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Chitosan microspheres of nifedipine and nifedipine-cyclodextrin inclusion complexes

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Abstract

Chitosan microspheres of nifedipine and nifedipine-cyclodextrin complexes were prepared by the glutaraldehyde cross-linking of chitosan. Microspheres having different degrees of swelling were made by varying the cross-linking density. Drug incorporation efficiencies exceeding 70% could be achieved for this drug. In vitro release rates were influenced by the cross-linking density, particle size and initial drug loading in the microspheres. While the solubility of nifedipine was enhanced by inclusion into a cyclodextrin complex, the drug release from the complex was significantly reduced. In addition the drug release mechanisms were discussed.

Keywords: Chitosan; Nifedipine; Microspheres; Cyclodextrin complexes; Sustained release

1. Introduction

Haemodynamic effects of sensitive drugs may be dependent on the pharmaceutical formulation used (Echizen and Eichelbaum, 1986). Nifedipine was chosen as a model drug in this investigation because it is highly crystalline, poorly soluble with a biological half-life of 3.43 h and also photosensitive, and therefore its novel delivery formulations may be worthwhile studying. Here chitosan microspheres with nifedipine (N) or nifedipine-cyclodextrin complexes (NC) were prepared by the cross-linking of chitosan with glutaraldehyde. Suspension cross-linking procedures are widely used in the preparation of polysaccharide-based microspheres for biomedical application. This technique could be applied to produce chitosan microspheres, a water-in-oil (w/o) suspension system with or without the use of any stabilizer. The aqueous phase is usually composed of a chitosan acetic acid solution while the oil phase contains soya oil, paraffin oil, or mineral oil. Glutaraldehyde is sometimes used as a cross-linking agent (Yao et al., 1995). The idea of this study would be to increase the drug solubility by applying cyclodextrin complexes while at the same time achieving the controlled release by means of microencapsulation.

Cyclodextrins are cyclic oligosaccharides, consisting of six (α), seven (β), or eight (γ)-1.4 linked glucose units. The size of the lipophylic cone-like cavity is a decisive criterion for the formation of stable inclusion complexes, since a guest molecule

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must fit at least partly into this opening. Recently, chemically modified cyclodextrins have received considerable attention, because their physical properties and inclusion behaviour are different from those of natural cyclodextrins. Hydroxypropyl-β-cyclodextrin is much more soluble in water and organic solvents in comparison with β-cyclodextrin. Solid complexes of nifedipine with hydroxypropyl-β-cyclodextrins in molar ratios of 1:1 were prepared using freeze-drying and spray-drying methods.

For the microencapsulation step chitosan, (1-4)2-amino-2-deoxy-β-D-glucose, was used because it is a non-toxic, biodegradable and biocompatible polysaccharide. It can be used as a material for the microspheres or drug delivery systems and has been extensively investigated for pharmaceutical and medical purposes (Li et al., 1992; Wan et al., 1994).

2. Materials and methods

2.1. Materials

Chitosans of different viscosity and degree of deacetylation (Seacure 123, 22 mPas, 88.7% and Seacure Cl 210, 60 mPas, 81%) were obtained from Protan Laboratories Inc. (Drammen, Norway) and were used as received. Hydroxypropyl-β-cyclodextrin (by an average MS-value of 0.9) was kindly supplied by Wacher-Chemie GmbH, Münich, Germany. Glutaraldehyde (biological grade, 25% aqueous solution) was from Sigma, USA. Nifedipine was from Pliva, Croatia. Since nifedipine is light sensitive all glassware used was wrapped in black paper in order to avoid photodecomposition. The other chemicals used were of analytical grade and were purchased from Kemika, Croatia.

2.2. Preparation of nifedipine-cyclodextrin complexes

Solid nifedipine-cyclodextrin inclusion complexes (NC) were prepared by complete or partial solubilization of N in the aqueous solutions of cyclodextrin using freeze-drying (NC_f) and spray-drying (NC_s) techniques. For NC_f an excess of N was added to an aqueous hydroxypropyl-β-cyclodextrin solution, the suspension was stirred for 5 days and following filtration the supernatant was lyophilized. NC_s was prepared by dissolving N and hydroxypropyl-β-cyclodextrin (1:1 molar ratios) in ethanol and water, respectively. The mixture of these solutions was then sonicated to produce clear solution which was subjected to spray drying. The amount of N incorporated in the complexes was determined spectrophotometrically. The total content of N was 5 and 32% (w/w) for NC_f and NC_s, respectively.

2.3. Preparation of microspheres

2.3.1. A samples

An amount of N or NC was suspended in aqueous solution of chitosan (1–3%, w/w). The suspension was then emulsified in liquid paraffin containing 2% (v/v) Span 85. The membrane was formed by interfacial polymerization of chitosan following addition of glutaraldehyde-saturated chloroform (1 ml) (Longo et al., 1982). The emulsion was formed in a 250-ml beaker, under constant stirring by means of simple paddle at 1750 rev./min at room temperature. Solidified microspheres were separated from the reaction mixture by vacuum filtration, washed several times with chloroform and methanol and air dried for 24 h at room temperature and stored in a desiccator.

2.3.2. B samples

A 4% solution of chitosan was prepared in 5% (v/v) acetic acid. N or NC was dispersed in this solution. The suspension was then emulsified in sunflower oil containing 2% (v/v) Span 85. The membrane was formed by interfacial polymerization of chitosan following addition of glutaraldehyde (0.6 ml, 25% aqueous solution) as cross-linking agent. The emulsion was formed in a 250-ml beaker, under constant stirring by means of simple paddle at 250 or 500 rev./min. The emulsification and reaction times were 10, 15 and 20 min, and the reaction was quenched by dilution with an aqueous detergent solution. Hardened microspheres were separated from the reaction mixture by filtration, washed several

times with ice-cold water and air dried for 24 h at room temperature and stored in a desiccator.

2.4. Determination of drug content

The drug content of the microspheres was determined spectrophotometrically after extracting the microspheres in methanol for 24 h or dissolving the microspheres in 0.1 M HCl. Each determination was carried out in triplicate.

2.5. Size distribution and swelling of microspheres

A microscopical imaging analysis technique for determination of particle size distribution was used. Microsphere size and distribution were determined with a Olympus BH-2 microscope, equipped with a computer-controlled image analysis system (Optomax V, Cambridge).

The swelling ability of microspheres in phosphate buffer (pH 7.4) was determined by suspending microspheres in buffer and measuring particle size and distribution of swollen microspheres. The concentration of the suspension was sufficient to allow the counting of approximately 1000 particles from one slide. Swelling ability of systems was estimated by comparing mean diameters of 'dry' and swollen microspheres.

2.6. In vitro release

In vitro release profiles of the N and NC from microspheres were examined in phosphate buffer, pH 7.4. The drug-loaded microspheres (5 mg) were added to 250 ml of the dissolution medium in an Erlenmeyer flask. The studies were performed at 37°C, in a bath incubator with agitation on a rotating shaker. Samples (3 ml) were withdrawn at time intervals and assayed spectrophotometrically in an UV-Vis spectrophotometer (Pharmacia LKB Ultrospec Plus) at 240 nm. To maintain a constant volume of the dissolution medium, an amount equal to the volume withdrawn was immediately added after each withdrawal. All experiments were carried out in triplicate and average values were plotted. The curvefit computer program, which consists of least-squares fit to a linear or non-linear function with linearization of the fitting function, was applied for calculation of the best-fit curves to the experimental data.

3. Results and discussion

The chitosan microspheres with N, as model substance poorly soluble in water, and NC were prepared by using the suspension cross-linking procedure. Nifedipine-cyclodextrin complexes were prepared to improve the drug dissolution rate and to evaluate the feasibility of using these complexes as a core for microcapsules in order to obtain controlled release. The solid complexes of nifedipine with hydroxypropyl-β-cyclodextrin were prepared by using NC_f or NC_s techniques.

The characteristics of microspheres prepared are given in Table 1. Microspheres were spherical and of well-defined shape. All the samples showed a tendency to form agglomerates after the drying step. The internal structure of the microspheres revealed that N had been crystallized and incorporated within the microporous structure of the polymer matrix. The N crystals were observed mainly inside of the matrix than at the outer surface, as is evident from Fig. 1. The microspheres with NC_s were transparent and the complex was of amorphous appearance with some presence of spherical small homogeneous particles distributed within the matrix. One noticeable characteristic of microspheres is their brownish colour. The intensity of colour increased as gelation proceeded. Roberts and Taylor (1989) reported that the chitosan/glutaraldehyde gels of yellow-brown colour were produced whether purified or unpurified glutaraldehyde was used. They also suggested that the production of colour is a consequence of inter-chain cross-link formation. Several variables that affect the gelation behaviour of the chitosan/glutaraldehyde system were found as follows. The rate of gelation is increased by the increase in concentration of either chitosan or glutaraldehyde, or by increase in temperature, and reduced by increase in the concentration of acetic acid (Roberts and Taylor, 1989).

Table 1
Charactetistics of N and NC complex microcapsules

Variable	A1	A2	A3	A4	A5	A6	A7	A8	B1	B2	B3	B4	B5	B6	B7
Chitosan concentration (%)	2	2.5	3	2	2.5	3	2.5	3	4	4	4	4	4	4	4
Nifedipine amount (mg)	10	10	10	10	10	10	10	10	20	20	20	20	30	60	30
Drug type	N	N	N	NC_f	NC_f	NC_f	NC_s	NC_s	N	N	N	N	N	N	NC_s
Glutaraldehyde reaction time (min)	120	120	120	120	120	120	120	120	10	10	15	20	10	10	10
Stirring rate (rev./min)	1750	1750	1750	1750	1750	1750	1750	1750	500	250	250	250	250	250	250
Total drug content (%)	3.4	5.0	4.6	0.22	0.23	0.26	0.8	1.1	45	63	51	46	73	88	79
'Dry' mean diameters (μm)	3.6	3.3	4.7	4.5	3.5	5.9	5.0	5.3	16.1	20.2	15.6	15.2	18.3	23.6	22.6
'Swollen' diameters (μm)									24.2	27.8	26.1	24.4	29.0	32.4	34.2

A samples prepared with Seacure 210 and liquid paraffin; B samples prepared with Seacure 123 and sunflower oil.

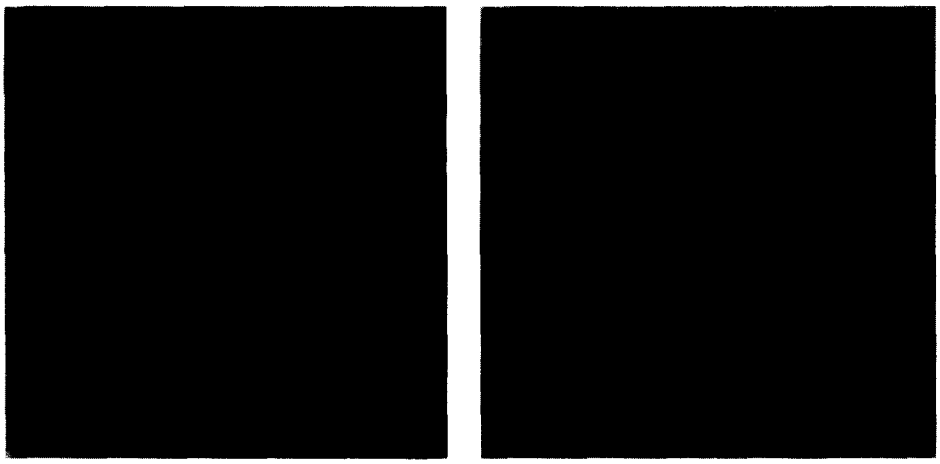

Fig. 1. Micrograph of chitosan microspheres with incorporated NC_s (a) and N (b).

Two types of chitosan were used for the preparation of microspheres, Seacure 210, and Seacure 123 which differs in viscosity and in the degree of deacetylation. Two series (A and B) of microspheres were prepared (Table 1). As has been explained in detail in a previous section, microspheres of series A were prepared with chitosan of higher viscosity (Seacure 210), varying its concentration in aqueous medium. Liquid paraffin was used as an oil phase while cross-linking was achieved with glutaraldehyde-saturated chloroform. The slow and uniform cross-linking of the droplets particularly on the surface was felt desirable to generate spheres of good sphericity. Glutaraldehyde-saturated chloroform, because of its solubility in the oil medium would be uniformly available for cross-linking the surface of the droplets. The surface hardening of the droplets by cross-linking thus stabilizes the shape and surface morphology of the microspheres. The denaturation time and stirring rate were kept constant. Chitosan of lower viscosity (Seacure 123) in acetic acid was used for the preparation of B samples. Sunflower oil was used as an oil phase and chitosan was cross-linked with aqueous solution of glutaraldehyde. The chitosan concentration, volume of oil phase, stabilizer concentration and amount of glutaraldehyde added were kept constant, while the denaturation time, the initial loading of N and stirring rate were varied. The microspheres were washed with detergent aqueous solution.

The drug incorporation efficiencies of the N, NC_f and NC_s in the microspheres are given in Table 1. The microspheres of series B showed better loading efficiency compared with the microspheres of series A. Drug amount taken into preparation can not solely be responsible for the differences in the total drug content found in the samples prepared. It should also be attributed to the method of preparation used. During the process of preparation, chitosan is cross-linked with glutaraldehyde which was in chloroform (A samples) or in water (B samples). As N is a lipophylic drug it could be assumed that some of the drug is lost in the outer oily phase at the beginning of preparation before the solid chitosan matrix is formed. This effect could be enhanced by the addition of organic solvent in the reaction mixture, as in the case of A samples. In addition to this, lower entrapment efficiencies for A samples could also be due to the final washing of microspheres with chloroform. Therefore for the preparation of B samples the aqueous solution of glutaraldehyde was used for cross-linking and microspheres were washed with the detergent solution.

The entrapment efficiency of the drugs for microspheres of series A increased with increasing the concentration of chitosan solution. Also, N showed better loading efficiency compared with the more soluble NC. This could be attributed to

the relatively high initial loading of NC in the preparation of microspheres needed to keep constant the initial loading of N. It is especially the case with the NC_f. A highly concentrated solution of chitosan made the dropping process difficult and microspheres could not readily be formed. Therefore, for the preparation of the B samples the chitosan with lower viscosity was used and NC_f was skipped. The entrapment efficiency of N for microspheres of series B increased with increasing the initial loading of N and decreasing the stirring rate, while it decreased with an increase in the denaturation time. With these microspheres, the more soluble NC_s showed better loading efficiency compared with N.

Particle size analysis of polydisperse samples of microspheres indicated logarithmic-normal distribution. Microsphere sizes were up to 10 and 50 μm for A and B samples, respectively. The samples were characterized by calculating mean diameters. The microsphere sizes are influenced by the stirring rate, extent of cross-linking induced, initial loading of drugs in preparation and by the type of substance incorporated. Table 1 shows the effect of these variables on the mean microsphere diameter. The effect of the extent of cross-linking induced is straightforward; the microspheres exposed for longer time to glutaraldehyde had a smaller mean diameter. The degree of swelling of microspheres in phosphate buffer was also dependent on the extent of cross-linking induced. The highly cross-linked microspheres swell to a lesser extent than microspheres with lower degree of chitosan denaturation. Also, the microspheres with incorporated N swell to a lesser extent than microspheres with incorporated NC.

The drug release from microspheres containing N or NC_s was compared to the drug dissolution from NC_s alone. The results are shown in Fig. 2. At given experimental set-up, N was dissolved very fast from NC_s: for instance about 25% of N was released in 1 h. However, the drug release from microencapsulated NC_s was the slowest (less than 15% in 20 h). Microencapsulated N gave an intermediate slow release (about 40% in 20 h). One should consider the relation between solubilities of pure N and its cyclodextrin form in water medium when discussing the release from microspheres. The solubility of N in water is 5×10^{-6} mol l^{-1}, while with cyclodextrin present in concentration of 1×10^{-2} mol l^{-1} its solubility is almost doubled (i.e. 9.8×10^{-6} mol l^{-1}). Therefore one would expect the N release from B7 sample to be faster than from B5 sample. The results presented in Fig. 2 show that N in NC_s is not readily available for the release when microspheres are subjected to water medium. Although the stability constant of the inclusion complex is not very high (647.3 M^{-1}) the release mechanism can be supposed as follows. N has to be liberated firstly from the cyclodextrin cavity, and then the free drug will permeate out of the sphere. This last step, as concluded above is controlled by matrix permeability, and it is possible that the presence of hydroxypropyl-β-cyclodextrin in system decreases the permeability of matrix for nifedipine. The effect of cyclodextrin could be attributed to the formation of more hydrophylic chitosan/cyclodextrin matrix layer around the lypophylic drug than in the case of chitosan matrix alone. It is highly unlikely that cyclodextrin molecule diffuses out of microspheres.

The dissolution rates of N were inversely related to the particle size as would be expected from surface area relationships (Fig. 3). Smaller

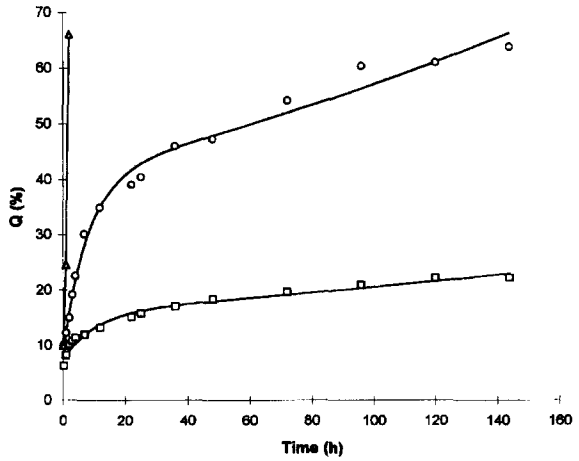

Fig. 2. The release profile of drug from chitosan microspheres with incorporated N, (○-○) B5, and micropsheres with incorporated NC_s (□-□) B7 in comparison to drug dissolution from NC_s alone (Δ-Δ). Points are experimental and curves are the best fit for first-order kinetics of the biphasic type.

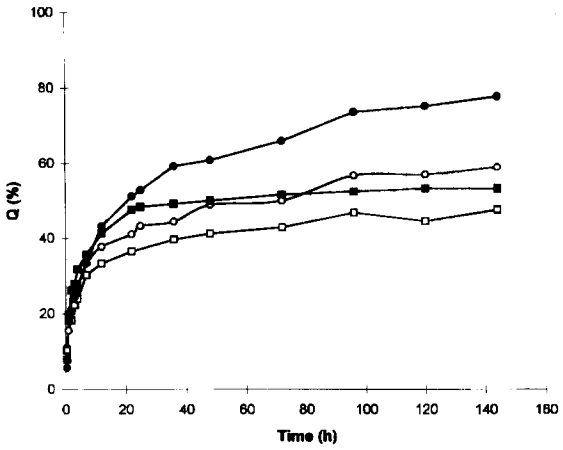

Fig. 3. The release profile of N from chitosan microspheres having different mean diameters in phosphate buffer, pH 7.4. (○-○) B1, (□-□) B2. Open symbols represents samples which were simply supended in buffer and closed symbols represent samples which were sonicated.

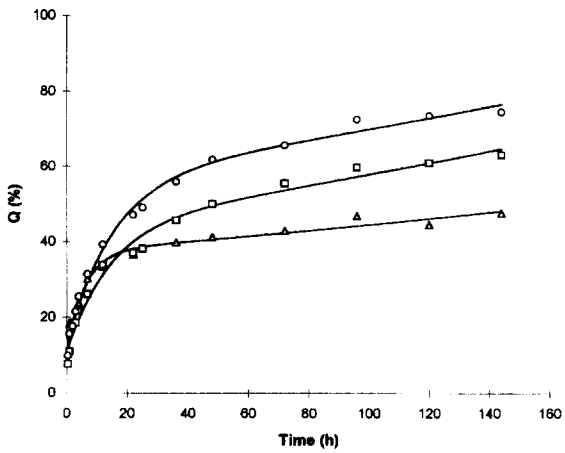

Fig. 4. The release profile of N from chitosan microspheres having different cross-linking density in phosphate buffer, pH 7.4. Points are experimental and curves are the best fit for first-order kinetics of the biphasic type: (○-○) B2, (□-□) B3, (Δ-Δ) B4.

spheres, because of larger area of contact with the dissolution medium, would release the drug more readily in comparison with larger spheres. The difference in the release properties observed would be even more significant if the smaller microspheres did not agglomerate. Therefore, at onset of the in vitro release study some of the samples were subjected to ultrasonication in order to redisperse the microspheres into separated entities. As can be seen from Fig. 3 the release rate of N from microspheres which were sonicated was faster as compared to microspheres which were simply suspended in the phosphate buffer.

The cross-linking of the microspheres influenced the release profiles of the drugs. The release profiles of N from chitosan microspheres cross-linked to three different extents is shown in Fig. 4. The least cross-linked microspheres released the drug at a faster rate than highly cross-linked microspheres. These findings are basically the same with previous findings of other authors. The permeability coefficient of chitosan membranes showed a definite decrease with increase in the degree of cross-linking (Thacharodi and Rao, 1993; Akbuga and Durmaz, 1994).

The rate of release was significantly influenced by the initial loading of nifedipine in the microspheres (Fig. 5). In contrast to findings of Thanoo et al. (1992) an increase in the release rate was observed when the drug loading was increased. It is possible that a higher proportion of drug inside the matrix structure of microspheres make the drug more available for the release once the original matrix structure had been exhausted of the drug.

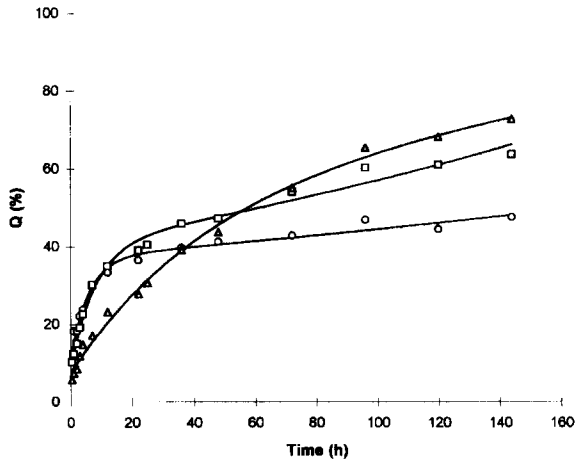

Fig. 5. The release profile of N from chitosan microspheres having different drug loading in phosphate buffer, pH 7.4. Points are experimental and curves are the best fit for first-order kinetics of the biphasic type: (○-○) B2, (□-□) B5, (Δ-Δ) B6.

Concerning the possible mechanism of drug release, the drug solubility of poorly soluble drugs might be a dominant factor, if the drug dissolution from the surface or interior of microspheres through water-filled pores governs the mechanism of drug release. In such cases, Hixson-Crowell kinetics (Hixon and Crowell, 1931) should be found for the release data. In the present study, the data did not conform to Hixson-Crowell kinetics, suggesting that drug solubility is not responsible for the mechanism of drug release. The release profiles were analyzed kinetically applying the models representing the diffusion-controlled release: i.e. zero-order, first-order and Higuchi's (Higuchi, 1963) square-root of time models. Zero-order release was not found even at the onset of the drug release. The first-order release of biphasic type was considered the best fit for all release data, but it was also found that a Higuchi model gives an even better correlation for the sample of microspheres with the highest loading of nifedipine. For instance, the correlation coefficient (r) for the first order (biphasic) release type was 0.995 and 0.977 for samples B6 and B7, respectively, while for Higuchi type they were 0.998 and 0.975. It appears that the release of nifedipine from microspheres is controlled by the permeability of the matrix. The drug molecules near to the microsphere surface diffuse first into the receiving medium creating a drug-depleted layer in the matrix. The transport of the drug from the interior of microspheres to depleted regions is much slower. This phenomenon was observed with other authors too. Thacharodi and Rao (1993) observed that the release of nifedipine from the chitosan membranes was altered significantly due to cross-linking. Because of the large decrease in the partition coefficient of chitosan membranes it is possible that both partition and pore mechanisms operate concurrently in the transport of nifedipine through chitosan/cross-linked chitosan membranes. Chandy and Sharma (1992, 1993) developed the theory of ampicillin and nifedipine release from chitosan microspheres. They stated that the outer core of the microspheres is first swollen, followed by diffusion of drug, then subsequent swelling in array of the inner core of the microspheres occurs, leading to internal mobilization of drug and uniform release rates of drugs.

In conclusion it can be stated that microspheres of N and NC can be prepared in order to modify the drug release and by variation of preparation variables drug formulations can be obtained having suitable kinetic profiles of release. In further work some in vivo experiments should be performed to investigate if the controlled activity of the drug would be obtained.

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