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CONTROLLED RELEASE STUDIES OF CALCIUM ALGINATE HYDROGELS

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Abstract. Controlled release of substances in many cases may be achieved from calcium alginate hydrogels. In this research, the time dependence of the mass of released model substance bovine serum albumin (BSA) from calcium alginate spherical hydrogels of three different types (G/M ratio) have been investigated. The hydrogels were prepared with the drop-wise method of sodium alginate aqueous solutions with concentration of 0.02 g/cm^3 with 0.01 g/cm^3 BSA and a gelling water bath of chitosan in 0.2 M CH_3COOH/0.4 M CH_3COONa with added 0.2 M $CaCl_2$.The hydrogel structures were characterized by dynamic light scattering and scanning electron microscopy. The controlled release studies were conducted by UV-Vis spectrophotometry of the released medium with pH=7 at 37 ^{0}C. The results showed that the model of osmotic pumping is the dominant mechanism of the release. Also, large dependences of the release profile on the homogeneity of the hydrogels were found.

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1. INTRODUCTION

Controlled release systems of active substances are widely used in many applications in medicine, chemical technology, food technology, etc. Although the active substances can be various, the matrix (or carrier of the active substance) is common for all of them. The type of the matrix mainly determines the effects of the controlled release. Most common phenomena that are used for choosing type of the matrix are: diffusion of the active substance through the matrix; erosion of the matrix caused by some chemical reaction; swelling of the matrix at contact with the surrounding fluid; and osmotic pumping of the active substance out of the matrix. Most important criterion that must be followed when choosing the type of the matrix is to develop a system with controlled release properties that do not depend significantly on the change of the physico-chemical conditions in the controlled release surroundings (media). At the same time the kinetics of release of the active substance should be reproducible and

predetermined [1]. When studying kinetics of controlled release of active substance from hydrogel particles of calcium alginate with chitosan membrane, three different types of controlled release systems have been investigated: reservoir with membrane, erosion type and osmotic type. Choosing the system type reservoir with membrane is based on the fact that the gel matrix of calcium alginate (CA) is wrapped in a membrane of chitosan that stays stable from the start of the release of the active substance to the manifestation of the "osmotic breaking" when the membrane is ruptured due to high internal osmotic pressure in the matrix. From the start of active substance release from CA particles with membrane of chitosan to the "osmotic breaking" of the matrix, the system becomes complicated. In between these two phases the active substances diffuse in the interior of the matrix because of the gel's erosion. The gel's erosion is caused from the fluid diffusion from the surrounding environment with ionic strength of 0.1 M NaCl or higher. When number ratio of Na^+ ions over against Ca^{2+} ions embedded to the guluronic monomers of the alginate macromolecules in the gel are less then 1:25, an exchange of the Ca^{2+} ions in the gel with the Na^+ ions occurs that destabilizes the gel structure and causes gel's dissolution (washing) [2].

The experimental investigation of the controlled release properties is based on determination of the kinetics of release, i.e. accumulative released mass of active substance in the releasing bath at different times, M_t, starting from the null time (after the moment of positioning the controlled release system into the releasing bath) and up to the moment of total dissolution (disintegration) of the release matrix. The second important quantity to investigate is the release rate, dM_t/dt – first derivative of the accumulative released mass over time t.

For the controlled release model of reservoir with membrane, when the reservoir is with definite volume V_1, and the releasing bath is considered as infinite because of the much larger volume of the reservoir, the mass of the released active substance and the release rate over time t is determined by the expressions [3]:

$$M_t = M_1\left[1 - e^{-\frac{A\cdot D_m K}{\delta\cdot V_1}t}\right], \tag{1}$$

$$\left(\frac{dM_t}{dt}\right)_r = \frac{M_1\cdot A\cdot D_m\cdot K}{\delta\cdot V_1}\cdot e^{-\frac{A\cdot D_m K}{\delta\cdot V_1}t}, \tag{2}$$

where M_1 is a mass of the active substance of the reservoir.

For the erosion model of controlled release of calcium alginate gel particles with radius a with chitosan membrane in large volume of releasing bath (infinite volume), the dependence of the released mass M_t of active substance over time t is given according to relation [3]:

$$\frac{M_t}{M_\infty} = 1 - \frac{6}{\pi}\sum\frac{1}{n^2}\cdot e^{-\frac{D_a n^2\pi^2}{a^2}t} \tag{3}$$

where M_∞ is a released mass at time of total erosion of the gel matrix, D_a is a diffusion coefficient of the active substance in the dissolved gel. In Eq (3), number of the summing parts

n used for calculation is usually taken to a value at which there is no significant divergence between the calculated at measured quantities M_t / M_∞ . For the osmotic pumping model for system with constant volume, the volume rate of release of active substance in releasing bath is equal to the volume rate of diffusion of water into the gel system. The dependence of the released mass M_t of active substance over time *t* for this model is given according to relation [3]:

$$\left(\frac{dM_t}{dt}\right)_r = \sigma RTA\frac{L_p}{\delta}C_a^2, \tag{4}$$

where σ - coefficient of fluid reflectivity of the gel membrane (equal to 1 for ideal semipermeabile membrane and equal to 0 for nonporous membrane), R – universal gas constant, T – temperature, A – surface area of membrane pores, L_p – coefficient of hydraulic membrane's permeability, δ - membrane thickness, C_a – active substance concentration in the gel matrix.

2. EXPERIMENTS

A bovine serum albumin (BSA) was used as an active substance in this research. The purpose of the research was to investigate the influence of the inhomogenity of the internal structure of calcium alginate gel particles with chitosan membrane on the controlled release properties. Three different types of sodium alginate were used in this research: type LF120M with weight-average molecular weight of $M_w = 671 \cdot 10^3$ g/mol and G/M ratio of 50/50, type LF200M with $M_w = 687 \cdot 10^3$ g/mol and G/M ratio of 55/45 and LF240D with $M_w = 497 \cdot 10^3$ g/mol and G/M ratio of 70/30. The gel spherical particles were obtained by the drop-wise method [4,5]. For each type of alginate the procedure for obtaining gel particles with membrane is the same as the following. The gelling solution of sodium alginate has a concentration of 2 % w/w in double distilled and deionized water in which BSA with concentration of 1 % w/w was added and well mixed for 24 h. The gelling bath was a solution of chitosan ($M_w = 5.67 \cdot 10^4$ g/mol, deacetlylation degree 0.88) with concentration of $0.25 \cdot 10^{-2}$ g/cm^3 in 0.2 M CH_3COOH/0.4 M CH_3COONa with added 0,2 M $CaCl_2$ as a gelling agent for ionic gelation. The CA gel particles were obtained by setting drops of the gelling solution from a needle in the gelling bath at 25 ^{0}C put on a magnetic stirrer with 300 rpm. After the first contact of the drops and the gelling medium, chitosan membrane is immediately formed. After 1 h of steering, the gel spheres were washed with distilled water and were ready for controlled release characterization. This is the procedure for obtaining homogenous gel particles. The only difference for the inhomogeneous gel particles was that in the gelling bath of sodium alginate 1 mM $CaCl_2$ and 10 mM $CaCl_2$ were added as inducer of aggregated structures that are spread through the gel matrix after finishing full gelation from such systems. The sizes of the aggregated structures (microgel domains) in the gelling solution were measured by dynamic light scattering techniques. The inhomogeneities of the gels are visible on SEM pictures obtained in this research (fig.1 and fig.2). We found that size of gel particles do not depend on

type of alginate. The size change depends only on the concentration of added $CaCl_2$ in the gelling solution. The diameter of gel particles is average value of measurements on ten different particles: - for particles without $CaCl_2$, d = (2.45 ± 0.35) mm; - for particles with 0.1 mM $CaCl_2$, d = (2.70 ± 0.55) mm; - for particles with 10 mM $CaCl_2$, d = (3.00 ± 0.75) mm.

Controlled release studies were conducted as the following [5]. A releasing bath is thermostated at 37 ^{0}C on a magnetic stirrer on 300 rpm. The releasing bath is a phosphate buffer solution with pH = 7.4. The releasing bath contains 0.1 M NaCl that breaks the calcium bridges in the gel matrix and thus disintegrate the gel because diffusion of releasing medium through chitosan membrane's pores. The volume of gel particles in the releasing bath is 1cm^3, and the volume of the releasing bath is 30 cm^3. The experimental condition of constant active substance concentration on the outer surface of the membrane is provided by constant steering of the releasing bath. Weight of gel particles is an important quantity for determination of the active substance loading coefficient (ratio of BSA mass and mass of gel particles).

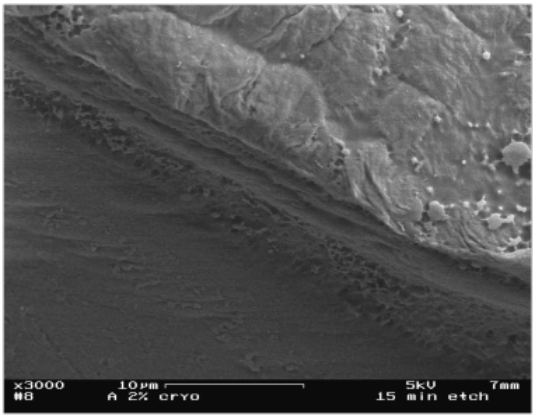

Fig.1. SEM on homogenous LF120M particles.

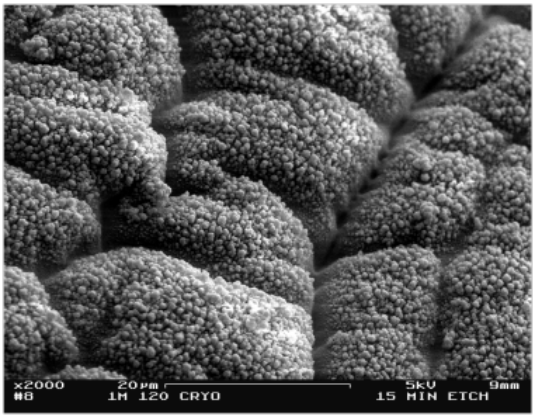

Fig.2. SEM on inhomogeneous LF120M particles

After putting the gel particles in the releasing medium, at the beginning on short time intervals and then on longer, 1 cm^3 of the releasing bath in which BSA is released is taken off and the same volume is replaced with pure releasing medium. The taken 1 cm^3 released volume is placed in CECIL series 2000 UV-Vis spectrophotometer to measure absorbance A at 280 nm, a wavelength at which BSA in phosphate buffer solution shows maximum absorption. The concentration of BSA is determined by the Lamber-Beer's law of light absorption. Before measuring the kinetics of controlled release of BSA, a calibration curve is constructed that represents the dependence of light absorbance at 280 nm of several BSA solutions with known concentrations c, $A = kdc$, where d is UV cell length and k is a calibration curve constant.

Scanning electron microscopy of gel particles is performed on the following way [5]. The particles were taken from water filled container for their transport and put on paper to wipe excess water from their surface. After that, the particles were put in nitrogen gas chamber and after that set in the preparation chamber at temperature – 110 ^{0}C. Here, gel particles were kept for 15 minutes for water sublimation from the gel. Six slices from center to the shell were cut from each particle on microtome machine and coated with 25 nm layer of Au/Pd. The slices

were investigated in Cryo FE-SEM, LEO Electron Microscopy, Germany) kept on -110 ^{0}C. Fig.1 and fig.2 presents two examples of SEM pictures from all nine investigated systems for visualizing differences between homogenous and inhomogeneous particles.

The size of microgel structures in the gelling solutions of the nine systems of CA gel particles was investigated by dynamic light scattering method [5,6]. The measure for the size is the characteristic correlation length ξ included in the Stokes-Einstein equation $D = kT/(6\pi\eta\xi)$, where η - dynamic viscosity of the gelling solution, k – Boltzmann's constant, T – temperature and D – coefficient of translational diffusion of the polymer chains in solution. The coefficient D is determined from the dynamic light scattering measurement of the autocorrelation function $G(\tau)$ that depends on the scattering wave vector $q = (4\pi/\lambda)\sin(\theta/2)$ and time τ of sampling intensity of the scattered light: $G(\tau) = 1 + e^{-2Dq^2\tau}$. The data for the characteristic length ξ is given on Table 1.

3. RESULT AND DISCUSSION

Figure 3 gives a graph of the measured kinetics of BSA controlled release for one of the nine systems. The other kinetics of release, are similar to the one given in fig.3. The results from the measuring kinetics of controlled release of BSA of the nine different gel particles systems are summarized in table 1. Fig.3 shows a percentage of released mass of BSA on the left ordinate m_t/m_0 %, where m_0 is the mass of BSA in the particles obtained after total disintegration of the particles, and m_t is mass of released BSA at any time during release. On the right ordinate, the releasing rate is given for the same system. In figure 3 (typical of all nine systems) is observable that BSA release starts with a burst effect.

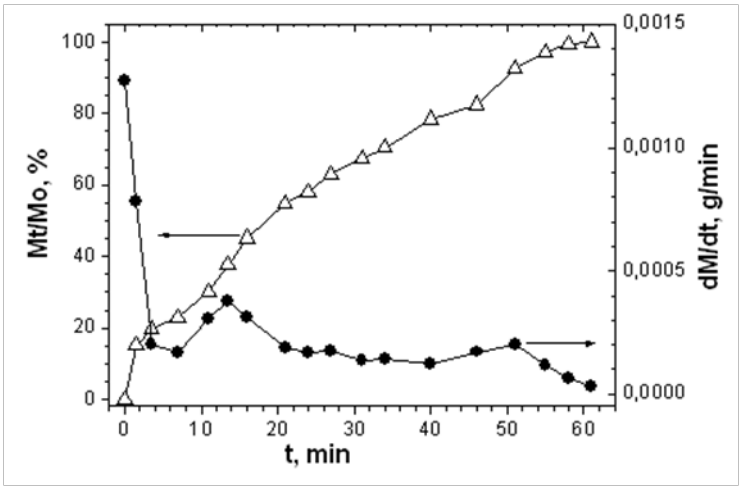

Fig.3. Release kinetics of BSA from CA system type LF240D (without added Ca^{2+} in gelling solution) at 37 ^{0}C.

The integral efficacy of controlled release is calculated by integration of release rate on time (upper boundary is end time of controlled release; lower boundary is time after end of the burst effect). The osmotic disintegration of gel particles is not pronounced equally for all systems. Time for start of osmotic disintegration effect at 37 ^{0}C for homogenous gel particles

without added $CaCl_2$ is: 52 minutes for system LF120M, 115 minutes for system LF200M, greater than 200 minutes for LF240D. The situation is different, for example, when added 10 mM $CaCl_2$. For those systems the time for start of osmotic disintegration effect is not much changed. Only for the system LF240D (with highest G/M ratio) and with added 10 mM $CaCl_2$, time for start of osmotic disintegration effect is much lower (16 minutes). From the three models of controlled release, we found that the osmotic pumping model is valid during all the time of controlled release. Taking the values of the thickness of chitosan membrane of all the particles measured on optical microscopy (22 ± 5) μm and thus knowing the surface area of the particles, BSA concentration in the particles C_a, and taking value 1 for the membrane's reflectivity, we calculated from Eq (4) the coefficient of the hydraulic permeability L_p and the values are given on Table 1 for the three gel systems. The existence of burst effect at the beginning of BSA release from the systems indicates on large concentration of BSA at the surface (membrane) of particles which is expectable to the reservoir model of release. Release profiles of the systems at the osmotic disintegration of particles are not sharp but times broaden, especially observed for inhomogeneous gels. This is an indication that the erosion model is taking place by the mechanism of gel's dissolution during BSA release.

Table.1. BSA release parameters in phosphate buffer solution at 37 0C for three types of CA systems.

	LF120M			LF200M			LF240D		
	0 mM	1 mM	10 mM	0 mM	1 mM	10 mM	0 mM	1 mM	10 mM
Release rate at controlled release stage, mg/min	0.05	0.05	0.12	0.01	0.16	0.09	0.05	0.19	0.27
Release rate at start time (with burst effect), mg/min	0.15	0.38	4.20	5.6	1.70	2.81	1.8	1.8	0.17
Release rate at 90 % release of BSA, mg/min	0.36	0.10	0.18	1.00	0.05	0	0.25	0.20	0.29
Time for release of 90 % BSA, min	58.5	25.5	45.0	119.0	72.5	92.0	25.5	29.0	27.0
Loading coefficient, %	1.0	1.7	2.6	3.3	1.9	1.7	`1.7	1.7	1.6
Loading efficacy	29.8	50.3	76.6	89.9	57.0	51.2	50.7	52.6	51.0
L_p, $m^3 \cdot s/kg$	4.9	1.8	1.6	0.3	5.1	1.9	-	6.7	6.0
Integral efficacy of release, %	29	18	30	3	35	27	0	31	32
Characteristic length ξ, μm	10.69	16.02	323.3	19.32	32.34	589.0	20.9	30.5	523.0

4. CONCLUSIONS

The research on the release properties of BSA from three types of calcium alginate particles with chitosan membrane, showed that the release rate increases with the increase of Ca^{2+} ions concentration in gelling solutions, i.e. by the increase of the inhomogeneity of the gel matrices reflected by the ξ parameter. The release profiles for all the systems showed flat release rate in a wider time domain. The release rates are higher for the system type LF240D

especially for the ones with added Ca^{2+} ions (inhomogeneous). Because of this, the time of 90 % release of BSA for this system is smaller than the other systems. From the analysis of the results, it can be concluded that CA gel particles with smaller *G*/*M* ratio showed optimal release properties expressed through high release rates, high time of 90 % release of BSA, optimal loading coefficient, high value of coefficient of the hydraulic permeability L_p, and optimal integral efficacy of release. We found that internal inhomogeneities of gel particles caused change in the structure of the gels that led to less pronounced controlled release properties.

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