Thermocontrolled release of some bioactive substances from poly(N-isopropylacrylamide) hydrogel matrix

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Introduction

Immobilization of biologically active substances, such as drugs, proteins, DNA, enzymes and living cells within stimuli-responsive hydrogels is of great interest for medicine, pharmaceutics, biotechnology, bio- and gene-engineering (Peppas, 2006). Of special interest is homo- and copolymers of N-isopropylacrylamide (NIPA) that undergo a sharp volume transition around the body temperature (Rzaev, 2007). Many researchers have examined the potential application of NIPA-based polymers for encapsulating and delivering purposes (Hoffman, 2004; Lee, 2007). The aim of this work is to encapsulate local anesthetic drug – richlocaine, proteins – bovine serum albumin (BSA) and lysozyme within NIPA-based hydrogels and to study the oscillating "on-off" release of bioactive substances in response to temperature change.

Materials and methods

Monolith hydrogels possessing pH- and thermoresponsibility have been synthesized by copolymerization of NIPA and 2-acrylamido-2-propanesulfonic acid (AMPS) (or acrylic acid (AA) in the presence richlocaine. BSA and lysozyme were immobilized within the PNIPA hydrogels by sorption of proteins from aqueous and buffer solutions. Diffusion parameters of bioactive substances into hydrogel matrices were determined by formula: $M_t/M_\infty = kt^n$ (where M_t is the mass uptake of the swelling medium at time t, M_∞ is the mass of hydrogel at equilibrium-swollen state, k is the characteristic swelling constant, n is the swelling exponent that describes the diffusion mechanism of bioactive agents).

Results and Discussion

Fig. 1 shows the temperature-dependent volume-phase transitions of NIPA-based hydrogels.

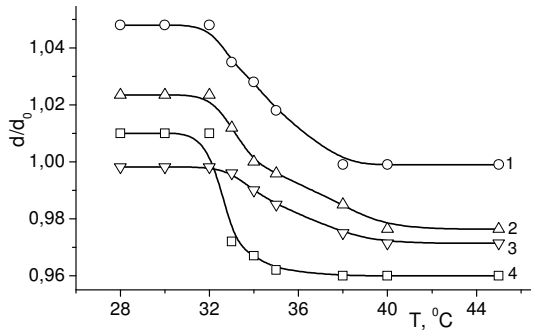

Fig.1. Change of diameter of NIPA-AA (1), NIPA-AA-Richlocaine (2), NIPA-AMPS (3) and PNIPA in water in dependence of temperature.

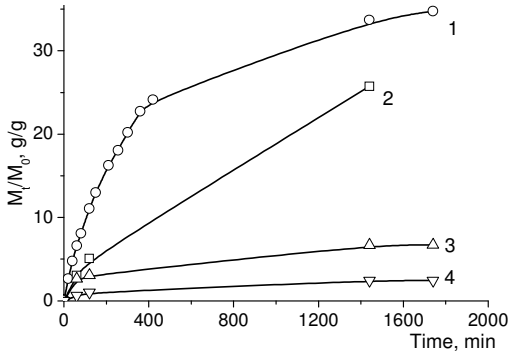

Fig. 2. Swelling of NIPA-AA (1,2) and PNIPAM (3,4) in aqueous solution of 10^{-3} mol/L richlocaine (1), phosphate buffer of 0.05% lysozyme (2), in aqueous solution (3) and phosphate buffer of 0.1% BSA (4).

It is suggested that complex formation between bioactive molecules and functional groups of hydrogels is responsible for immobilization. The lower critical solution temperature (LCST) of PNIPA can be tuned to the desired value by introduction of hydrophobic or hydrophilic fragments. The swelling-deswelling behavior of hydrogels is changed in the following order: NIPA-AMPS (35 °C) > NIPA-AA (34.4 °C) > NIPA-AA/Richlocaine (33.8 °C) > PNIPA (32.7 °C). Swelling dynamics of PNIPA, NIPA-AMPS and NIPA-AA in aqueous solution of richlocaine and phosphate buffer solutions of BSA and lysozyme are shown in Fig.2. Dynamic swelling parameters of bioactive substances into hydrogel matrices are summarized in Table 1.

Hydrogel/Bioactive substances	[Bioactive substances]	In water		In phosphate buffer	
		n	$k \cdot 10^2$	n	$k \cdot 10^2$
NIPA-AMPS (1:1 mol/mol)	-	1.01	1.4	-	-
NIPA-AMPS (1:1)/Richlocaine	0.001 mol/L	0.93	9.1	-	-
NIPA-AMPS (3:1 mol/mol)	-	0.82	8.9	-	-
NIPA-AMPS (3:1)/Richlocaine	0.001 mol/L	0.84	2.5	-	-
NIPA-AA (3:1 mol/mol)	-	0.90	2.7	-	-
NIPA-AA (3:1)/Richlocaine	0.001 mol/L	1.03	2.1	-	-
PNIPA/BSA	0.1%	0.42	18	0.63	10
NIPA-AA (3:1)/BSA	0.1%	0.68	5.3	0.74	4.5
PNIPA/Lysozyme	0.05%	0.71	9.9	-	-
NIPA-AA (3:1)/Lysozyme	0.05%	-	-	0.53	19.2

Table 1: The values of *n* and *k* for NIPA-based hydrogels within of which the bioactive substances are embedded.

The values of *n* ranging between 0.42 and 0.93 indicate that the loading mechanism of bioactive substances deviates from Fickian diffusion. For NIPA-AMPS and NIPA-AA/Richlocaine systems the diffusion mechanism is relaxation-controlled because their *n* values are close to 1. Temperature-dependent release of richlocaine from the NIPA-AA hydrogels is shown in Figs. 3 and 4.

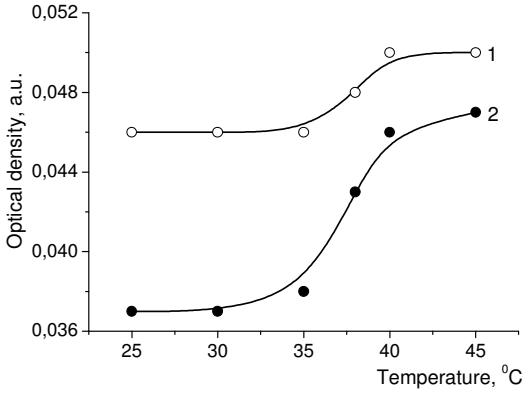

Fig. 3. Temperature-dependent richlocaine release from the NIPA-AA (3:1 mol/mol) hydrogel matrix into pure water pH=5.5 (1) and phosphate buffer (2). pH = 7.4. $C_{Richlocain} = 1 \cdot 10^{-3}$ mol/L.

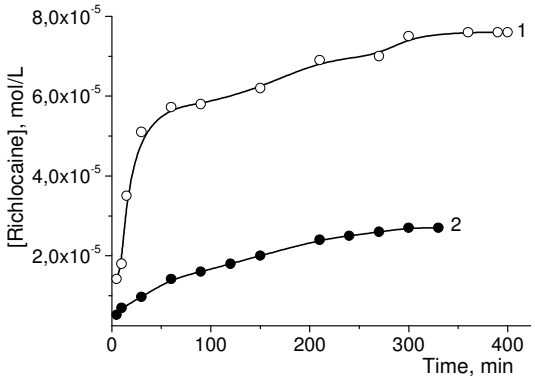

Fig. 4. Time-dependent richlocaine release from NIPA-AA (3:1 mol/mol) hydrogel matrix at 40 ^{0}C (1) and 35 ^{0}C (2). Phosphate buffer, pH = 7.4, μ = 0.15 M NaCl.

As seen from Fig.3, a sharp increase of optical density of richlocaine coincides well with volume transition of NIPA-AA (see Fig.1). The amount of released richlocaine was equal to 20% and 7% at 40 ^{0}C and 35 ^{0}C respectively. The *n* value that is equal to 0.52 at 40 ^{0}C reflects the Fickian diffusion while the *n* = 0.26 at 35 ^{0}C is characteristic for anomalous diffusion of richlocaine. Drug release rate from NIPA-AA is minimal around pH 5 then it gradually increases and levels off at pH 8 (Fig.5).

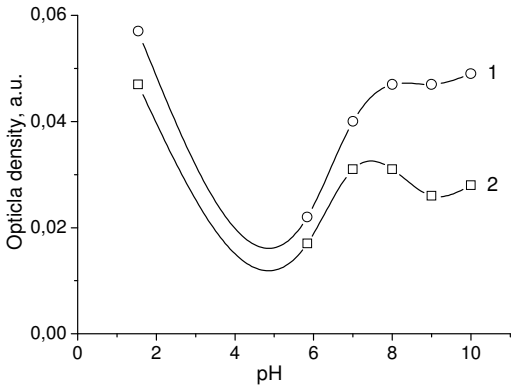

Fig. 5. pH-dependent release of richlocaine from NIPA-AA hydrogel volume. $C_{Richlocain}$ = 3·10^{-3} (1) и 1·10^{-3} (2) mol/L.

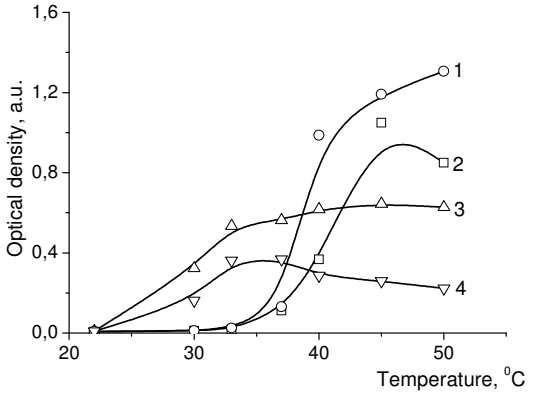

Fig. 6. Temperature-dependent release of lysozyme (1,4) and BSA (2,3) from PNIPA into water (1,2) and phosphate buffer (3,4). C_{BSA} = 0.1%; $C_{Lysozyme}$ = 0.05%.

This is accounted for strong electrostatic interactions between COO^- groups of NIPA-AA and NH^+ groups of richlocaine because at pH 5 the carboxylic groups of AA are partly ionized while the richlocaine molecules are in salt form. In strong acidic region the ionization of carboxylic groups is fully suppressed and the electrostatic interactions between COOH and NH^+ groups are vanished. This is in favor of richlocaine release from hydrogel volume. In basic region the carboxylic groups are in fully ionized while the richlocaine molecules are in deprotonated states. This leads to destruction of ionic bonds between NIPA-AA and richlocaine and consequently to release of drug to environment medium. In aqueous solution the optical density of proteins sharply increases at the range 35-45 ^{0}C. In phosphate buffer the release of proteins has discontinuous character and lies between 22 and 32 ^{0}C. The latter is probably accounted for charge effects of proteins. The response kinetics of the oscillating swelling-deswelling behavior to the temperature cycles across the LCST between 25 and 40 ^{0}C is shown in Fig. 7. The hydrogels are in swollen and shrunken states at 25 ^{0}C and 40 ^{0}C respectively. The drug and proteins release profile exhibits a similar trend with swelling-deswelling behavior of hydrogels. The initial release of bioactive substances is due to presence of surface encapsulated components which are squeezed out during the first temperature pulse. Release of richlocaine, BSA and lysozyme at T < LCST is governed by diffusion. At T > LCST the surface of the hydrogel shrunk immediately and forms impermeable "skin" layer restricting the release of immobilized bioactive molecules. The second and third temperature pulses lead to decreasing of the release rate due to decreasing the concentration of bioactive substances in hydrogel volume.

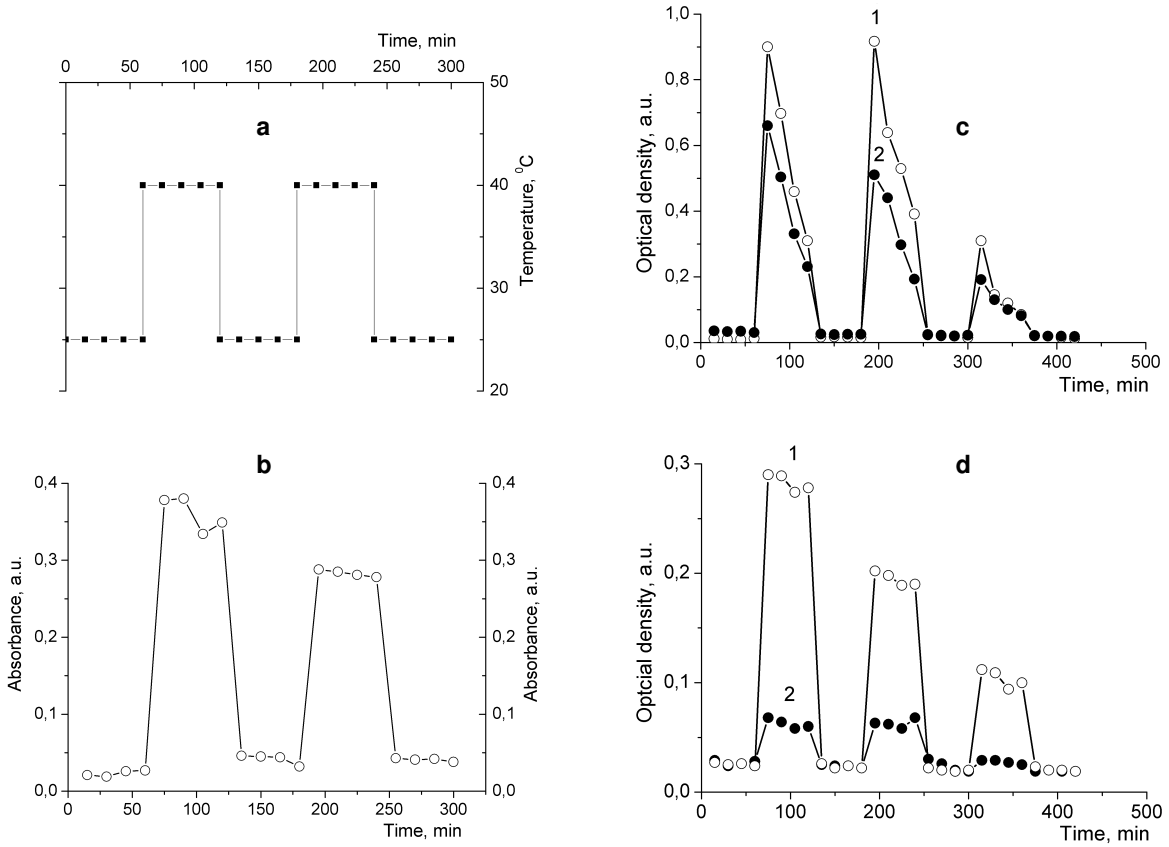

Fig.7. Oscillating change of temperature (a) and time-dependent pulsatile release of BSA (b), richlocaine (c) and lysozyme (d) from PNIPAM hydrogel into phosphate buffer (1) and water (2) at 25 and 40 ^{0}C.

Conclusions

Thus richlocaine, bovine serum albumin (BSA) and lysozyme were immobilized within the thermo- and pH-sensitive hydrogel matrix – PNIPA, NIPA-AMPS and NIPA-AA. The values of *n* and *k* characterizing the sorption mechanism of bioactive substances by hydrogels were calculated. Oscillating "on-off" release mechanism of drug and proteins from the volume of PNIPA and NIPA-AA hydrogels was observed in the course of cyclic shrinking and swelling of hydrogels in water and phosphate buffer at 25 and 40 ^{0}C.

Bibliography

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