

Angela Shurshina, Elena Kulish and Roman Lazdin

STUDY OF KINETICS OF MEDICINAL SUBSTANCES RELEASE FROM CHITOSAN FILMS

Bashkir State University,
32, Zaki Validi str., 450074 Ufa, Russia; alenakulish@rambler.ru

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Abstract. The kinetics of controlled release of medicinal substances from chitosan films has been investigated. Correlation between values of diffusion coefficients and the sedate indicator characterizing diffusion of medicinal substance from a film and shares of medicinal substance connected with a polymeric chain has been established. With the increase in amount of the entered medicinal substance the rate of release of medicines decreases.

Keywords: chitosan, medicinal substance, diffusion, the abnormal character.

1. Introduction

Lately a considerable interest to studying the diffusion phenomena in polymers has been observed. The reason is that the diffusion plays a primary role in such processes as dialysis, permeability of biomembranes and tissues, prognostication of the protective properties of polymeric coatings, *etc.* [1]. Studies of the diffusion of substances from polymer matrix are also necessary in development of medicinal polymeric films with controllable release of medicinal substances.

The aim of our study was to examine the fundamental aspects of processes of diffusion of medicinal substance in polymeric films based on a natural polysaccharide chitosan and to determine opportunities of medicinal preparations release monitoring.

2. Experimental

We used a sample of chitosan (ChT) manufactured at Bioprogress ZAO (Russia) by alkaline deacetylation of crab chitin (degree of deacetylation ~ 84 %) with $M_{sd} = 334\ 000$ (MS) antibiotics of the amino glycoside (gentamicin, GM) and cephalosporin series (cefatoxim, CFT) served as the medicinal substance.

To form chitosan acetate film samples, a 1 % polymer solution in 1 % acetic acid was poured on a glass surface. An antibiotic aqueous solution was added to the chitosan solution immediately before film formation. The drug content in a film was as high as 0.01, 0.05, or 0.1 mol/mol chitosan. Throughout all experiments, the film thickness was constant and equal to 100 μm . The film samples were subjected to isothermal annealing at 393 K for a fixed period of time.

The kinetics of drug release under thermally controlled conditions ($T = 3019\ \text{K}$) was studied *via* placement of a sample in a cell with distilled water. An antibiotic released into the aqueous phase was recorded with a spectrophotometer at a wavelength corresponding to the maximum of drug absorption in the UV region. The amount of drug G_s , released from the film up to time t was estimated from the calibration curve. The time at which a constant concentration of drug, G_∞ , was established was taken as the instant of equilibrium attainment. The weight fraction of a drug capable of diffusion, α , was estimated as the ratio of the maximum amount of the antibiotic released from a film to the amount of drug introduced in the film.

The mechanism of mass transfer was analyzed through an equation describing the kinetics of release:

$$\frac{G_s}{G_\infty} = kt^n \quad (1)$$

where G_∞ is the value of G_s at $t \rightarrow \infty$; k is a constant related to the parameters of polymer-diffusate interaction; n is a parameter characterizing the mechanism of transport of a low-molecular mass substance in the film. Exponent n in Eq. (1) was found from the tangent of the slope of the $\ln(G_s/G_\infty) - \ln t$ dependence.

The interaction of MS with ChT was studied by IR and UV spectroscopy. The IR spectra of the samples were recorded with a Shimadzu spectrometer (KBr pellets, films) in the spectral range of 700–3600 cm^{-1} . UV spectra of all the samples were recorded in 1 cm thick quartz

cuvettes relative to water with a Specord M-40 spectrophotometer in the range of 220–350 nm.

To determine the weight fraction of a drug bound to a polymer matrix β , the products of the interaction of chitosan with the antibiotic were isolated *via* double precipitation from a solution in acetic acid into a NaOH solution followed by washing of the precipitate with ethanol. The precipitate was dried up to a constant weight. The content of the drug bound with the chitosan matrix was determined from the data of elemental analysis (for sulfur) on a EUKO EA-3000 analyzer.

3. Results and Discussion

Now the established fact that at controlled release of MS from polymeric systems diffusion processes dominate is standard.

Fig. 1 shows typical experimental curves of CFT release from chitosan films with different drug contents. All kinetic curves level off with a clearly defined limit value corresponding to equilibrium drug yield G_∞ .

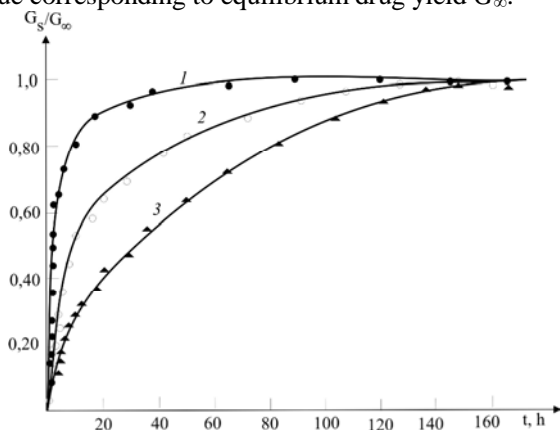


Fig. 1. Kinetic curves of drug release for ChT-CFT films with CFT contents of 0.01 (1), 0.05 (2) and 0.1 (3) mol/mol chitosan. The isothermal annealing time is 30 min

The diffusion coefficients of low-molecular mass components in films were determined with the use of Fick's second law [2]:

$$\frac{\partial c_s}{\partial t} = D_s \frac{\partial^2 c_s}{\partial x^2} \quad (2)$$

The solutions to the above equation for long ($G_s/G_\infty > 0.5$) and short ($G_s/G_\infty \leq 0.5$) experiments have dissimilar forms.

$$\text{At } G_s/G_\infty \leq 0.5 \quad \frac{G_s}{G_\infty} = \left[\frac{16D_s t}{pL^2} \right]^{0.5} \quad (3a)$$

$$\text{and at } G_s/G_\infty > 0.5 \quad \frac{G_s}{G_\infty} = 1 - [8/p^2 \exp(-\frac{p^2 D_s t}{L^2})] \quad (3b)$$

where $G_s(t)$ – concentration of the desorbed substance at time t and $G_\infty - G_s$ value at $t \rightarrow \infty$; L – thickness of the film sample.

In case of the molecular diffusion submitting to the classical Fick's equation, it is necessary to expect equality of coefficients of $D_s^a = D_s^b$ [3]. However, as can be seen from the data presented in Table 1, for all analyzed cases, value of diffusion coefficients calculated at initial and final stage of diffusion do not coincide.

It indicates to a deviation of the mechanism of diffusion from classical type and allows assuming the so-called pseudo-normal mechanism of diffusion of MS from a chitosan matrix.

The type of the kinetic curves constructed in coordinates of $G_s/G_\infty - t^{1/2}$ (Fig. 2) also testifies to pseudo-normal type of diffusion of MS. In case of simple diffusion, dependence of release of MS from film samples in coordinates $G_s/G_\infty - t^{1/2}$ would have to be straightened at all times of experiment. However, as can be seen from Fig. 2, the linear site is observed only at $G_s/G_\infty < 0.5$; then the rate of release of an antibiotic significantly decreases.

Table 1

Parameters of cefatoxim, gentamicin desorption from chitosan films

Film composition	Drug concentration in film, mol/mol ChT	Annealing time, min	$D_s^a \cdot 10^{11}, \text{cm}^2/\text{s}$	$D_s^b \cdot 10^{11}, \text{cm}^2/\text{s}$	n	α
ChT-GM	1:0.01	30	25.30	1.99	0.31	0.97
		60	23.70	1.92	0.24	0.92
		120	22.00	1.50	0.21	0.88
	1:0.05	30	24.60	1.87	0.20	0.84
ChT-CFT	1:0.01	30	23.50	1.59	0.16	0.73
		30	91.40	6.46	0.38	0.97
		60	83.90	5.43	0.36	0.95
	120	77.60	5.18	0.31	0.94	
	1:0.05	30	56.80	6.13	0.32	0.94
1:0.1	30	40.90	5.79	0.28	0.92	

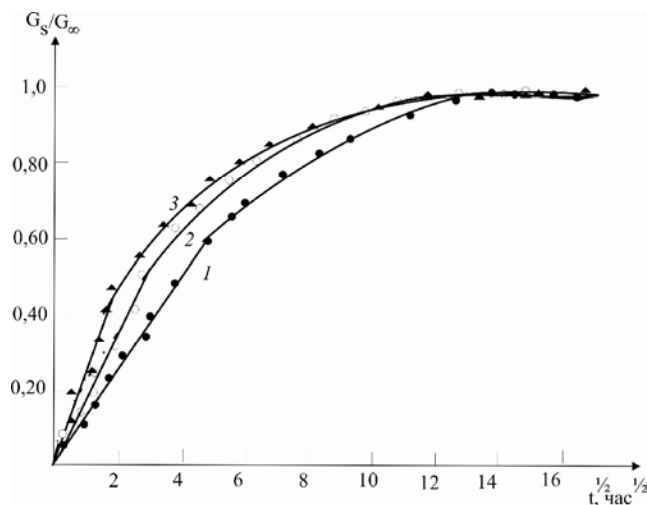


Fig. 2. Kinetic curves of drug release for ChT-GM films with GM contents of 0.01 (1), 0.05 (2) and 0.1 (3) mol/mol chitosan. The isothermal annealing time is 30 min

At MS diffusion from films abnormally low values of the parameter n estimated on a tangent of angle of an inclination in coordinates of $\ln(G_s/G_\infty) - \ln t$ take place. Increases in the drug concentration and isothermal annealing time are accompanied by an additional decrease in n . The change in α value is symbate to that of n .

All the specific features inherent in the anomalous (non-Fick's) diffusion are well described in terms of the relaxation model [4]. In contrast to the Fick's diffusion, in which an instantaneous attainment of the surface concentration of the sorbate and its change is assumed, the relaxation model presumes variation of the concentration in the surface layer (*i.e.*, variable boundary conditions) in accordance with the first-order equation [5]. One of the main reasons causing change of boundary conditions is called non-equilibrium of the structural-morphological organization of a polymeric matrix [4]. It is noteworthy that the anomalously small values of n have also been observed by other researchers and attributed to the strong interaction of the MS with the polymer [6].

The structure of the medicinal substances used in the study suggest that they interact with ChT to give, *e.g.*, ChT-antibiotic complexes *via* hydrogen bonds and/or polymeric salts formed as a result of an exchange interaction. The interaction of antibiotics with ChT is evidenced by UV spectroscopic data. The maximum absorption of CFT at its concentration of 10^{-5} M in 1 % acetic acid is observed at 261 nm. Upon addition of an equivalent amount of ChT to the solution, the intensity of the absorption peak of the medicinal preparation noticeably grows and its position is bathochromically shifted by approximately 5–10 nm. The UV spectrum of GM at a concentration of 10^{-2} M in 1 % acetic acid shows an absorption peak at 286 nm. Addition of ChT solution to

the GM solution results in precipitate formation; however, analysis of the supernatant fluid shows that the peak of the corresponding absorption band in its spectrum is shifted by 5–7 nm. The observed changes unambiguously indicate that ChT affects the electron system of MS and adducts are formed. The binding energies in the complexes, evaluated by the shift of the absorption peaks in the UV spectra, are about 10 kJ mol^{-1} . This suggests that the complexation occurs *via* hydrogen bonds.

The interaction of the antibiotics with ChT is also confirmed by IR spectroscopic data. IR-spectrum of ChT has diffusion character that testifies to its polymeric nature and gives the general information on a polymer molecule. In IR-spectrum of ChT the wide strip of absorption in the field of $3700\text{--}3100 \text{ cm}^{-1}$, belonging to valent fluctuations OH- and NH- bonds (not resolved structure) is observed. Its situation and width testify to existence of intermolecular hydrogen bond with participation of these functional groups. Also the widened strip in the field of $2960\text{--}2920 \text{ cm}^{-1}$, to which valent fluctuations of CH-bonds of methylene and metilny groups get, is observed. In spectrum a strip in the field of 1593 cm^{-1} and 1647 cm^{-1} corresponding to deformation fluctuations of amino and amide group are shown. In the field of $1500\text{--}1200 \text{ cm}^{-1}$ strip with maxima are registered at 1462 cm^{-1} of deformation fluctuation of CH₂- or CH₃- groups and 1377 cm^{-1} – deformation fluctuation of OH- bonds. For area of the spectrum of $900\text{--}1200 \text{ cm}^{-1}$ the intensive strip, on which three peaks with frequencies are allocated, is characteristic: 1153 , 1074 and 1033 cm^{-1} which are possibly belonging to valent fluctuations of C-O, C-N and C-C bonds of a skeleton of a molecule.

In IR-spectrum of CFT there are strips characteristic of fluctuations of bonds of C=O in lactam group, fluctuations of bonds in C=O in carboxyl group, and fluctuations of bonds in C=O in ester at 1760 , 1728 and 1184 cm^{-1} , respectively. At a spectrum of CFT there are strips 1620 cm^{-1} , characteristic of deformation fluctuations of amino group and a strip at 1643 cm^{-1} , characteristic of fluctuations of amide group. In the field of $1384\text{--}1354 \text{ cm}^{-1}$ there are strips characteristic of fluctuations of -O-CO-CH₃ group. In the spectrum strips in the field of 1608 cm^{-1} and 1537 cm^{-1} correspond to fluctuations of -C=N- and -C(O)N- groups, respectively.

In IR-spectrum of adduct of ChT with CFT reaction there are strips at 1745 cm^{-1} , characteristic of fluctuations of bond of C=O in lactam group, and also a shoulder at 1675 cm^{-1} , characteristic of fluctuations of bond of C=O in carboxyl group, unambiguously testifying that MS is present at reaction adduct.

In comparison with ChT spectrum in spectrum of adducts of reaction there is a shift of strips of absorption, characteristic of deformation fluctuations OH- groups (by $5\text{--}7 \text{ cm}^{-1}$) and amino groups of ChT (Table 2).

Table 2

**Influence of complex formation on values of characteristic strips
of ChT absorption in IR-spectra of analyzed compounds of ChT-MS**

Analyzed bond	Shift of characteristic strip of absorption of bonds deformation fluctuations in analyzed compounds (in cm^{-1})	
	ChT-CFT	ChT-GM
N–H bond in amino group in ChT	-8	-35
C=O bond in amide group in ChT	-47	+3
OH– bonds in ChT	-6	0
C=O bond in lactam group in MS	-15	-
C=O bond in carboxyl group in MS	-53	-
N–H bond in amino group in MS	-35	-60
C=O bond in amide group in MS	-60	-

Table 3

Mass fraction β of an antibiotic in the reaction adducts obtained from 1 % acetic acid

Antibiotic	MS concentration in a film, mol/mol ChT	β
GM	1.00	0.69
	0.10	0.30
	0.05	0.20
	0.01	0.07
CFT	1.00	0.16
	0.10	0.09
	0.05	0.05
	0.01	0.02

In IR-spectrum of GM there are strips at 1618 and 1643 cm^{-1} characteristic of NH_2^- and NH^- group, respectively. Strips with maxima at 1433 cm^{-1} of deformation fluctuation of CH_3^- groups and 1377 cm^{-1} deformation fluctuation OH– bonds are registered also. Also there is a strip of absorption of high intensity at 619 cm^{-1} characteristic of SO_4^{2-} group.

In IR-spectrum of adduct of reaction of ChT-GM in comparison with a spectrum of ChT there is a strip of absorption of high intensity at 619 cm^{-1} characteristic of SO_4^{2-} group. It is possible to note that in IR-spectrum of adduct of ChT with GM there is a considerable shift of strips of absorption, characteristic of amino groups, both ChT, and MS. The strip characteristic of amino group fluctuations moves by 35 cm^{-1} .

Thus, it is possible to note that shift of characteristic strips of absorption both in MS and in ChT (Table 2) in most cases happens towards smaller values of wave numbers, which allows to speak about a complex formation of ChT taking place with MS, occurring due to hydrogen bonds with participation of amino groups of ChT, CFT carbonyl groups and GM amino groups.

Of no lesser importance for interpretation of the diffusion data are exchange interaction between ChT and MS, especially for GM. Because the sulfate-anions are dibasic, it can be assumed that two kinds of salts are formed, which provide cross-linking of ChT molecules, with loss of solubility: (i) a water-insoluble “double” salt,

ChT–GM sulfate, and (ii) a mixture of salts, water-insoluble ChT sulfate and soluble GM acetate.

In the case of CFT, the exchange reaction between ChT acetate and CFT yields soluble salts. Accordingly, the ChT–MS reaction product will be then composed of only the H-complex of ChT–CFT.

The data on the fraction β of the antibiotic bound into polymeric adducts formed in acetic acid solutions are presented in Table 3.

One can see that the ability of GM to crosslink the ChT chains results in a significantly higher amount of drug tightly bound to macromolecules than in the case of CFT.

Thus, the structural changes in the polymer matrix, including those resulting from its modification *via* the interaction with drugs, are most probably responsible for the deviations of the mechanism of the mass transfer processes from the classical Fick’s mechanism.

4. Conclusions

Via investigation of the kinetics of release of antibiotics from films, a correlation has been found between the exponent n , which characterizes the mechanism of antibiotic transport from films, and the fraction of a drug β , bounded with a polymer chain.

The values of the fraction of a drug involved in the diffusion process α and exponent n , which determines the diffusion mechanisms, decrease regularly with the isothermal annealing time.

It has been suggested that the interaction of the studied antibiotics with the polymer matrix is responsible for the deviations of the diffusion mechanism from the classical Fick's mechanism.

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ВИВЧЕННЯ КІНЕТИКИ ВИВІЛЬНЕННЯ ЛІКАРСЬКИХ РЕЧОВИН З ХІТОЗАНОВИХ ПЛІВОК

Анотація. Досліджено кінетику контрольованого вивільнення лікарських речовин з хітозанових плівок. Встановлено взаємозв'язок між значеннями коефіцієнту дифузії, показником, що характеризує режим дифузії, та часткою лікарської речовини, зв'язаної з полімерною матрицею. Показано, що зі збільшенням кількості лікарської речовини швидкість їх вивільнення зменшується.

Ключові слова: хітозан, лікарська речовина, дифузія, аномальний характер.