Ophthalmic drug delivery system based on the complex of gellan and ofloxacin

Ophthalmic drug delivery system based on the complex of gellan and ofloxacin

G.S. Tatykhanova[1,2]*, V.O. Aseyev[3], M. Vamvakaki[4], V.V. Khutoryanskiy[5,6], S.E. Kudaibergenov[1]

[1]Institute of Polymer Materials and Technology, Almaty, Kazakhstan
[2]Satbayev University, Almaty, Kazakhstan
[3]University of Helsinki, Finland
[4]University of Crete, Greece
[5]Reading School of Pharmacy, University of Reading, UK
[6]Al-Farabi Kazakh National University, Almaty, Kazakhstan
*E-mail: *gulnur-ts81@yandex.kz*

Complex formation between a natural polysaccharide – gellan and an antimicrobial drug – ofloxacin was studied in aqueous solution. Conductimetric and potentiometric titration curves revealed that gellan and ofloxacin forms a water-soluble complex of composition 2:1 mol/mol stabilized by ionic and hydrogen bonds. The formation of the gellan-ofloxacin complex was confirmed by FTIR spectroscopy, dynamic light scattering, zeta-potential and thermogravimetric analysis. The average hydrodynamic size of the complex was found 307±5 nm and its zeta-potential was negative and equal to -15 mV. Thin films of the gellan-ofloxacin complex, gelled in 0.3 wt.% of $CaCl_2$, were used to study the release kinetics of ofloxacin in distilled water and phosphate buffer. The drug release kinetics evaluated by UV-Vis spectroscopy at λ_{max} = 289 nm and calculated by the Ritger-Peppas model correspond to non-Fickian diffusion in distilled water and Case II transport (zero-order kinetics) in phosphate buffer. The cumulative release of ofloxacin from the gellan-ofloxacin films was equal to 96±2% and 36±2% in phosphate buffer and distilled water, respectively. It is expected that the gellan-ofloxacin complex is able to form *in situ* gel on the surface of the eye and to prolong the drug residence time in the tear fluid.

Keywords: gellan; ofloxacin; complexation; drug delivery.

Геллан және офлоксацин кешеніне негізделген офтальмологиялық дәрі-дәрмектерді жеткізу жүйесі

Г.С. Татыханова[1,2]*, В.О. Асеев[3], М. Вамвакаки[4], В.В. Хуторянский[5,6], С.Е. Кудайбергенов[1]

[1]Полимерлік материалдар және технологиялар институты, Алматы, Қазақстан
[2]Сәтбаев Университеті, Алматы, Қазақстан
[3]Хельсинки Университеті, Финляндия
[4]Крит Университеті, Грекия
[5]Рединг қаласының фармациялық мектебі, Рединг Университеті, Ұлыбритания
[6]Әл-Фараби атындағы Қазақ ұлттық университеті, Алматы, Қазақстан
*E-mail: *gulnur-ts81@yandex.kz*

Табиғи полисахарид – геллан мен микробқа қарсы препарат – офлоксациннің сулы ерітінділерінің арасындағы кешеннің түзілуі зерттелді. Кондуктометрлік және pH-метрлік титрлеу қисықтарының нәтижелері бойынша геллан офлоксацинмен иондық және сутектік байланыстар арқылы 2:1 моль/моль қатынаста кешен түзетіндігі анықталды. Кешеннің орташа гидродинамикалық өлшемі 307±5 нм, дзета-потенциалы теріс, яғни -15 мВ тең. Геллан-офлоксацин кешенінің түзілетінін ИК-Фурье спектроскопия, динамикалы жарықты шашыратқыш, дзета-потенциал және термогравиметриялық анализдермен расталды. Геллан-офлоксациннің өте жұқа пленкасы 0,3% $CaCl_2$ ерітіндісінің қатысында алынып, су және фосфат буферінің ерітіндісіне пленкадан офлоксациннің шығу кинетикасы зерттелді. Дәрілік заттың шығу кинетикасы УК-көрінетін спектроскопиясында $\lambda_{макс}$ = 289 нм толқын ұзындығында анықталып, Ритгер-Пеппас моделімен есептелді. Суда және фосфат буферінің ерітіндісінде дәрілік заттың шығу кинетикасы Фик заңына бағынбайды және аномальды (II жағдайда) диффузия байқалады. Геллан-офлоксацин пленкасынан офлоксациннің кумулятивті босап шығуы фосфат буферінде 96±2%, ал дистилденген суда 36±2% тең болды. Зерттеулерден күтетін нәтиже, геллан-офлоксацин кешені көз жасымен әрекеттескенде in situ гель түзіп, дәрілік заттың әсер ету мерзімін ұзартады.

Түйін сөздер: геллан; офлоксацин; комплекс түзу; дәрі-дәрмекті босату.

Лекарственная офтальмологическая форма на основе комплекса геллан-офлоксацин

Г.С. Татыханова[1,2]*, В.О. Асеев[3], М. Вамвакаки[4], В.В. Хуторянский[5,6], С.Е. Кудайбергенов[1]

[1]Институт полимерных материалов и технологий, Алматы, Казахстан
[2]Университет Сатбаева, Алматы, Казахстан
[3]Университет Хельсинки, Финляндия
[4]Университет Крит, Греция
[5]Рединская школа фармации, Университет Рединга, Великобритания
[6]Казахский национальный университет им. аль-Фараби, Алматы, Казахстан
*E-mail: *gulnur-ts81@yandex.kz*

Комплексообразование между природным полисахаридом – гелланом и антимикробным препаратом – офлоксацином изучено в водном растворе. Кривые кондуктометрического и pH-метрического титрования показали, что геллан образует с офлоксацином водорастворимый комплекс состава 2:1 моль/моль, стабилизированный ионными взаимодействиями и водородными связями. Среднегидродинамический размер комплекса составляет 307±5 нм, а его дзета-потенциал отрицателен и равен -15 мВ. Образование комплекса геллан-офлоксацин подтверждено данными ИК-Фурье спектроскопии, динамического светорассеяния, дзета-потенциала и термогравиметрического анализа. Тонкая пленка геллан-офлоксацин, приготовленная в 0,3% растворе $CaCl_2$ была использована для изучения кинетики выхода офлоксацина в воду и фосфатный буфер. Кинетика выхода лекарства, изученная методом УФ-видимой спектроскопии при $\lambda_{макс}$ = 289 нм и вычисленная по модели Ритгера-Пеппаса, в воду и фосфатный буфер соответствует нефиковской и аномальной (случай II) диффузии. Кумулятивное высвобождение офлоксацина из пленок геллан-офлоксацин соответственно равно 96±2% и 36±2% в фосфатном буфере и дистиллированной воде. Ожидается, что комплекс геллан-офлоксацин способен образовать in situ гель в слезной жидкости и пролонгировать время удержания лекарства.

Ключевые слова: геллан; офлоксацин; комплексообразование; высвобождение лекарства.

(2022) Chem Bull Kaz Nat Univ 2:4-12

CHEMICAL BULLETIN
of Kazakh National University
http://bulletin.chemistry.kz/

UDC 541.64+678.744 https://doi.org/10.15328/cb1239

Ophthalmic drug delivery system based on the complex of gellan and ofloxacin

G.S. Tatykhanova[1,2]*, V.O. Aseyev[3], M. Vamvakaki[4], V.V. Khutoryanskiy[5,6], S.E. Kudaibergenov[1]

[1]Institute of Polymer Materials and Technology, microdistrict "Atyrau 1" 3/1, 050019 Almaty, Kazakhstan
[2]Satbayev University, Satpaev str. 22a, 050013 Almaty, Kazakhstan
[3]University of Helsinki, Yliopistonkatu 4, 00100 Helsinki, Finland
[4]University of Crete, Heraklion 700 13, Greece
[5]Reading School of Pharmacy, University of Reading, Whiteknights RG6 6APUK, Reading, UK
[6]Al-Farabi Kazakh National University, al-Farabi av. 71, 050040 Almaty, Kazakhstan
*E-mail: *gulnur-ts81@yandex.kz*

1. Introduction

Over the past few decades, microbial polysaccharides have attracted the attention of researchers due to their advantageous physicochemical properties. Currently, one of the most widely studied and comprehensively described member of this group is gellan – a linear polymer consisting of a tetrasaccharide repeating unit of 1,3-linked β-D-glucose, 1,4-linked β-D-glucuronic acid, 1,4-linked β-D-glucose, and 1,4-linked α-L-rhamnose produced by *Sphingomonas elodea* [1]. The fermentative production and manufacturing of gellan at industrial scale is described in many reviews [2,3].

Due to its unique structure and beneficial properties, gellan is currently described as a potent multifunctional additive for various pharmaceutical products. Its specific gelling properties in different media has led to the development of controlled release formulations based on gellan. Various formulations have been studied including oral, ophthalmic, nasal and other [4, 5]. A recent report [4] suggests that gellan-based materials can also be used in regenerative medicine, dentistry or gene delivery. Gellan gum-based hydrogels exhibit excellent *in vivo* and *in vitro* biocompatibility [6], tunable physical mechanical and injectable properties [7-9] for application in the regeneration of cartilage [7,8], tissue engineering [10], cell encapsulation [11], nucleus pulposes regeneration [12]. Recent progress in the design of multi-functional hydrogels based on gellan gum in the context of biomedical engineering and regenerative medicine is discussed and summarized in recent reviews [4,13-15].

Gellan-based systems for the sustained delivery of ophthalmic drugs are of great interest because the sol-gel transition of gellan in response to mono- (Na^+, K^+) and bivalent (Ca^{2+}, Mg^{2+}) cations present in tear fluid makes it suitable for ophthalmic formulations as a thickening or gelling component [14, 16]. Gellan is nontoxic and not harmful for human organisms and is therefore widely used as a viscosifying additive in the food industry [17]. The most popular ocular formulation based on gellan is Timoptic XE® which is administered topically to the eye and increases the drug bioavailability by 3-4 times, while it considerably decreases the unfavorable effect in comparison with the standard solution of timolol [18,19]. The systems consisting of the model drug Gatifloxacin (0.3%) and gellan or a mixture of gellan-sodium alginate-carboxymethylcellulose exhibit mucoadhesive properties. *In vivo* experiments on ocular delivery demonstrated that the drug retention was exceeding 12 h [20]. Combination of a Gerlite®:alginate containing matrix also shows the prolonged effect *in vivo*. The rheological measurements showed that such mixtures exhibit pseudoplastic character after contact with tear liquids [21]. The viscosity of a microemulsion system containing terbinafine hydrochloride in combination with mucin confirms the interaction of gellan and mucin justifying the possibility of adhesion to biosurfaces [22]. Gellan gum and its methacrylated derivatives were tested as in situ gelling mucoadhesive formulations of pilocarpine [23]. A recent review [24] highlights the stimuli-responsive in-situ gelling systems based on gellan gum studied using *in vivo* models for glaucoma and various ocular infections. In spite of the wide applications of gellan-drug combinations in medicine,

pharmacy and biotechnology, the application of novel eye drops of prolonged action based on gellan and ofloxacin in ophthalmology was not described in the literature yet. Only one information that we have found is ofloxacin loaded gellan/poly(vinyl alcohol) nanofibers possessing gastroretentive/mucoadhesive drug delivery potential [25].

The present communication describes the complexation of gellan with ofloxacin to develop prolonged dosage eye drops by the immobilization of the antibacterial drug – ofloxacin within a matrix of biocompatible and biodegradable natural polymer – gellan in the form of thin film. It is expected that the eye drops consisting of gellan and ofloxacin will form a thin gel-like film on the eye surface due to the presence of inorganic ions in tear fluid and will provide delivery of ofloxacin for a definite time.

2. Experiment

2.1 Materials

Commercial low acyl gellan with the molecular weight in the range of (1-5)·10^5 Da was purchased from "Zhejiang DSM Zhongken Biotechnology Co., Ltd.", China, and used without further purification. Antimicrobial drug – ofloxacin purchased from Sigma-Aldrich (UK) was used as received. Reagent grade phosphate buffer with pH 7.4 was purchased from Sigma-Aldrich (UK).

2.2 Methods

FTIR spectra of samples were recorded using Carry 660 (Agilent, USA) using dried samples. The thermal characteristics of samples were determined using TGA «Labsys EVO» Setaram (France). The average hydrodynamic sizes and ζ-potentials were measured using Malvern Zetasizer Nano ZS90 (UK). Absorption spectra were registered by UV-Vis spectroscopy (Specord 210 plus BU, Germany). Conductimetric and pH-metric titrations were carried out on Modular conductivity meter 856 Conductivity Module Metrohm and Automatic Titrator 905 Titrando Metrohm (Switzerland). Mini-magnetic stirrer IKA Topolino (Germany) was used for gentle stirring of the solution (around of 50 rpm) in order not to damage the hydrogel film.

2.3 Preparation of gellan-ofloxacin films

Gellan-ofloxacin films were prepared by mixing of a 10 mL 10^{-3} mol·L^{-1} gellan solution and 5 mL 10^{-3} mol·L^{-1} ofloxacin (2:1 mol/mol) and adding 1 mL 0.3 wt.% $CaCl_2$ to this mixture to achieve a uniform dispersion and gelation. Casting of the fluid gel into Petri dishes and allowing it to stand at room temperature and afterwards drying for 24 h, leads to the formation of an insoluble in water gellan-ofloxacin flexible film. Circular pieces of each film, 4.0±0.2 mm in diameter and 0.5±0.1 mm thick, were cut with a cork borer and were used for drug delivery studies. Each piece of weighed film was approximately equal to 10±1 mg.

2.4 Evaluation of release rate of ofloxacin from gellan-ofloxacin films

The release rate of ofloxacin from the gellan-ofloxacin films was studied as follows. Small pieces of gellan-ofloxacin films (d = 4.0±0.2 mm, h = 0.5±0.1 mm) were placed in glass vials at room temperature (25±1°C) and soaked in either 8 mL distilled water or phosphate buffer under gentle stirring (around of 50 rpm) in order not to damage the hydrogel film [26]. From time to time 2 mL of the solution were taken to measure the absorbance of ofloxacin at 289 nm. These experiments were performed under sink conditions and to keep the volume of the solution constant, the 2 mL solution taken for UV-Vis measurement were compensated by adding 2 mL of distilled water or phosphate buffer, respectively [25]. The accumulative release was calculated by formula [27]:

$$\text{Cumulative percentage release, \%} = \\ = \frac{\text{Volume of sample withdrawn, mL}}{\text{Bath volume, mL}} \cdot P_{t-1} + P_t,$$

where P_t is the percentage release at time t; P_{t-1} is the percentage release previous to "t"

and the results were shown as mean ± SD. The cumulative release of ofloxacin is the total amount (in percentage) of ofloxacin released from the gel matrix during the full time of experiments until the full or partly release of drug will be reached.

3. Results and Discussion

Addition of an aqueous solution of ofloxacin to an aqueous solution of gellan leads to the decrease in the electrical conductivity and pH of the system due to the formation of both ionic and hydrogen bonds between the two components (Figure 1). Partial neutralization of glucuronic acid of gellan by tertiary amine groups of ofloxacin and formation of hydrogen bonds with participation of carboxylic groups of drug and polymer chain is responsible for sharp decrease in conductivity and gradually decrease the pH of the solution. After formation of gellan-ofloxacin complex of definite composition, the excess of drug molecules significantly increases the conductivity and slightly decreases pH of the system. Such phenomenon is usually specific for interpolymer or polymer-drug complexes stabilized by ionic and hydrogen bonds or both [28,29]

The composition of gellan-ofloxacin determined from the minimum and the bend in the conductimetric and pH-metric titration curves was approximately equal to [gellan]:[ofloxacin] = 2:1 mol/mol. Taking into account that the molar composition of gellan-ofloxacin determined by the conductimetric and pH-metric titration curves is close to 2:1 mol/mol, the speculative structure of the complex can be represented as shown in Figure 2.

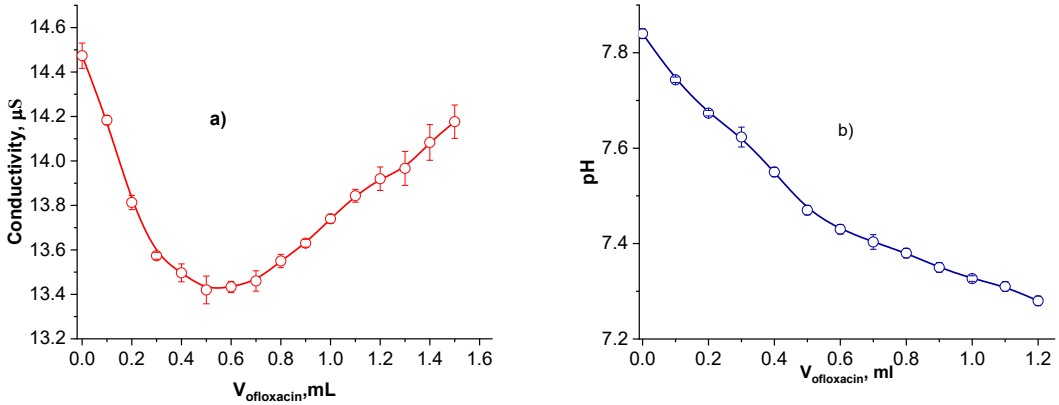

Figure 1 – Conductimetric (a) and pH-metric (b) titration curves of gellan with ofloxacin. [Gellan] = $1 \cdot 10^{-4}$ mol·L^{-1}, [Ofloxacin] = $1 \cdot 10^{-3}$ mol·L^{-1}. The volume of gellan solution used for the titration is 10 mL.

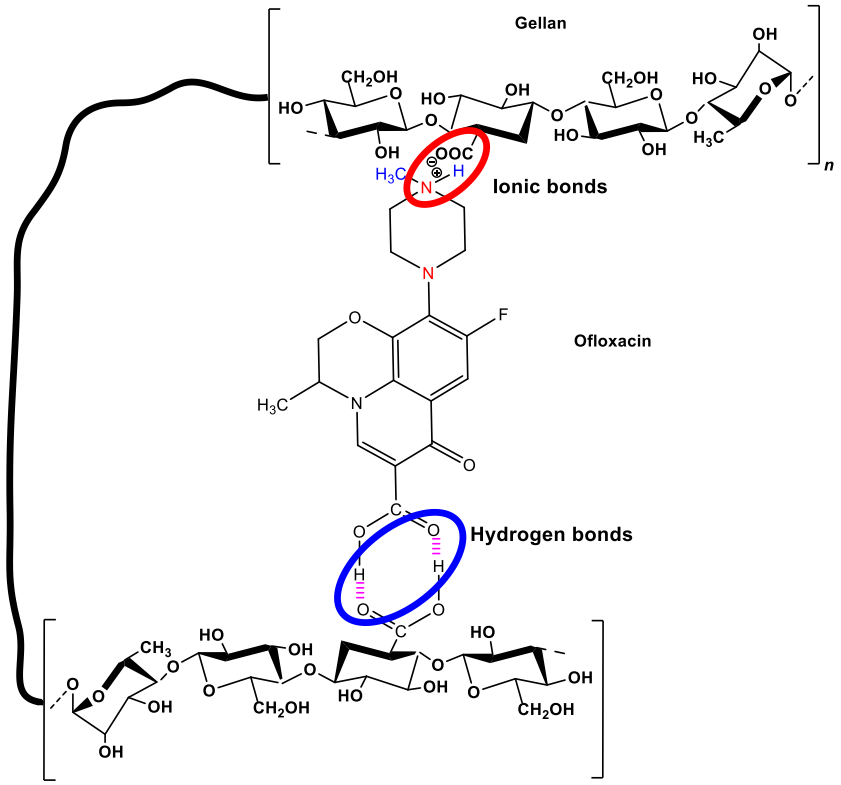

Figure 2 – Schematic representation of the intra-macromolecular gellan-ofloxacin complex of composition 2:1 mol/mol

It should be noted that ofloxacin can be involved into both intra-macromolecular and inter-macromolecular complexation with both hydroxyl and carboxyl groups of gellan. Formation of a gellan-ofloxacin complex of composition 2:1 mol/mol is also confirmed by DLS measurements (Table 1).

The minimal values of average hydrodynamic size and zeta-potential of the complex gellan-ofloxacin confirm the composition of 2:1 mol/mol. The negative charges of gellan, ofloxacin and the complex particles is due to the presence of carboxylic groups in the structure of all substances. The minimum value of zeta-potential (− 14.9 mV) in case of the gellan-ofloxacin complex 2:1 mol/mol is probably related to the partial neutralization of the carboxylic groups of gellan by the amine groups of ofloxacin and the involvement of the carboxylic groups of gellan and ofloxacin in the formation of hydrogen bonds as schematically shown in Figure 2.

Table 1 – The average hydrodynamic size and zeta-potential of gellan, ofloxacin and a mixture of gellan-ofloxacin of different composition

Composition of gellan-ofloxacin, mol/mol	Average hydrodynamic size, nm	Zeta-potential, mV
4:1	336±5	-43.0±2
2:1	307±5	-15.0±1
1:1	315±5	-40.0±2
1:2	338±4	-21.0±5
1:4	370±2	-25.0±5
Gellan	605±10	-41.0±2
Ofloxacin	187±3	1.0±3

The FTIR spectra of dry gellan, ofloxacin and the complex of gellan-ofloxacin (2:1 mol/mol) are shown in Figure 3 together with the identification of some characteristic bands (Table 2).

As revealed from the FTIR spectra, the intensive peak at 1712 cm^{-1} disappears in the complex of gellan-ofloxacin because of the hydrogen bonding of the carboxylic groups of ofloxacin with the hydroxyl or carboxyl groups of gellan. In addition, the C-N stretching vibration of ofloxacin at 1549 and 1522 cm^{-1} are shifted to 1537 cm^{-1} confirming the involvement of the amine groups of ofloxacin in the formation of ionic bonds.

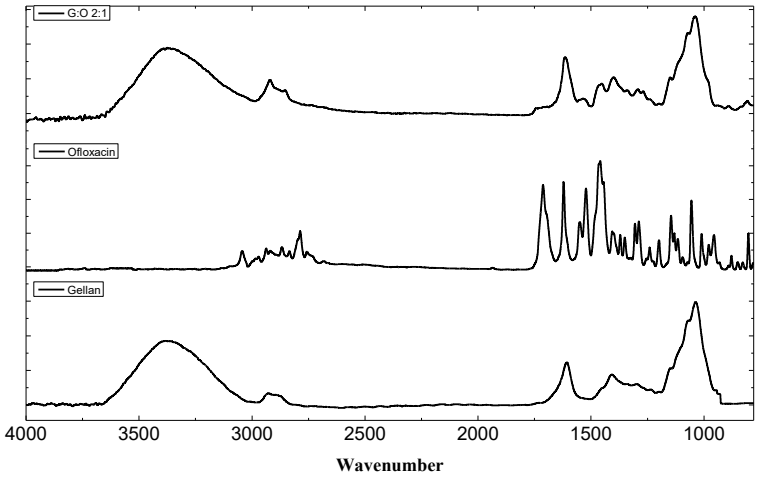

Figure 3 – FTIR spectra of dry gellan, ofloxacin and the gellan-ofloxacin complex of composition 2:1 mol/mol

Table 2 – Identification of the characteristic bands of gellan, ofloxacin and the gellan-ofloxacin complex (2:1 mol/mol)

Wavenumber, cm^{-1}			
Gellan	Ofloxacin	Gellan-ofloxacin complex (2:1 mol/mol)	Band assignments
3369	-	3376	OH stretching
2928	3044-2786	2920, 2857	CH stretching
-	1712	-	C=O stretching of COOH groups
1607	1621	1615	COO^- stretching
-	1549, 1522	1537	C-N stretching
	1459	1453	C-C stretching in benzene ring
1408	1406	1399	CH deformation
1037	-	1039	C-O-C stretching

DTA curves of the individual components and the complex are shown in Figure 4. Decomposition of the gellan backbone takes place between 200 and 300°C. The sharpest weight loss is observed at 249°C that coincides well with the literature [16] which reports the thermal decomposition of ofloxacin at 243°C. In the case of the gellan-ofloxacin complex (2:1 mol/mol) the decomposition peaks of the individual components (gellan and ofloxacin) fully disappear demonstrating a weight loss for the complex at 338 and 379°C. This is related to the formation of the gellan-ofloxacin complex as the sole compound.

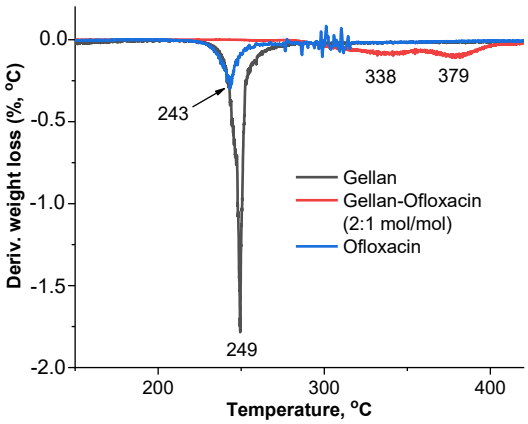

Figure 4 – DTA curves of gellan, ofloxacin and the gellan-ofloxacin (2:1 mol/mol) complex

The release kinetics of ofloxacin from the gellan-ofloxacin thin films, gelled by addition of 0.3 wt.% $CaCl_2$, were evaluated using UV-Vis spectroscopy by detecting the time dependent absorption spectra of ofloxacin at λ = 289 nm in distilled water and phosphate buffer at room temperature (Figure 5).

As seen from Figure 6 the release kinetics of ofloxacin from the gellan-ofloxacin (2:1 mol/mol) thin films into distilled water and phosphate buffer is different. During 30-40 min, the

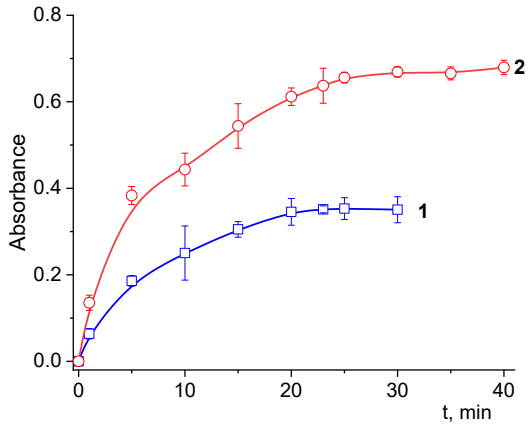

Figure 6 – Release kinetics of ofloxacin from the gellan-ofloxacin thin films, gelled by the addition of 0.3 wt.% $CaCl_2$, into distilled water (1) and phosphate buffer (2) at 25±1°C

release of ofloxacin in phosphate buffer is 2 times faster compared to that in distilled water; this is related to the presence of inorganic ions in the buffer solution that reduce the strength of the polymer-drug ionic bonds. Calculation of the release kinetics using the Ritger-Peppas model [30] expressed as $A_t/A_\infty = kt^n$ (where A_t is the absorbance of ofloxacin at time t, A_∞ is the absorbance of ofloxacin at infinite time t_∞, k is a structural/geometric constant for a gelled film, n is the release exponent representing the release mechanism) corresponds to non-Fickian diffusion (n = 0.59) in distilled water and Case II transport (n = 0.95) in phosphate buffer. The percentage release of ofloxacin into distilled water after 30 min and phosphate buffer after 40 min was leveled off at ≈ 12±1% and ≈ 4±0.5% respectively (Figure 7). The cumulative release of ofloxacin from the gellan-ofloxacin films calculated by procedure [27] is equal to 36±2% and 96±2% into distilled water and buffer solution respectively (Figure 8). In the case of distilled water, the release of ofloxacin may be influenced by the hydration and swelling of the gellan film, the penetration of water into the gel matrix

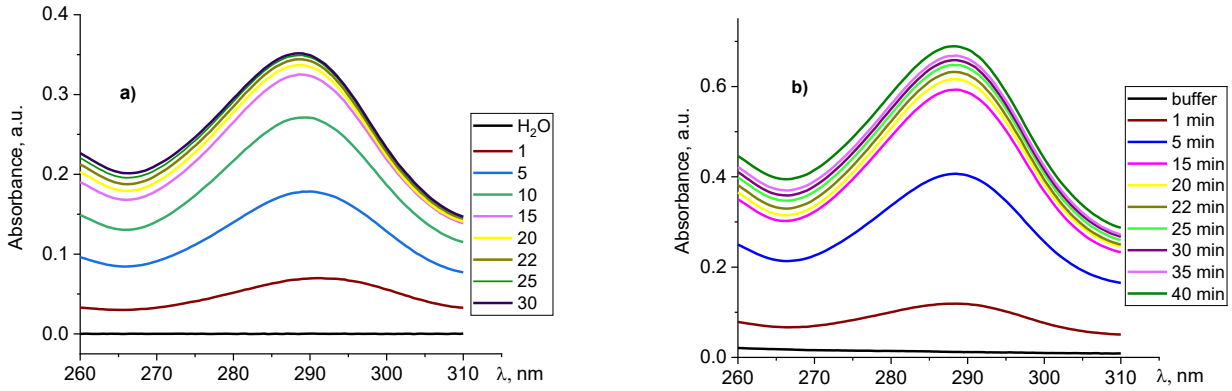

Figure 5 – Time-dependent absorbance of ofloxacin from the gellan-ofloxacin (2:1 mol/mol) thin films gelled by the addition of 0.3 wt.% $CaCl_2$ into distilled water (a) and phosphate buffer (b) at 25±1°C

resulting in the passive diffusion of ofloxacin from the gel matrix. The faster release of ofloxacin into the buffer solution was attributed to the destruction of the ionic bonds, formed between the carboxylic groups of gellan and the amine groups of ofloxacin, by the ionic species of the solution, which is accompanied by an enhanced diffusion of ofloxacin from the gel matrix.

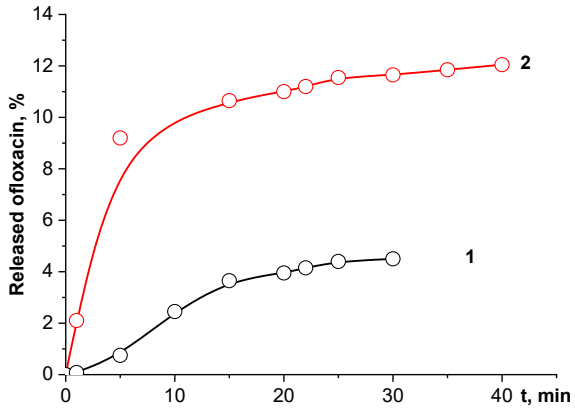

Figure 7 – Percentage of released ofloxacin into distilled water (1) and phosphate buffer (2) from the gellan-ofloxacin thin films, gelled by the addition of 0.3 wt.% $CaCl_2$ at 25±1°C

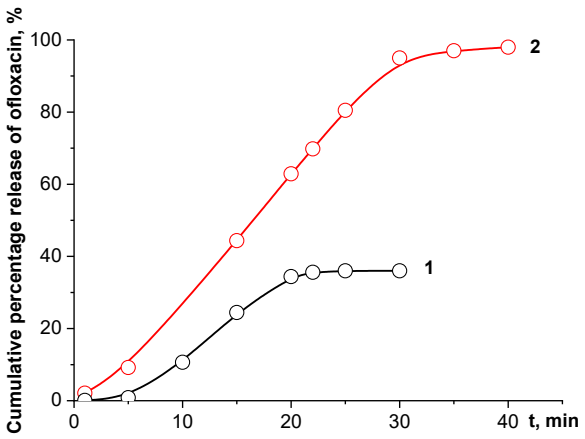

Figure 8 – Cumulative percentage release of ofloxacin from gellan gel matrix into distilled water (1) and phosphate buffer (2) from the gellan-ofloxacin thin films, gelled by the addition of 0.3 wt.% $CaCl_2$ at 25±1°C

Authors [31] have studied in vitro release of covalently bounded and physically incorporated methylprednisolone (MP) from the gellan film and found that approximately 75% and 95% of MP is released into phosphate buffer at 32°C during 24 h. The gellan-MP films exhibited zero-order (or Case II transport) release kinetics with n=0.93. Since the gellan-MP film contains esterified MP, its release involves penetration of water into the matrix, swelling of the matrix, hydrolysis of the covalent ester bonds and diffusion of the drug through the matrix. The gellan film with physically entrapped methylprednisolone exhibited anomalous release kinetics with n = 0.7. The release mechanism of physically entrapped MP from the gellan gel matrix is explained by hydration and swelling of the network, penetration of water and diffusion of drug to the outer solution. The behavior of the gellan-ofloxacin system studied by us is also similar to the results of above authors.

4. Conclusion

A water-soluble gellan-ofloxacin complex of composition 2:1 mol/mol is stabilized by both ionic and hydrogen bonds, formed by the carboxylic, hydroxyl and amine groups of the two interacting components. The formation of the gellan-ofloxacin complex is confirmed by conductimetric and potentiometric titrations, DLS and zeta-potential measurements in addition to FTIR spectroscopy and TG analysis. The gellan-ofloxacin complex is gelled upon addition of 0.3 wt.% $CaCl_2$ and forms thin gel films. The release kinetics of ofloxacin from the gellan-ofloxacin complex were described by the Ritger-Peppas model ($A_t/A_\infty = kt^n$) and corresponds to non-Fickian diffusion (n = 0.59) in distilled water and Case II transport (n = 0.95) in phosphate buffer. The amount of cumulative ofloxacin release from the gellan-ofloxacin films was 96±2% in phosphate buffer and 36±2% in distilled water. To assess the effectiveness of the gellan-ofloxacin system as a sustainable drug delivery system, experiments, both *in vitro* and *in vivo*, should be carried out in tear fluids at various temperatures, and pH values of the medium.

Acknowledgments

This research was supported by the Horizon 2020 research and innovation program of the European Union Maria Sklodowska-Curie (grant agreement 823883-NanoPol-MSCA-RISE-2018) and was funded by the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan (Grant No. AP13067773).

References (GOST)

1 Morris E.R., Katsuyoshi N., Rinaudo M. Gelation of gellan. – A review // Food Hydrocolloids. – 2012. – Vol.28, Is.2. – P.373-411.

2 Bajaj I.B., Survase S.A., Saudagar P.S., Singhal R.S. Gellan gum: Fermentative production, downstream processing and applications // Food Technol Biotechnol. – 2007. – Vol.45, Is.4. – P.341-354.

3 Giavasis I., Harvey L.M., Neil Mc. B. Gellan gum // Critical Reviews in Biotechnology. – 2000. – Vol.20. – P.177-211.

4 Osmalek T., Froelich A., Tasarek S. Application of gellan

gum in pharmacy and medicine // International Journal of Pharmaceutics. – 2014. – Vol.466, Is.1-2. – P.328-340.

5 Gan L., Gan Y., Zhu X., Zhu J. Novel microemulsion in situ electrolyte–triggered system for ophthalmic delivery of lipophilic cyclosporine A: In vitro and in vivo results // International Journal of Pharmaceutics. – 2009. – Vol.365, Is.1-2. – P.143-149.

6 Correa S. J., Zavan B., Vindigni V., Silva T.H., Oliveira J.M., et al. Biocompatibility evaluation of ionic- and photo-crosslinked methacryalted gellan gum hydrogels: In vivo and in vitro study // Advanced Healthcare Materials. – 2013. – Vol.2, Is.4. – P.568-575.

7 Gong J., Wang C., Lai R.C., Su K., Zhang K., et al. An improved injectable polysaccharide hydrogel: Modified gellan gum for long–term cartilage regeneration in vitro // Journal of Materials Chemistry. – 2009. – Vol.19. – P.1968-1977.

8 Oliveira J.T., Gardel L.S., Rada T., Martins L., Gomes M.E., et al. Injectable gellan gum hydrogels with autologous cells for the treatment of rabbit articular cartilage defects // Journal of Orthopaedic Research. – 2010. – Vol.28, Is.9. – P.1193-1199.

9 Coutinho D.F., Sant S., Shin H., Oliveira J.T., et al. Modified gellan gum hydrogels with tunable physical and mechanical properties // Biomaterials. – 2010. – Vol.31, Is.2. – P.7494-7502.

10 Correa S.J., Oliveira J.M., Caridade S.G., Oliveira J.T., Sousa R.A., et al. Gellan gum–based hydrogels for intervertebral disc tissue engineering applications // Tissue Engineering and Regenerative Medicine. – 2011. – Vol.5, Is.6. – P.e97-e107.

11 Tsaryk R., Correia S. J., Oliveira J.M., Unger R.E., Landes C., et al. Biological performance of cell-encapsulated methacrylated gellan gum-based hydrogels for nucleus pulposes regeneration // Tissue Engineering and Regenerative Medicine. – 2017. – Vol.11, Is.3. – Vol.637-648.

12 Correa S. J., Goncalves M.V., Salgado A.J., Sousa N., Oliveira J.M., et al. Angiogenic potential of gellan-gum-based hydrogels for application nucleus pulposes regeneration: In vivo study // Tissue Engineering and Regenerative Medicine. Part A. – 2012. – Vol.18, Is.11-12. – P.1203-1212.

13 Hacker M.C., Nawaz H.A. Multi–functional macromers for hydrogel design in biomedical and regenerative medicine // International Journal of Molecular Sciences. – 2015. – Vol.16, Is.11. – P.27677-27706.

14 Tatykhanova G.S., Aseyev V.O., Kudaibergenov S.E. Mucoadhesive properties of gellan and its modified derivatives // Review Journal of Chemistry. – 2020, –Vol.10, Is.3-4. – P.140-157.

15 Kudaibergenov S.E., Nurakhmetova Zh.A., Tatykhanova G.S. Immobilized anticancer agents and metal nanoparticles in a matrix of gellan: achievements and prospects // Chemical Bulletin of Kazakh National University. – 2020. – Vol.99, Is.4. –P.32-41.

16 Lavikainen J., Dauletbekova M., Toleutay G., Kaliva M., Chatzinikolaidou M., et al. Poly(2–ethyl–2–oxazoline) grafted gellan gum for potential application in transmucosal drug delivery // Polymers for Advanced Technologies. – 2021. – Vol.32, Is.7. – P.2770-2780.

17 Singh S.R., Carreiro S.T., Chu J., Niesman M.R, Collette W.W., Younis H.S., Sartnurak S., Gukasyan H.J. L–Carnosine: multifunctional dipeptide buffer for sustained-duration topical ophthalmic formulations // Journal of Pharmacy and Pharmacology. – 2009. – Vol.61, Is.6. – P.733-742.

18 Rozier A., Mazuel C., Grove J., Plazonnet B. Gelrite: a novel, ion–activated, in situ gelling polymer for ophthalmic vehicles – effect on bioavailability of timolol // International Journal of Pharmaceutics. – 1989. – Vol.57. – P.163-168.

19 Arthur Sh.H., Laurence J., Barrish A., et al. Plasma timolol concentrations of timolol maleate: Timolol gel–forming solution (TIMOPTIC–XE(R)) once daily versus timolol maleate ophthalmic solution twice daily // Documenta Ophthalmologica. – 2001. – Vol.103, Is.1. – P.73-79.

20 Kesavan K., Nath G., Pandit J. K. Preparation and in vitro antibacterial evaluation of gatifloxacin mucoadhesive gellan system // DARU Journal of Pharmaceutical Sciences. – 2010. – Vol.18, Is.4. – P.237-246.

21 Liu Y., Liu J., Zhang X., et al. In situ gelling Gelrite/Alginate Formulations as Vehicles for ophthalmic drug delivery // AAPS Pharmscitech. – 2010. – Vol.11, Is.2. – P.610-620.

22 Tayel S.Ah., El-Nabarawi M.Ah., Tadros M.I., et al. Promising ion–sensitive in situ ocular nanoemulsion gels of terbinafine hydrochloride: Design, in vitro characterization and in vivo estimation of the ocular irritation and drug pharmacokinetics in the aqueous humor of rabbits // International Journal Pharmaceutics. – 2012. – Vol.443, Is.1-2. – P.293-305.

23 Agibayeva L.E., Kaldybekov D.B., Porfiryeva N.N., Garipova V.R., Mangazbayeva R. A., Moustafine R. I., Semina I. I., Mun G. A., Kudaibergenov S. E., Khutoryanskiy V. V. Gellan gum and its methacrylated derivatives as in situ gelling mucoadhesive formulations of pilocarpine // International Journal of Pharmaceutics. – 2020. – Vol.577. – P.119093.

24 Pandey M., Choudhury H., Azila binti Abd Aziz A., Bhattamisra S.K., Gorain B., Su J.S.T., Tan C.L., Chin W.Y., Yip K.Y. Potential of stimuli-responsive in situ gel system for sustained ocular drug delivery: Recent progress and contemporary research // Polymers. – 2021. –Vol.13. – P.1340.

25 Vashisth P., Raghuwanshi N., Srivastava A.K., Singh H., Nagar H., Pruthi V. Ofloxacin loaded gellan/PVA nanofibers – synthesis, characterization and evaluation of their gastroretentive/mucoadhesive drug delivery potential // Materials Science and Engineering: C. – 2017. –Vol.71. – P.611.

26 Zhu L., Ao J., Li P. A novel in situ gel base of deacetylase gellan gum for sustained ophthalmic drug delivery of ketotifen: in vitro and in vivo evaluation // Drug Design, Development and Therapy – 2015. – Vol.9. – P.3943-3949.

27 Chandrasekaran A. R., Jia Ch. Y., Theng Ch. Sh., Muniandy T., Muralidharan S., Dhanaraj S. A. In vitro studies and evaluation of metformin marketed tablets–Malaysia // Journal of Applied Pharmaceutical Science. –2011. – Vol.1, Is.5. – P.214-217.

28 Bekturov E.A., Bimendina L.A. Interpolymer Complexes // Adv. Polym. Sci. – 1981. – Vol.45. –P.1-119.

29 Bekturov E.A., Legkunetz R.E. Association of polymers with small molecules. – Nauka, Alma-Ata, 1982. – 207 pp.

30 Ritger P.L., Peppas N.A. A simple equation for description of solute release I. Fickian and non–fickian release from non–swellable devices in the form of slabs, spheres, cylinders or discs // Journal of Controlled Release. –1987. – Vol.5. – P.23-36.
31 Sanzgiri Y.D., Maschi S., Crescenzi V., Callegaro L., Topp E. M., Stella V.J. Gellan–based systems for ophthalmic sustained delivery of methylprednisolone // Journal of Controlled Release –1993. – Vol.26. – P.195-201.

References

1 Morris ER, Katsuyoshi N, Rinaudo M (2012) Food Hydrocolloids 28:373-411. *https://doi.org/10.1016/j.foodhyd.2012.01.004*
2 Bajaj IB, Survase SA, Saudagar PS, Singhal RS (2007) Food Technol Biotechnol 45:341-354.
3 Giavasis I, Harvey LM, Neil McB (2000) Crit Rev Biotechnol 20:177-211. *https://doi.org/10.1080/07388550008984169*
4 Osmalek T, Froelich A, Tasarek S (2014) Int J Pharm 466:328-340. *https://doi.org/10.1016/j.ijpharm.2014.03.038*
5 Gan L, Gan Y, Zhu X, Zhu J (2009) Int J Pharm 365:143-149. *https://doi.org/10.1016/j.ijpharm.2008.08.004*
6 Correa SJ, Zavan B, Vindigni V, Silva TH, Oliveira JM, et al (2013) Adv Healthc Mater 2:568-575. *https://doi.org/10.1002/adhm.201200256*
7 Gong J, Wang C, Lai RC, Su K, Zhang K, et al (2009) J Mater Chem 19:1968-1977. *https://doi.org/10.1039/b818090c*
8 Oliveira JT, Gardel LS, Rada T, Martins L, Gomes ME, Reis LR (2010) J Orthop Res 28:1193-1199. *https://doi.org/10.1002/jor.21114*
9 Coutinho DF, Sant S, Shin H, Oliveira JT, et al (2010) Biomaterials 31:7494-7502. *https://doi.org/10.1016/j.biomaterials.2010.06.035*
10 Correa SJ, Oliveira JM, Caridade SG, Oliveira JT, Sousa RA, et al (2011) J Tissue Eng Regen Med 5:e97-e107. *https://doi.org/10.1002/term.363*
11 Tsaryk R, Correia SJ, Oliveira JM, Unger RE, Landes C, et al (2017) J Tissue Eng Regen Med 11:637-648. *https://doi.org/10.1002/term.1959*
12 Correa SJ, Goncalves MV, Salgado AJ, Sousa N, Oliveira JM, et al (2012) Tissue Eng Part A 18:1203-1212. *https://doi.org/10.1089/ten.tea.2011.0632*
13 Hacker MC, Nawaz HA (2015) Int J Mol Sci 16:27677-27706. *https://doi.org/10.3390/ijms161126056*
14 Tatykhanova GS, Aseyev VO, Kudaibergenov SE (2020) Rev J Chem 10:140-157. *https://doi.org/10.1134/S207997802003005X*
15 Kudaibergenov SE, Nurakhmetova ZhA, Tatykhanova GS (2020) Chem Bull Kaz Nat Univ 99:32-41. *https://doi.org/10.15328/cb1169*
16 Lavikainen J, Dauletbekova M, Toleutay G, Kaliva M, Chatzinikolaidou M, et al (2021) Polym Adv Technol 32:2770-2780. *https://doi.org/10.1002/pat.5298*
17 Singh SR, Carreiro ST, Chu J, Niesman MR, Collette WW, et al (2009) J Pharmacy Pharmaco 61:733-742. *https://doi.org/10.1211/jpp.61.06.0005*
18 Rozier A, Mazuel C, Grove J, Plazonnet B (1989) Intern J Pharmaceutics 57:163-168.
19 Arthur ShH, Laurence J, Barrish A, et al (2001) Documenta Ophthalmologica 103:73-79. *https://doi.org/10.1023/A:1017962731813*
20 Kesavan K, Nath G, Pandit JK (2010) Daru J Pharm Scien18:237-246.
21 Liu Y, Liu J, Zhang X, et al (2010) AAPS Pharmscitech 11:610-620. *https://doi.org/10.1208/s12249-010-9413-0*
22 Tayel SAh, El-Nabarawi MAh, Tadros MI, Wessam HAE (2012) Int J Pharm 443:293-305. *https://doi.org/10.1016/j.ijpharm.2012.12.049*
23 Agibayeva LE, Kaldybekov DB, Porfiryeva NN, Garipova VR, Mangazbayeva RA, Moustafine RI, Semina II, Mun GA, Kudaibergenov SE, Khutoryanskiy VV (2020) Int J Pharm 577:119093. *https://doi.org/10.1016/j.ijpharm.2020.119093*
24 Pandey M, Choudhury H, Azila binti Abd Aziz A, Bhattamisra SK, Gorain B, Su JST, Tan CL, Chin WY, Yip KY (2021) Polym 13:1340. *https://doi.org/10.3390/polym13081340*
25 Vashisth P, Raghuwanshi N, Srivastava AK, Singh H, Nagar H, Pruthi V (2017) Mater Sci Eng C 71:611. *https://doi.org/10.1016/j.msec.2016.10.051*
26 Zhu L, Ao J, Li P (2015) Drug Design, Development and Therapy 9:3943. *https://doi.org/10.2147/DDDT.S87368*
27 Chandrasekaran AR, Jia CY, Theng Ch Sh, Muniandy T, Muralidharan S, Dhanaraj SA (2011) J Appl Pharm Sci 01:214 Corpus ID: 759716.
28 Bekturov EA, Bimendina LA (1981) Adv Polym Sci 45:1-119
29 Bekturov EA, Legkunetz RE (1982) Association of polymers with small molecules. Nauka, Alma-Ata, Kazakhstan.
30 Ritger PL, Peppas NA (1987) J Control Release 5:23-36.
31 Sanzgiri YD, Maschi S, Crescenzi V, Callegaro L, Topp EM, Stella VJ (1993) J Control Release 26:195-201. *https://doi.org/10.1016/0168-3659(93)90186-9*