DOI 10.31489/2020Ch4/128-134

UDC 661.122:615.281:615.453.6

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Development of the orodispersible films based on CO_2 extract of *Ziziphora bungeana* with antimicrobial activity

The present work was intended to develop the new drug in the form of film soluble in the oral cavity: development of its composition, production technology, the study of its antimicrobial activity. The relevance of the problem is caused by the absence of drugs in the form of films on the domestic pharmaceutical market. The optimal composition of films was selected by evaluating a number of physical, chemical and technological indicators of the obtained films. The article indicates the materials used to obtain the drugs in question, presents the technology for their preparation and quality determination methods: unit measurement methods and potentiometric determination of pH, tensiometric and conductometric methods, thin-layer and gas chromatography and others. Antimicrobial activity of the resulting films has been proven in vitro. Staphylococcus aureus ATCC 6538-p and Escherichia coli ATCC 8739 have been used as the test microorganisms in order to study them by disk diffusion method in agar. The introduction of CO_2 extract of *Z. bungeana* from medicinal plant raw materials as active ingredients in medicinal films will expand the range of complex phytopreparations of the domestic pharmaceutical market for the treatment of inflammatory diseases of the oral mucosa.

Keywords: CO_2 extract of *Ziziphora bungeana*, orodispersible phytofilm, film composition, phytofilms, the parameters of the technological mode, technological process, antimicrobial activity, test microorganisms.

Introduction

Currently, the main spectrum of pharmaceutical research is aimed at finding new medications improving the existing ones and creating drug delivery systems. One of the most promising therapeutic agents are medicinal films based on biologically active compounds of plant origin and informally called "phyto films" [1]. The oral mucosa has recently become increasingly important as an alternative route of administration for individual, controlled drug delivery. Multilayer films on mucous membranes, referred to the monograph of oromucosal drugs in the European Pharmacopoeia, have the advantages of a dosage form and satisfy the requirements associated with this place of drug delivery [2].

Orally dispersible films (ODF) have recently become one of the most popular forms of drug administration due to their superior convenience and patient compliance. The main advantage of the dosage form arises from its rapid disintegration: it can be taken without water. Compared to conventional oral dosage forms, ODF generally provides a better drug bioavailability with a faster onset of action. In addition, ODFs are flexible [3].

Medicinal films (MF) are polymeric elastic plates of oval or rectangular shape with equal edges and a flat surface of various sizes and thicknesses. They are a type of transdermal therapeutic systems (TTS). The difference between Phytofilms and synthetic polymeric therapeutic systems are following: phytofilms are made in the form of a TTS (transdermal therapeutic system) matrix on carriers of natural origin (gelatin, collagen, sodium alginate, agar-agar, etc.), which makes them safer and more compatible with a living organism (1).

Despite the above advantages of films, only one of the drugs in the form of a film belonging to the ATC classification, G04BE03, is registered in the State Register of the Republic of Kazakhstan with the trade name Viniks, produced by si.L.Farm Co., LTD, Republic of Korea. In other groups of the ATC classification, there are no drugs in the form of a film [4]. The development of films is the most pressing problem in dentistry. Their use makes it possible to treat diseases associated with inflammatory periodontal diseases, such as gingivitis, periodontitis, periodontal disease, etc.

In this regard, the purpose of our activity was to develop the composition, technology and quality assessment of films with antibacterial and anti-inflammatory properties, thanks to the use of a CO_2-extract of

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the plant *Z. bungeana*Juz family *Lamiaceae*as their basis [5]. *Ziziphora* phytochemicals include monoterpene essential oils, triterpenes, and phenolic substances belonging to flavonoids. In Kazakh traditional medicine, *Ziziphora* species are used for several medicinal purposes. In particular, *Z. bungeana* Juz. and *Z. clinopodioides* Lam. are used for treating diseases associated with the cardiovascular system, or to fight various infections [6]. To emphasize the natural origin of the base of the films, we named them orodispersephyto films (hereinafter referred to as films).

Experimental

The CO_2 extract of the medicinal plant material *Z. bungeana* was used as an active substance. To select the optimal composition, we studied about 54 film-forming compositions of biodegradable polymers and plasticizers. The latter were of natural and synthetic origin and represented different ratios of film-forming agents and plasticizers.

The selection of the optimal composition of the film, providing the necessary technological and consumer properties, was carried out in 2 stages [7]. At the first stage, an experiment was carried out to select excipients (films and plasticizers) that can form a matrix film for the subsequent administration of drugs. The film-forming properties of polymer solutions at various concentrations were studied during the experiment. The polymers were of various origins (natural and synthetic) and production (domestic and foreign). The criterion for choosing film compositions at the initial stage was a satisfactory appearance: transparency, elasticity, uniformity, the absence of microcracks and tears in the film. Glycerin was added to all bases as a plasticizer. Based on a preliminary study, 6 compositions out of 54 were selected. The rest of the models did not correspond to such technological parameters as mechanical strength, solubility, elasticity, etc. The selected models are presented in Table 1.

Table 1

Selected films forming compositions

Base	No. 1 model	No. 2 model	No. 3 model	No. 4 model	No. 5 model	No. 6 model
MC, g	–	–	15	–	–	–
PVA, g	–	15	–	–	–	–
PVP, g	30	–	–	–	–	–
Sodium alginate, g	–	–	–	–	–	4.0
Sodium CMC, g	–	–	–	3.0	–	–
Natrosol250G, g	–	–	–	–	3.0	0
CO_2 extract of *Z. bungeana*, g	1.5	1.5	1.5	1.5	1.5	1.5
Glycerin, g	1.5	1.5	1,5	1.5	1.5	1.5
an aqueous solution of gelatin, %	15	15	15	15	15	15
40 % ethanol	15	15	15	15	15	15
Purified water up to 50 ml	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml

The films were evaluated according to the following criteria: physical characteristics (color, odor, size and shape); average weight; solubility; pH of the aqueous solution; microbiological purity; weight loss on drying; authenticity. In addition, the criteria for evaluating the quality of the films included such technological parameters as vapor permeability, mechanical tensile strength, shell thickness, weight loss on drying, and dissolution time. Harrington's method of generalized preference function was used to statistically process the experimental results. Based on the results of the final activity, 3 compositions of bases No. 1, No. 3 and No. 6 were selected according to the characteristics of the films.

The second stage of the study was the selection of the optimal composition of the matrix film by the method of mathematical planning. The selection criteria were the following parameters of the film quality: the pH value of the aqueous solution, thickness and moisture content (Table 2).

According to the results of the second stage of research, the composition of samples No. 1 and No. 6 did not correspond to the main technological parameters — the indicator of dissolution and pH of the aqueous solution.

A certain contribution to the kinetics of drug release is made by the process of edema of high molecular weight substances (HMS). In the films based on natrosol 250G, Sodium CMC and Sodium alginate, small edema is observed, and in films based on PVA, PVP, moderate edema of the film based on MC is observed. At the same time, model No. 3 based on MK demonstrated the optimal composition. On the basis of this

model, an elastic, homogeneous, colorless and transparent film corresponding to the technological parameters was obtained (Table 3). The film sample contains the active pharmaceutical ingredient — CO_2-extract of the plant *Z. bungeana*, the antimicrobial properties of which are determined by the high content of pulegon.

Table 2

Technological parameters of dispersed films

No. model	Thickness, mm	Dissolution time, min	Humidity, %	pH	Mechanical strength, kg/m^2	Average weight, g
1	0.15 ± 0.015	3.45 ± 0.1	9.81 ± 0.1	8.05 ± 0.07	8.7±0.2	0.049 ± 0.003
2	0.3 ± 0.1	4.30 ± 0.2	5.52 ± 0.1	5.09 ± 0.07	4.7±0.2	0.349 ± 0.002
3	0.25 ± 0.015	2.50 ± 0.1	9.93 ± 0.1	6.14 ± 0.03	9.2±0.1	0.251 ± 0.015
4	0.169 ± 0.016	6.47 ± 0.1	10.47 ± 0.1	4.2 ± 0.01	5.6±0.3	0.153 ± 0.015
5	0.159 ± 0.015	4.10 ± 0.1	18 ± 0.1	8.0	3.1±0.3	0.142 ± 0.004
6	0.2 ± 0.01	4.37 ± 0.1	10.12 ± 0.1	7.3 ± 0.5	9.8±0.3	0.047 ± 0.004

Table 3

The optimal composition of the film based on CO_2 extract of *Z. bungeana*

The name of the ingredient	ND (Normative document)	Quantity, %	Functional purpose
Water solution of gelatin 30 %	SPRK (State pharmacopoeia of Republic of Kazakhstan)	30 %	Film-forming
Water solution of MC5 %	SPRK	30 %	The adhesive component
40 % ethanol	SPRK	30 %	Solvent
Glycerin	SPRK	2 %	Plasticizer
CO_2 extract of *Z. bungeana*	SPRK	3 %	Active substance
Purified water	SPRK	up to 100 ml	Solvent

The selection criterion for the films based on *Z. bungeana* CO_2 extract was a satisfactory appearance (transparency, elasticity, uniformity, absence of microcracks), sufficient, flexible and satisfactory bending strength. The films are uniform in thickness and weight.

The method of making a long-acting medicinal film consisted of 3 stages: preparation of the composition; deaeration and pouring into a special mold; drying and cutting.

Description of the technological process. Aqueous solutions of gelatin 30 % and MC5 % were mixed with the prepared components at a temperature of 40 °C, and thoroughly mixed with the addition of glycerin. The resulting mixture was heated to 40 °C and the drug substance was added there to. Then 40 % ethanol was added. By thoroughly mixing the components (exposure in a water bath at 40 °C for 30 minutes, rotation of the blades 20 ± 5 rpm), we obtained a homogeneous composition. To avoid the formation of air bubbles, centrifugation was carried out for 2 hours. The mixture was poured into a special mold, dried at a temperature of 40–45 °C to 10 % residual moisture. After drying, the films were cut into medium therapeutic doses of 1×2 cm. In practice, films dried at room temperature acquire a high-quality appearance. The films were packed with aluminum foil. The final product was placed in a box along with instructions for medical use. In appearance, the films are elastic plates of light yellow color with a specific odor, without mechanical damage and air bubbles: width (1 ± 0.2) cm, length (2 ± 0.2) cm, thickness (0.253 ± 0.015) mm (Fig. 1).

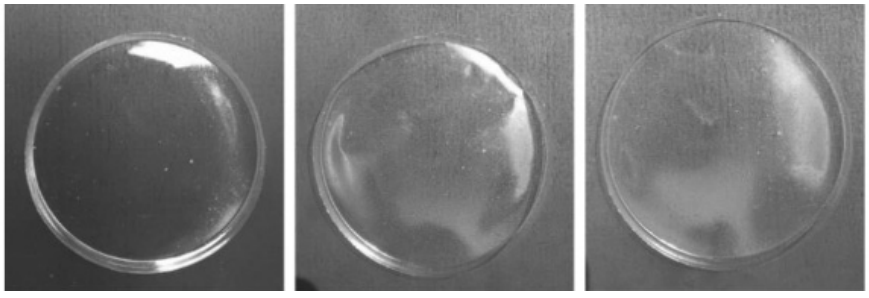

Figure 1. Orodispersible film obtained on the basis of *Z. bungeana* extract by pour method

Determination of the average mass. The average mass of a 1×2 cm film sample (an average therapeutic dose) was determined by measuring 10 films with an accuracy of 0.0002 g. The mass of an individual film was determined by a single measurement of 20 samples with an accuracy of 0.0002 g.

The permissible deviation in weight for films is up to 0.1 g and not less than ± 10 % [9].

Determination of thickness. The film thickness was measured with a MKTs-25 micrometer (GOST 6507–90).

Film disintegration time. The solubility of polymer films is the main criterion for the functional suitability of this dosage form and characterizes the ability of the film to be completely absorbed by biological body fluids. The process of dissolution of polymer films should be limited by the time required for the gradual release of the active substance and ensuring its constant concentration over a given period. The dissolution time of the films depends on the hydrophilicity of the selected polymer. A sample of 1×2 cm film was dissolved at room temperature in 2.5 ml of purified water to avoid adhesion to the wall of the glass tube, and the time of complete dissolution of the film was measured using a stopwatch [10].

The pH of the aqueous solution was determined by the method of potentiometric determination of pH (IP RK I, vol. 1, 2.2.3.). A film sample weighing 0.5 g was dissolved at room temperature in 50 ml of purified water, stirred, and the pH of an aqueous film solution was recorded.

This indicator allows predicting the irritant effect of the dosage form. The pH of the film should be closer to that of an aqueous solution of fluid from a wound, fluid of the oral mucosa, pH of an aqueous fluid, which is 6.5–7.2 [11].

Microbiological testing of film materials based on Z. bungeana CO_2-extract. There are a number of requirements for biomedical polymer matrices. One of them is the antimicrobial activity of the material, which is determined by its ability to slow down the growth of microorganisms. The comparative microbiological tests were carried out in order to study the bactericidal activity of the obtained films on the basis of the CO_2 extract of *Z. bungeana*. As a control model, we used CPF, which exhibits antibacterial activity against *Staphylococcus aureus* ATCC 6538-p and *Escherichia coli* ATCC 8739: these strains are sensitive to this antibiotic. The test microorganisms were investigated by the agar diffusion method using *Staphylococcus aureus* ATCC 6538-p and *Escherichia coli* ATCC 8739. Figure 2 shows the results of the microbiological tests.

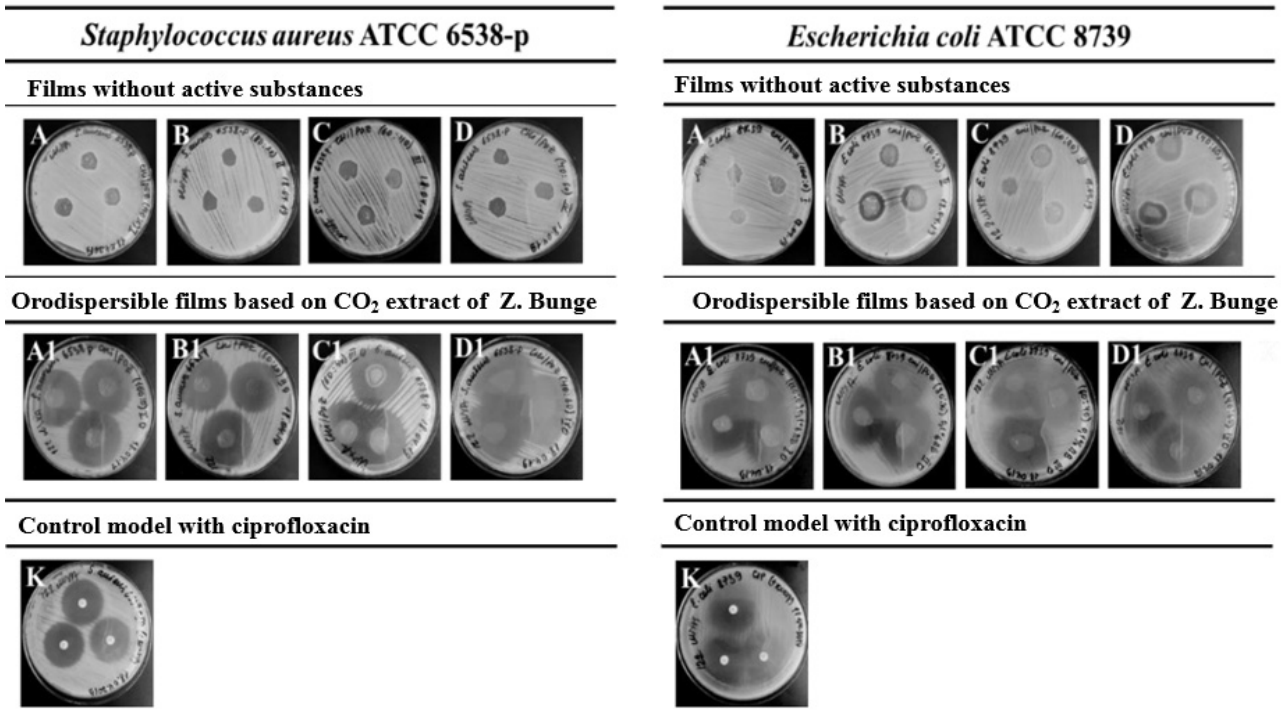

Figure 2. Zone of inhibition of the films against *Staphylococcus aureus* and *Escherichia coli* bacteria

Results and Discussion

As a result of the conducted research aimed at creating orodispersephy to films, matrices were obtained, which in appearance were films characterized by colorless or transparent, odorless, mechanical inclusions and air bubbles, good elasticity and good adhesion. The established experimental values of the indicators prove a satisfactory quality of the matrices as a rationality criterion for the composition of the developed films. An optimal composition of the films with gelatin as a polymer material has been developed and quality indicators have been determined.

Based on the transdermal therapeutic system, polymeric water-dispersible films have been obtained, which have a long-term therapeutic effect.

The results of experimental studies on the creation of medicinal films based on products of natural origin, i.e., phytofilms dispersed in water, make it possible to develop an optimal composition of medicinal preparations in the form of films with prolonged release of the active substance.

An elastic, homogeneous, colorless, transparent film corresponding to the technological parameters was obtained on the basis of model No. 3 (based on MC), which has an optimal composition with respect to edema of high molecular weight substances (HMS). In films on a different basis, either slight or moderate edema was observed.

The film compositions based on PVA, based on Sodium CMC and based on Natrosol 250G (formulations No. 2, No. 4, No. 5) did not meet the uniformity requirements due to the uneven distribution of particles and agglomerates formed in the sample based on sodium CMC. In addition, the degree of adhesion to the mucous layer was low.

Conclusions

The studies carried out during the development of new drug form and obtaining the product, as well as evaluating a number of physical, chemical and technological indicators allow making next conclusions:

– The choice of the optimal composition of the films and the assessment of the obtained orodisperse phytofilms should be done on the basis of a number of physicochemical and technological parameters. It is advisable to focus on the following indicators studied during biopharmaceutical research on the choice of the films composition and during step-by-step control during the production process: film thickness — 0.15–0.30 mm, pH of an aqueous solution — 6.0–7.2, the dissolution time is not less than 3 minutes [8], the mass loss (moisture content) is 8–10 % during drying.

– It is necessary to take into account the relationship between the kinetics of drug release and swelling of high molecular substances (HMS) during the choice of films optimal composition.

– Films based on *Z. bungeana* CO_2 extract have increased antimicrobial activity against the gram-negative strain of *Escherichia coli* and the gram-positive strain of *Staphylococcus aureus*.

– Films with MC (0.1 % w/v CPF) have a high antibacterial activity of *Staphylococcus aureus* ATCC 6538-p and *Escherichia coli* ATCC 8739.

– The high antibacterial activity of the film is explained by the presence of an active pharmaceutical ingredient in it — CO_2-extract of the *Z. bungeana* plant with a high content of pulegon.

– The developed films have the duration of action and the accuracy of the dosage of the active substance which is advantage of such dosage forms.

– The introduction of *Z. bungeana* CO_2 extract from medicinal plant materials as the active ingredient in medicinal films will expand the range of complex phytopreparations of the domestic pharmaceutical market for the treatment of inflammatory diseases of the oral mucosa.

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Ziziphora bungeana өсімдігінің сығындысы негізінде антимикробты қасиетке ие ородисперсті қабықшаларды әзірлеу

Мақалада ауыз қуысында еритін (ородисперсті) пленка түріндегі жаңа дәрілік препараттың жасалу жолы ұсынылған: препараттың құрамын жасау, пленканы алу технологиясын әзірлеу және оның микробқа қарсы белсенділігін зерттеу. Зерттеу жұмысының өзектілігі Қазақстанның фармацевтикалық нарығында пленка түріндегі дәрілік препараттардың болмауымен түсіндіріледі. Алынған қабықша физико-химиялық және технологиялық көрсеткіштер бойынша бағаланды, олардың нәтижелері бойынша фитоқабықшаның оңтайлы құрамы таңдалды. Мақалада дәрі-дәрмектерді алу үшін пайдаланылған материалдар, қабықшаны алу технологиясы және сапаны анықтау әдістері: пленканың pH-н анықтау, жеке өлшеу, потенциометриялық анықтау, тензиометриялық және кондуктометриялық әдістер, жұқа қабықты және газды хроматография және басқа әдістер ұсынылған. Алынған фитоқабықшаның микробқа қарсы белсенділігі In vitro жағдайында дәлелденді. Тест-микроорганизмдер ретінде *Staphylococcus aureus* ATCC 6538-p және *Escherichia coli* ATCC 8739 пайдалана отырып, агарда дискті-диффузды әдіспен зерттелді. Белсенді зат дәрілік өсімдік шикізаты *Z. bungeana* CO_2 сығындысы негізінде жасалған дәрілік қабықшаларды отандық фармацевтикалық нарыққа енгізу ауыз қуысының қабыну ауруларын емдеуге арналған кешенді фитопрепараттарын ассортиментін кеңейтеді.

Кілт сөздер: Зизифора Бунгенің CO_2-сығындысы, ауыз қуысында еритін дисперсті фитопленка, пленка құрамы, фитопленка, технологиялық режимнің параметрлері, технология, микробқа қарсы белсенділігі, тест-микроорганизмдер.

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Разработка ородисперсных пленок на основе CO_2-экстракта *Ziziphora bungeana* с антимикробной активностью

В статье представлена разработка нового лекарственного препарата в форме растворимой в полости рта (ородисперсируемой) пленки: разработка состава, технологии получения, изучение ее антимикробной активности. Актуальность разработки данной лекарственной пленки объясняется отсутствием лекарственных препаратов в форме пленок на фармацевтическом рынке Казахстана. Полученную пленку оценивали по ряду физико-химических и технологических показателей, по результатам которых был выбран оптимальный состав фитопленок. Авторами указаны использованные материалы для получения лекарственных препаратов, представлена технология их получения и методы определения качества: методы единичного измерения и потенциометрического определения pH, тензиометрический и кондуктометрический методы, тонкослойная и газовая хроматография и др. Противомикробная активность полученных фитопленок доказана *in vitro*. Тест-микроорганизмы изучали дисково-диффузионным методом в агаре. В качестве тест-микроорганизмов использовали *Staphylococcus aureus* ATCC 6538-p и *Escherichia coli* ATCC 8739. Внедрение CO_2 экстракта *Z. bungeana* из лекарственного растительного сырья в качестве активного ингредиента лекарственных пленок позволит рас-

ширить ассортимент комплексных фитопрепаратов отечественного фармацевтического рынка для лечения воспалительных заболеваний слизистой оболочки полости рта.

Ключевые слова: CO_2-экстракт зизифоры Бунге, фитопленка, диспергируемая в полости рта, состав пленки, параметры процесса технологического режима, технология, антимикробная активность, тест-микроорганизмы.

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