In vitro Investigation of a Controlled Release of Ophthalmic Drug from Thermo-sensitive Gel

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Introduction

We propose the design of a new potential drug for ophthalmology. We have found that peptide inhibitor (current commercial name "Lisophtal") of a distinct peptidase improves microcirculation and reparation in eye tissues when used topically in the treatment of the eyes of laboratory animals (rabbits). Thus, this compound can help to reconstruct ocular surface and can be used for the improvement of healing processes after eye injuries. It is worth noting that about 80% of the active substances in usual eye drops are pretty quick eluted with tears, and the proper eye treatment often requires high concentration of active compound and/or frequent drop application. Prolongation of the efficiency of eye drops is often achieved by the enhancement of the viscosity of the medium [Maichuk, U.F., 1975]. However, another approach seems to be very promising at the moment. The active substances can be included in thermo-sensitive gels allowing prolonged release of the drug as described in [Gutowska, A. et al., 1995].

Poly-N-isopropylacrylamide (NIPAA) hydrogels exhibit reversible volume phase transition upon decreasing/increasing of the temperature in aqueous solutions, i.e. reversible swelling and deswelling below and above a lower critical solution temperature (LCST), respectively [Schilld, H.G., 1992]. At low temperatures NIPAA shows full-hydrated and extended conformation. Over 32°, it extensively dehydrates and changes to compact chain conformation. Such phase transition is accompanied by the changes in NIPAA hydrophilic/hydrophobic properties: the gel is hydrophilic in character below LCST, but becomes hydrophobic as the temperature rises above LCST [Wu, X.S. et al., 1992]. The change in gel properties strongly affects the interactions of cells with the NIPAA surface, as cells adhere and grow on slightly hydrophobic surfaces, but do not adhere to highly hydrophilic surfaces. Such effects were observed for many types of cells, including of hepatocytes [Yamada, N. et al., 1990], endothelial cells [Kukuchi, A. et al., 1998], lymphocytes [Takei, Y.G. et al., 1995], monocytes, macrophages and foreign body giant cells [Collier, T.O. et al., 2002], CV1 cells [Kono, K. et al., 1999], and bacteria [Cunliffe, D. et al., 2000]. Thus, we can expect potential high adhesion of the gel on eye surface cells.

Here we describe swelling/deswelling processes of the NIPAA-gel at two different temperatures and present the data on the release of Lisophtal from thermo-sensitive gel.

Materials and methods.

N-Isopropylacrylamide (NIPAA), N-acetyl-L-cysteine and o-phtalaldehyde (OPA) were obtained from Sigma (USA). N,N'-methylene-bis-acrylamide (MBA), ammonium persulfate and N,N,N',N'-tetramethylethylenediamine (TEMED) were purchased from Reanal (Hungary). Components of buffer solutions ("pure for analysis" grade) were obtained from Reakhim (Russia).

Synthesis of NIPAA-gel preparations. 150 mg of NIPAA and 0.43 mg of cross-linking agent (MBA) were dissolved in 1 ml 0.05 M Na-phosphate buffer, 0.15 M NaCl, pH 7.5 (buffer A). Block copolymerisation was started by adding 0.02 ml of 0.78 M ammonium persulfate in water and 0.01 ml of TEMED and was carried out at 20° for 1 h. Prepared block copolymers were cut into 5 mm x

5 mm x 3 mm slices. The slices of gel were weighed and incubated at 20° in an excess of buffer A during 24 h.

Determination of relative hydration degree of NIPAA-gel. The slices of prepared gel were gently blotted by filter paper, weighed (W_0), placed into buffer A at given temperature for 24 h and weighed again (W_t). Relative hydration degree of gel was determined as W_t/W_0 ratio (per cents).

Kinetics of hydrohel swelling-deswelling. The slices of prepared gel were gently blotted by filter paper and weighed. For deswelling, the slices of gel were incubated at 37° (physiological temperature), the weight of each slice was determined at given time intervals. The slices of deswelled gel were swelled again at 20° as described above. The ratio $(W_s+W_p)/W_p$ (percents) was used as a weight swelling-deswelling parameter, where W_s is the absorbed water weight and W_p is the dry polymer weight.

Hydrogel swelling in the presence of Lisophtal. The slices of dry gel were incubated in 5ml of 1-10 mM Lisophtal in buffer A at 20° for 24 h. Then swelled slices were gently blotted, weighed and used for drug release experiments. The amount of drug loaded into gel was calculated from the material balance.

Drug release. Each slice of the gel preliminarily swelled in Lisophtal solution was placed into 5 ml of buffer A and incubated at 20° or 37°. Aliquots (0,125 ml) of the aqueous solution above the gel were taking at specific time points and drug concentration was determined.

Drug release control. Peptide Lisophtal contains free amino group that allowes using the method [Svedas, V.-J.K., et al., 1980] for estimation of drug concentration in the reaction media. The method is based on the reaction of free amino groups of a ligand with OPA in the presence of SH-containing compounds, the product being a chromophoric compound. Drug concentration was determined by color product appearance at λ = 336 nm due to the reaction with 0.875 ml of 4 mM OPA and 2,6 mM acetyl-cysteine in 0,1 M borate buffer, pH 9,6, on UV-265 FW spectrophotometer (Shimadzu, Japan). The molar extinction coefficient of the chromophore, 6200 $M^{-1}sm^{-1}$, was determined in a preliminary experiment. The absence of gel color reaction was shown in a preliminary experiment as well.

Results and discussion.

The temperature dependence of the relative hydration degree of NIPAA-gel in buffer A at equilibrium conditions is shown at Fig.1. The LCST was found to be close to 33° that is in accordance to the literature data [Schilld, H.G., 1992].

The swelling-deswelling kinetics of thermosensitive gel, when the temperature was cycled between 20° and 37° at 24 h intervals, is shown at Fig.2. The results indicate that time interval 5 h is enough to reach the equilibrium both in swelling and deswelling processes. The changes in relative percent of swelling and deswelling from square root of time according to [Tanaka, T. et al., 1985] are presented in Fig.3. One can see that the rates of swelling-deswelling processes are the same, the constant being equal to 52,5%/$h^{1/2}$. It is worth noting that the swelling and deswelling of the NIPAA-gel at 24 h intervals are reversible throughout an overall experimental period of 720 h.

Lisophtal has a low molecular weight (405 Da) and one could expect that only deswelling behaviour of polymer matrix should control the drug release with the increasing of temperature. The results on the kinetics of Lisophtal release from gel at 20° and 37° are presented at Fig.4.

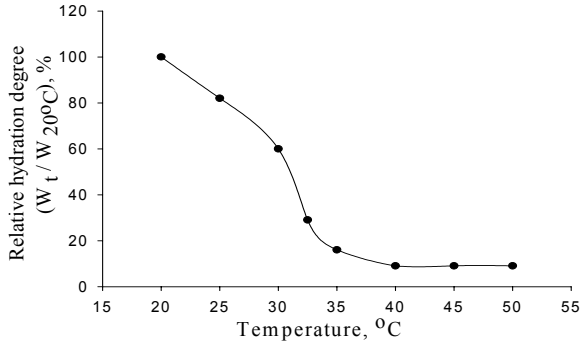

Fig.1. The temperature dependence of the relative hydration degree of NIPAA-gel in buffer A at equilibrium conditions

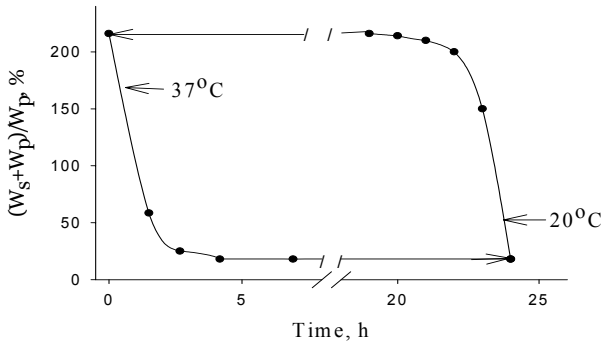

Fig.2. The swelling-deswelling kinetics of thermosensitive gel when temperature was cycled between 20° and 37°

Relatively slow and approximately linear release of the drug at 20° is changed upon rise of temperature to 37° to initial burst of drug followed by a slow rise (approximately 70% of loaded drug releases from the fist 0,5 h). The initial ejection of Lisophtal from the gel can be easily explained by deswelling process of the thermosensitive polymer matrix due to transfer of gel from 20° to the buffer with 37°. However the relatively long residual period of drug release does not correlate with the deswelling behaviour of NIPAA-gel (Fig.5). Evidently such release profile is connected with sharp increase in hydrophobicity of NIPAA at 37° in comparison to 20° and with appearance of strong interactions between the polymer chains and hydrophobic parts of Lisophtal.

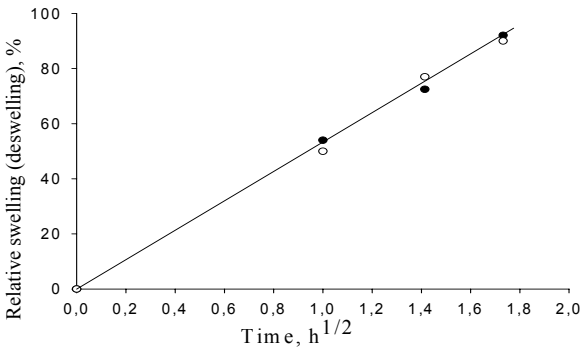

Fig.3. The changes in relative percent swelling (black symbols) and deswelling (opened symbols) from square root of time (calculated from Fig.2).

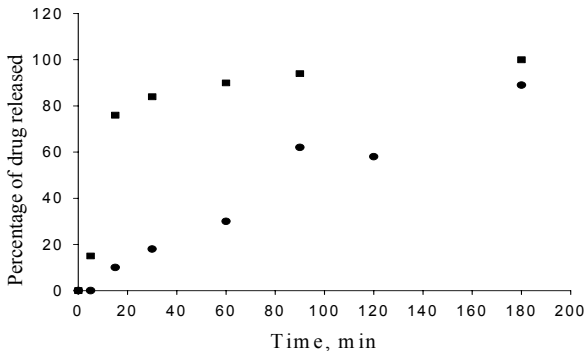

Fig.4. Kinetics of Lisophtal release from gel at 20° (circles) and 37° (squares).

Conclusions

Thus, the results obtained *in vitro* demonstrate a possibility of using NIPAA-gel as a base for Lisophtal-containing eye drops with prolonged action.

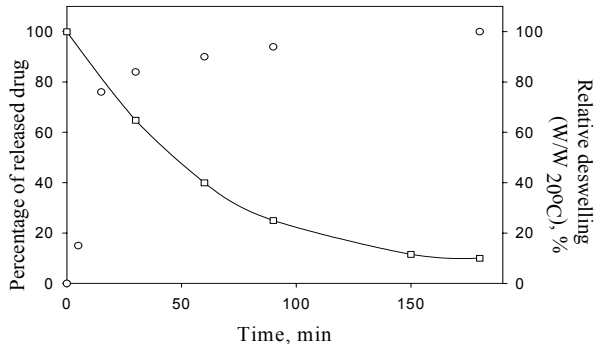

Fig.5. Kinetics of Lisophtal release (o) and deswelling kinetics of NIPAA gel (□) at 37°.

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