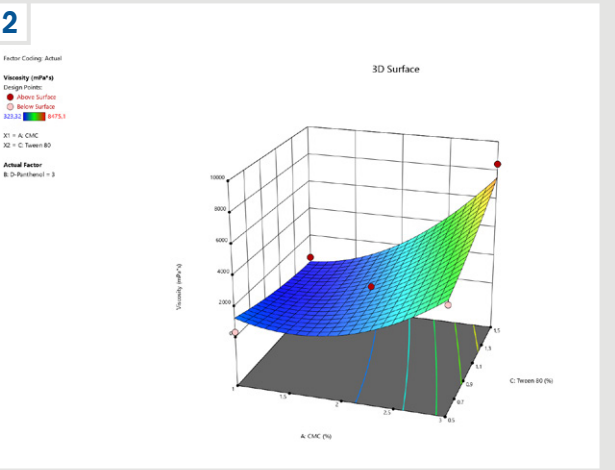
Source	Sum of squares	df	Mean square	F-value	p-value	
Model	7.330E+07	9	8.144E+06	**9.94**	**0.0105**	significant
A: CMC	5.387E+07	1	5.387E+07	**65.76**	**0.0005**	–
B: D-Panthenol	83019.98	1	83019.98	0.1013	0.7631	–
C: Tween-80	2.281E+06	1	2.281E+06	**2.78**	**0**.1561	–
AB	90132.05	1	90132.05	0.1100	0.7536	–
AC	5.226E+06	1	5.226E+06	6.38	0.0528	–
BC	54990.25	1	54990.25	0.0671	0.8059	–
A^2	1.069E+07	1	1.069E+07	13.05	0.0153	–
B^2	4.916E+05	1	4.916E+05	0.6001	0.4736	–
C^2	2.319E+05	1	2.319E+05	0.2831	0.6175	–
Residual	4.096E+06	5	8.192E+05	–	–	–
Lack of Fit	4.016E+06	3	1.339E+06	33.38	0.0292	significant
Pure Error	80201.39	2	40100.69	–	–	–
Cor Total	7.739E+07	14	–	–	–	–

$$y = 1303.97 + 2594.87 \times A + 101.87 \times B + 533.93 \times C + 150.11 \times AB + 1143.00 \times AC - 117.25 \times BC + 1701.62 \times A^2 - 364.89 \times B^2 + 250.62 \times C^2.$$

The oscillation mode offers distinct benefits over the rotational mode in rheological studies, as it avoids any additional mechanical damage to test samples and ensures measurements consider the initial state of the sample [17,18]. Utilizing the oscillation mode, we conducted rheological studies on experimental samples of anti-inflammatory oromucosal gel (st1-st15), enabling us to accurately determine key indicators such as linear viscoelastic range and loss coefficient *(Fig. 4–9)*.

The sample's behavior can be divided into two parts: the elastic (solid) part characterized by the modulus of elasticity G' and the viscous (liquid) part characterized by the modulus of viscosity G". When the curve for G' goes above the curve for G", it is assumed that the sample has viscoelastic properties within the linear viscoelastic range. The viscous behavior arises from internal friction between the components of the system, which converts the energy obtained from deformation into heat energy. This energy is gradually consumed and cannot be used by the sample. In contrast, the elastic part of the energy characterized by the modulus G' is stored in the deformed material, while the structure of bonds in the system remains intact. As a result, the material returns to its original shape when at rest [19,20,21].





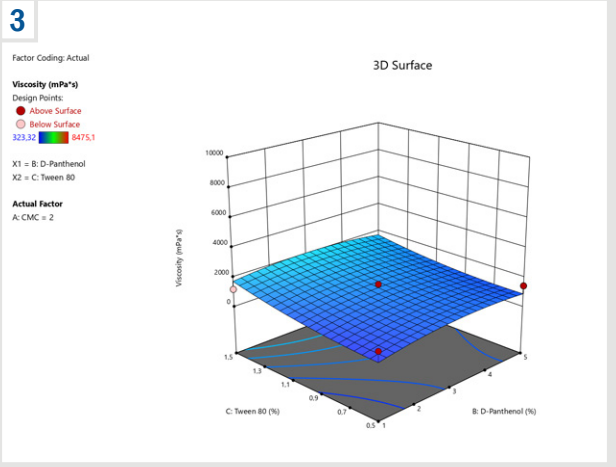
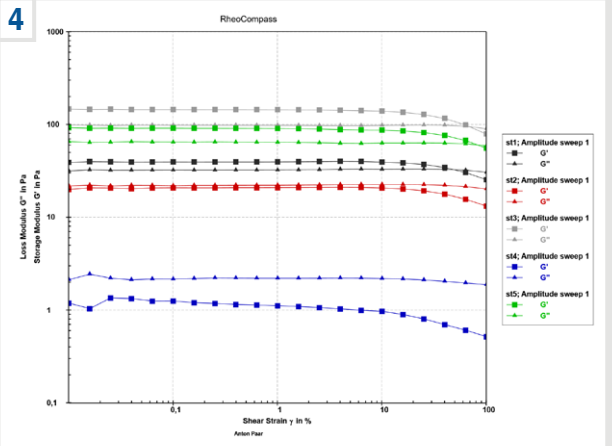
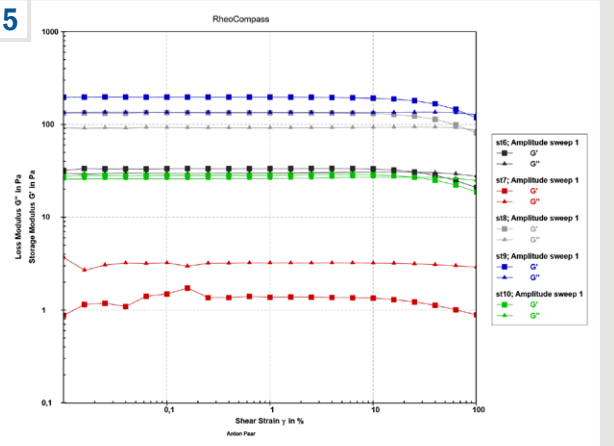


Fig. 1. 3D image of the relationship between variable factors (Na CMC, D-panthenol) and viscosity characteristics of oromucosal gels.

Fig. 2. 3D image of the relationship between variable factors (Na CMC, Tween-80) and viscosity characteristics of oromucosal gels.

Fig. 3. 3D depiction of the relationship between variable factors (D-panthenol, Tween-80) and viscosity characteristics of oromucosal gels.





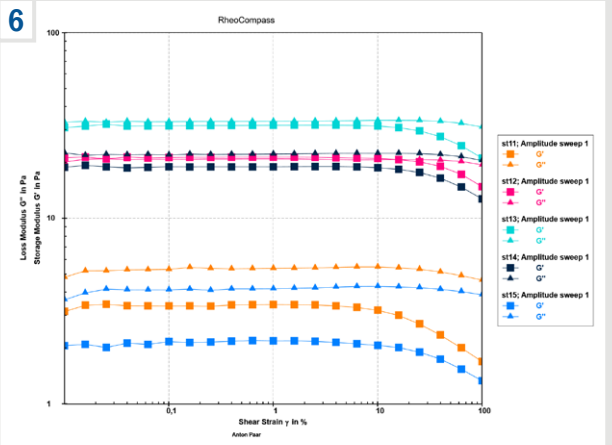
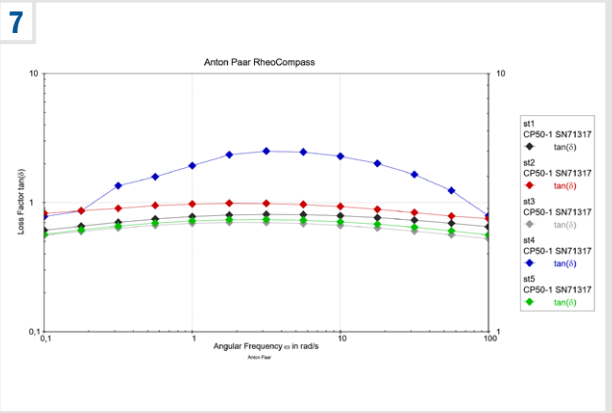
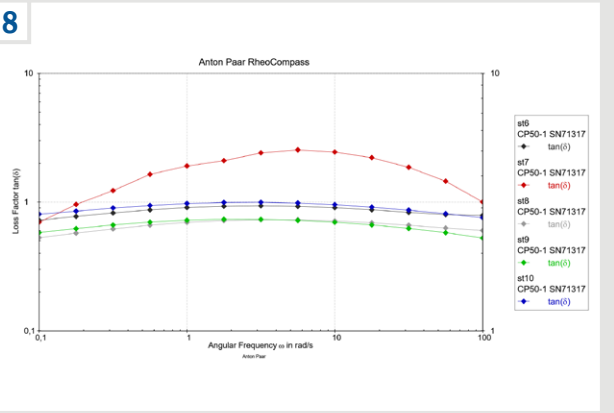


Fig. 4. Linear viscoelastic range (LVER) of oromucosal gels st1-st5.

Fig. 5. Linear viscoelastic range (LVEr) of oromucosal gels st6-st10.

Fig. 6. Linear viscoelastic range (LVER) of oromucosal gels st11-st15.





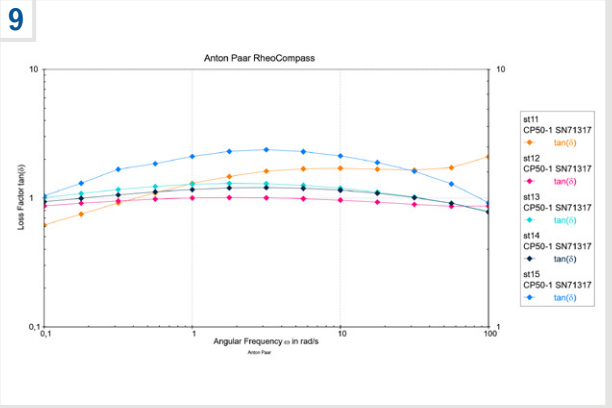
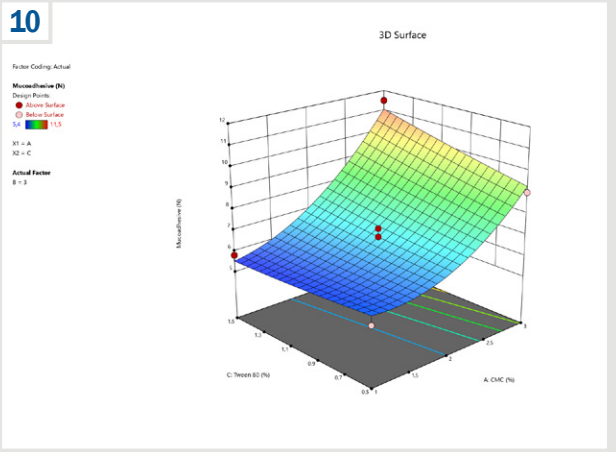
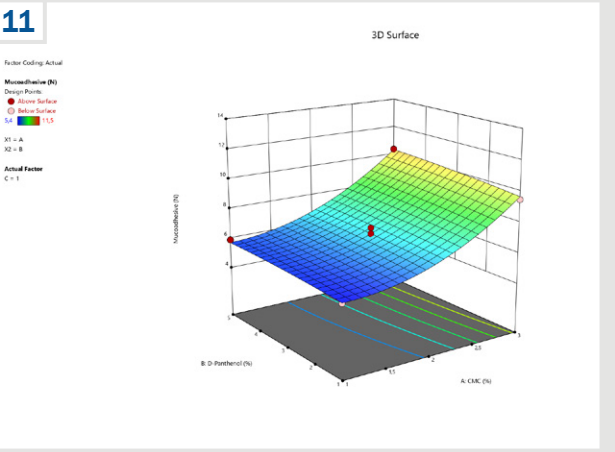


Fig. 7. Mechanical spectrum of dependence of loss coefficient (tanδ) on frequency (ω), at amplitude constant y = 0.5 % in samples of oromucosal gels st1-st5.

Fig. 8. Mechanical spectrum of dependence of loss coefficient (tanδ) on frequency (ω), at amplitude constant y = 0.5 % in samples of oromucosal gels st6-st10.

Fig. 9. Mechanical spectrum of dependence of loss coefficient (tanδ) on frequency (ω), at amplitude constant y = 0.5 % in samples of oromucosal gels st11-st15.





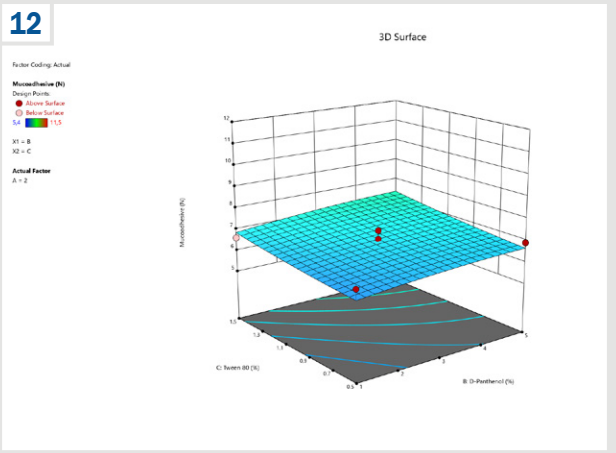


Fig. 10. 3D image of the relationship between variable factors (Na CMC, Tween-80) and mucoadhesive characteristics of oromucosal gels.

Fig. 11. 3D image of the relationship between variable factors (Na CMC, D-panthenol) and mucoadhesive characteristics of oromucosal gels.

Fig. 12. 3D image of the relationship between variable factors (Tween-80, D-panthenol) and mucoadhesive characteristics of oromucosal gels.

Table 5. Influence of variable factors on the value of mucoadhesive characteristics of oromucosal gels

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	46.70	9	5.19	17.10	0.0030	significant
A: CMC	39.60	1	39.60	**130.49**	**< 0.0001**	–
B: D-panthenol	0.4513	1	0.4513	**1.49**	**0.2771**	–
C: Tween-80	0.9113	1	0.9113	**3.00**	**0.1437**	–
AB	0.0400	1	0.0400	0.1318	0.7314	–
AC	1.21	1	1.21	3.99	0.1024	–
BC	0.0025	1	0.0025	0.0082	0.9312	–
A^2	4.37	1	4.37	14.39	0.0127	–
B^2	0.0283	1	0.0283	0.0931	0.7725	–
C^2	0.0144	1	0.0144	0.0475	0.8360	–
Residual	1.52	5	0.3035	–	–	–
Lack of Fit	1.20	3	0.3992	2.49	0.2990	not significant
Pure Error	0.3200	2	0.1600	–	–	–
Cor Total	48.21	14	–	–	–	–

Through rheological studies of oromucosal gels st1-st15 (as shown in *Fig. 4–6*), the limits of the linear viscoelastic range in the test gels were determined. Additionally, the type of systems present were characterized: those with pseudoplastic flow (G' > G''), which regain their structure after force application, and viscoelastic fluids (G'' > G'), with greater loss modulus values than elasticity modulus values.

In scientific literature, a useful indicator is employed – the loss factor, or TG δ = G'' / G'. This tool provides an additional way to evaluate the behavior of a sample. If Tg δ is less than 1, the sample is considered viscoelastic; if Tg δ equals 1, the sample is in a state of gelation; and if Tg δ is greater than 1, the sample is a viscoelastic liquid [22,23]. For experimental oromucosal gel samples, the mechanical spectra of the loss coefficient (tanδ) dependence on frequency (ω) *(Fig. 7–9)* are provided at a constant amplitude.

Analysis of the characteristics of experimental oromucosal samples of st1-st15 gels according to the indicators of amplitude and frequency tests made it possible to classify systems with pseudoplastic and other types of flow, these results are included in *Table 2*, in the response column (type) marked 1 – a pseudoplastic type of flow, in which G' at rest (frequency ω = 0.1 rad/s) > G'', 0 – another type.

The effectiveness of a topical oromucosal medication depends on the active ingredients in the dosage and how long they stay in the mouth. This can be challenging due to the constant production of saliva and mechanical actions. Mucoadhesion, or the adhesion of the medication to the oral mucosa, can increase the retention of the active substance and improve its effectiveness [24,25,26]. To compare the mucoadhesive properties of oromucosal gels st1-st15, we used dynamic mechanical analysis.

The results of statistical analysis *(Table 5)* of the ANOVA for Quadratic model highlight the significant influence of factors A – Na CMC, B – D-panthenol, C – Tween-80 on the mucoadhesive characteristics of oromucosal gels

Table 6. Proposed formulations of prescription oromucosal gel with IL-1β

Number	CMC, %	D-panthenol, %	Tween-80, %	pH	Viscosity, mPa × s	Prob (Type system = 1)	Mucoadhesive, N	Desirability, unit
1	3.000	5.000	1.500	6.520	7297.503	1.000	11.337	0.987
2	3.000	4.971	1.500	6.520	7306.290	1.000	11.335	0.986
3	3.000	4.935	1.500	6.520	7316.729	1.000	11.331	0.986
4	3.000	4.914	1.500	6.520	7322.709	1.000	11.329	0.986
5	3.000	5.000	1.496	6.520	7281.275	1.000	11.329	0.986
6	3.000	4.860	1.500	6.520	7337.815	1.000	11.324	0.985
7	3.000	5.000	1.490	6.520	7256.215	1.000	11.316	0.985
8	3.000	4.767	1.500	6.520	7362.068	1.000	11.315	0.985
9	3.000	4.737	1.500	6.520	7369.806	1.000	11.311	0.984
10	3.000	4.699	1.500	6.520	7379.011	1.000	11.307	0.984
11	3.000	5.000	1.485	6.520	7234.593	1.000	11.306	0.984
12	3.000	4.628	1.500	6.520	7395.784	1.000	11.300	0.983
13	3.000	4.573	1.500	6.520	7408.204	1.000	11.293	0.983
14	3.000	4.541	1.500	6.520	7415.132	1.000	11.290	0.983
15	2.993	4.861	1.500	6.520	7284.789	1.000	11.288	0.982
16	3.000	4.392	1.499	6.520	7440.515	1.000	11.270	0.981
17	2.984	5.000	1.500	6.520	7183.620	1.000	11.258	0.980
18	3.000	5.000	1.457	6.520	7123.544	1.000	11.249	0.979
19	3.000	5.000	1.452	6.520	7100.375	1.000	11.237	0.978
20	3.000	4.209	1.481	6.520	7398.133	1.000	11.212	0.976
21	3.000	3.925	1.500	6.520	7512.255	1.000	11.211	0.976
22	3.000	5.000	1.437	6.520	7042.262	1.000	11.208	0.976
23	3.000	3.892	1.500	6.520	7515.527	1.000	11.207	0.976
24	3.000	3.859	1.500	6.520	7518.268	1.000	11.202	0.975
25	3.000	3.718	1.500	6.520	7529.306	1.000	11.181	0.974
26	3.000	3.639	1.500	6.520	7533.754	1.000	11.169	0.973
27	3.000	3.531	1.500	6.520	7538.011	1.000	11.153	0.971
28	3.000	3.509	1.500	6.520	7538.628	1.000	11.149	0.971
29	3.000	3.398	1.500	6.520	7540.321	1.000	11.131	0.969
30	2.984	5.000	1.439	6.520	6933.969	1.000	11.131	0.969
31	3.000	3.177	1.500	6.520	7537.051	1.000	11.094	0.966
32	3.000	5.000	1.367	6.520	6768.538	1.000	11.066	0.964
33	3.000	2.969	1.500	6.520	7525.794	1.000	11.057	0.963
34	3.000	5.000	1.357	6.520	6729.925	1.000	11.046	0.962
35	3.000	5.000	1.347	6.520	6691.711	1.000	11.026	0.960
36	3.000	5.000	1.334	6.520	6641.243	1.000	11.000	0.958
37	3.000	2.622	1.500	6.520	7489.483	1.000	10.991	0.957
38	3.000	5.000	1.315	6.520	6569.181	1.000	10.962	0.955
39	3.000	5.000	1.294	6.520	6491.687	1.000	10.921	0.951
40	3.000	2.111	1.500	6.520	7396.071	1.000	10.884	0.948
41	3.000	2.044	1.500	6.520	7380.188	1.000	10.869	0.947
42	3.000	1.956	1.500	6.520	7358.189	1.000	10.849	0.945
43	3.000	1.720	1.500	6.520	7292.266	1.000	10.795	0.940
44	3.000	1.617	1.500	6.520	7260.403	1.000	10.770	0.938
45	3.000	5.000	1.194	6.520	6128.935	1.000	10.725	0.934
46	3.000	5.000	1.188	6.520	6110.762	1.000	10.715	0.933
47	3.000	1.389	1.500	6.520	7182.837	1.000	10.714	0.933
48	3.000	5.000	1.167	6.520	6034.996	1.000	10.673	0.930
49	3.000	1.065	1.500	6.520	7055.968	1.000	10.630	0.926
50	3.000	2.878	1.284	6.520	6619.360	1.000	10.614	0.925
51	3.000	4.997	1.130	6.520	5911.950	1.000	10.604	0.924
52	3.000	5.000	1.079	6.520	5740.745	1.000	10.509	0.915
53	3.000	1.073	1.403	6.520	6625.196	1.000	10.443	0.909
54	3.000	5.000	0.972	6.520	5399.737	1.000	10.311	0.897
55	3.000	5.000	0.861	6.520	5073.974	1.000	10.114	0.879
56	3.000	5.000	0.847	6.520	5034.596	1.000	10.090	0.877
57	3.000	1.000	1.194	6.520	5718.391	1.000	10.032	0.871
58	3.000	5.000	0.747	6.520	4762.298	1.000	9.917	0.860
59	3.000	5.000	0.741	6.520	4746.112	1.000	9.906	0.859
60	3.000	5.000	0.690	6.520	4617.738	1.000	9.821	0.851
61	3.000	2.593	0.869	6.520	5106.140	1.000	9.814	0.851
62	3.000	5.000	0.635	6.520	4483.658	1.000	9.730	0.843
63	3.000	5.000	0.560	6.520	4308.558	1.000	9.607	0.831
64	3.000	1.320	0.777	6.520	4388.400	1.000	9.393	0.809
65	3.000	1.000	0.634	6.520	3804.097	1.000	9.089	0.778

Table 7. Pharmaceutical characteristics of optimized anti-inflammatory oromucosal gel formulation

Metric type	Characteristics
Appearance	Gel with a thick consistency of yellowish-white color and odorless
pH	6.5500 ± 0.0334
Kinetic stability, H_k	0
Yield strength, Pa	353.4000 ± 4.2107
Restoration of structure (thixotropic properties), %	After 180 seconds after application, not less than 85 %

Table 8. Study of acute toxicity of il-1β blocker oromucosal gel in rats with oral administration

Volume, ml/100 g	Dose, mg/kg	Number of rats			Lethality, %
		total	dead	surviving animals	
1.0	1	6	0	6	0

(F-value > p-value). The relationship between the value of mucoadhesive characteristics and factors is shown in *Fig. 10–12* and covered in the equation:

$$y = 6.8 + 2{,}225 \times A + 0.2375 \times B + 0.3375 \times C + + 0.1 \times AB + 0.5 \times AC + 0.025 \times BC + 1.0875 \times A^2 – – 0.0875 \times B^2 + 0.0625 \times C^2.$$

Subsequently, the composition of the oromucosal gel formulation was optimized using Box–Behnken Design according to numerical characteristics to predict the optimal characteristics of the oromucosal gel formulation with IL-1β. The optimization procedure was configured in the Design expert software for the following purposes: type system – goal maximize, mucoadhesive – goal maximize, the results of forecast options are shown in *Table 6*.

To conduct additional research, we opted for Formulation Composition No. 1 *(Table 6)*, as it demonstrated the highest levels of desirability (0.987), mucoadhesive characteristics (11.337), and type system (type system = 1).

Following the production of the anticipated composition for the oromucosal gel formulation, which included IL-1β (1 %), Na CMC (3 %), D-panthenol (5 %), Tween-80 (1.5 %), benzalkonium chloride (0.02 %), and phosphate buffer (up to 100 %), we analyzed its technological attributes.

Based on the results of the thixotropy test, it has been determined that the experimental oromucosal gel possesses thixotropic properties. This means that its structure is able to recover after an applied force. Specifically, the restoration of structure was observed at 69.5 % after 10 seconds, 76.1 % after 30 seconds, and 85.4 % after 180 seconds. These findings provide insight into the stability of the dosage form, both pre and post-application *(Table 7)*.

After conducting pharmacotoxicological studies, it was discovered that administering the oromucosal gel in the appropriate volume did not lead to any animal fatalities during the observation period. Additionally, there were no noted changes in the behavior or appearance of the animals *(Table 8)*.

Based on the results, the experimental oromucosal gel appears to fall into the VI toxicity class. The study of its localized irritant effect revealed that only 1 out of 10 animals exhibited slight redness of the conjunctiva within 2 hours of application, with no further adverse reactions detected in subsequent observation periods. These findings suggest that the experimental oromucosal gel does not have an irritant effect.

Additionally, the study of its allergizing effect demonstrated that rats did not experience anaphylactic shock after 5 days of application on a sheared skin area. Therefore, it can be concluded that the oromucosal gel with IL-1β does not cause an allergizing effect.

Discussion

In the contemporary understanding of how inflammatory diseases develop in the oral cavity, a hopeful approach involves using medication that has metabolic, endothelial, antioxidative and anti-inflammatory effects. Studies have revealed that a crucial factor in the pathogenesis of inflammation in the oral mucosa is the expression of pro-inflammatory cytokines such as IL-1β and TNF-a, increased activity of iNOS, and the activation of nitrosating stress, resulting in the proliferation of cytotoxic forms of NO [27].

Clinicians and pharmacologists are highly interested in the use of an IL-1β receptor or antibody antagonist. However, medicinal products within this category have a short half-life of only 4–6 hours when administered parenterally. To maintain proper concentration, daily subcutaneous injections are required. Fortunately, our team has developed a gel specifically for dentistry that improves its pharmaco-technological characteristics. The development of new dosage forms for oromucosal medicine involves the use of software and mathematical technologies within pharmaceutical development. These advancements not only reduce the time and resources required by researchers but also allow for the creation of oromucosal medicines with controlled pharmaco-technological properties [28].

Our results are consistent with our previous studies on the development of formulations with the IL-1β antagonist. Thus, the developed 0.5 % gel with IL-1β, for intranasal use, highlights the long-term neuroprotective and nootropic effect and has a good safety profile [29,30].

Conclusions

1. The composition of the anti-inflammatory oromucosal gel with the interleukin antagonist IL-1β for the complex treatment of inflammatory periodontal diseases is developed.

2. It has been established that the consistency and mucoadhesive properties of the oromucosal gel are significantly influenced by the components of Na CMC, Tween-80 present in the formulation.

3. The developed composition of the resulting oromucosal gel has satisfactory indicators of kinetic stability and thixotropic properties.

4. The developed gel for dentistry meets all the requirements for harmlessness and safety of dosage forms of this group – low toxicity, absence of locally irritating and allergizing effects.

Prospects for further research. Prospective further research of the new oromucosal gel to study its specific activity.

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