THE USE OF THERMORESPONSIVE HYDROGEL MEMBRANE AS MODULATED DRUG DELIVERY SYSTEM

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ABSTRACT

Stimuli-sensitive polymers are suitable candidates for novel drug delivery systems, since they release drugs in a controlled manner in response to a stimulus such as temperature. In the present study temperature-sensitive polymer of N-isopropylacryamide (NIPAAm) was evaluated to modulate release of drugs with different molecular weights. Membranes of poly NIPAAm and its copolymers with acryl amide (AAm) were prepared by casting monomers, cross linker, and initiator between two glass plates with a defined spacer thickness. These thermo sensitive hydrogels that cross linked with N,N-methylene-bis-acrylamide (MBAAm) showed a swelling transition temperatures (37°C) that was used in the permeation control of hydroxy urea (HU) and erythromycin (Er). Permeation rates of the drugs in various temperatures were investigated. It was shown that the diffusion rate of HU and Er through membranes is increased with a decrease in temperature. This phenomenon may be explained by the swelling (hydration) properties of the polymers and the thermodynamic influence of temperature and may be used as on-off switching key for controlled release of different molecules.

Keywords: Thermosensitive hydrogels, Membranes, Hydroxyurea, Erythromycin, Poly NIPAAm, Copoly NIPAAm/AAm.

INTRODUCTION

Stimuli-sensitive polymers which their structure and physical properties change in response to external signals have a potential in the design of drug delivery systems. Particularly, self-regulation or auto feedback drug release systems may be achieved by utilization of stimuli-sensitive polymers (1). Non-responsive controlled drug delivery systems release drugs in a fashion similar to IV infusion with a zero or nearly zero order kinetic profile. Since blood concentration of endogenic substances such as hormones fluctuates in response to a stimulus, the best drug delivery system would be the one that releases its contents in response to stimuli in a similar fashion. Central to this investigation is the science of polymers which is involved in the fabrication of the devices to achieve zero order or modulated drug release. Approaches such as swelling controlled, biodegradable, stimuli sensitive polymers have received much attention (2). Stimuli-responsive polymers have been developed by considerable efforts to regulate the degree of swelling of polymers in relation to external environmental conditions such as pH changes (3-6), magnetic field (7,8), temperature changes (9,10), ultrasonic stimuli (11,12), electrical energy (13,14), photoelectric stimuli (15), and chemical changes (16-18). The major application of functional polymers in drug delivery system is to control the permeation of drugs. Stimuli-sensitive polymeric membranes in drug delivery systems act simultaneously as a sensor for detection of the biological conditions or external stimuli and as a rate controller for drug release (18). Covalently cross-linked temperature sensitive hydrogels such as co-poly NIPAAm/AAm are perhaps the most extensively studied class of environmentally sensitive polymer systems in drug delivery (19-21). At least one component of the hydrogel should possess temperature–dependent solubility in water. In order to obtain a hydrogel that dramatically changes its swelling degree in water, the gel constituents must be insoluble above or below a certain temperature, called the lower or upper critical solution temperature (LCST or UCST, respectively). For drug release applications, LCST systems are mainly relevant. The abrupt shrinking of temperature-responsive gels above the LCST results in such a drastic change in their swelling degree that have called forth a rather extensive research effort for application of hydrogels to the

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controlled release of drugs. Poly NIPAAm hydrogels show negative temperature sensitivity i.e. they tend to uptake water and swell at temperatures below a phase transition temperature (32°C). They can be co-polymerized with hydrophilic co-monomers such as AAm to have a higher phase transition temperature and when cross-linked, they show a phase transition temperature higher than 32 °C above which they shrink and below that they absorb water. This hydrogel has been applied as a functional material for control of drug release rate and for temperature responsive intelligent drug delivery system (22). In this study the possibility of "on-off" regulation of drug release was investigated by the use of co-poly NIPAAm/AAm hydrogels in response to temperature change with stepwise temperature change above and below the phase transition temperature. Two substances, one, Hydroxy urea (HU) with low molecular weight of 76 and the other, erythromycin (Er) with high molecular weight of 452 were used as model drugs to study the effect of temperature on the diffusion and permeation of these drugs through membranes. The use of Hydroxy urea in cancer therapy and erythromycin in infectious diseases may be regarded as a possible application of these systems.

MATERIALS AND METHODS

Materials

N-isopropylacrylamide (NIPAAm), Acrylamide (AAm), N,N-methylene-bis-acrylamide (MBAAm), N,N,N',N-tetra-methyl-ethylenediamine (TEMED), and Ammonium persulfate (AP) were obtained from Aldrich, Hydroxyurea (HU) and Erythromycin (Er) were obtained from Sigma, UK. Iodine, Potassium iodide, Sodium bicarbonate, Sodium hyposulfite, Starch, Potassium mono hydrogen phosphate and Potassium dihydrogen phosphate were obtained from Merck, Germany. All other chemicals used were of analytical reagent purity and water was double distilled and freshly prepared.

Methods

Preparation of thermoresponsive hydrogel membranes

Thermoresponsive membranes of 0.75 mm thickness with a phase transition temperature of 37 °C were prepared by employing a free radical polymerization method. A solution of 10% (w/v) NIPAAm (monomer), 1% (w/v) acrylamide (co-monomer), and 0.2% (w/v) MBAAm (cross-linker) in 60 ml of distilled water were stirred under nitrogen gas for 30 minutes. Then 40 mg of AP and 40 µl of TEMED as co-initiators were added to the solution and stirring under nitrogen gas was continued for one more minute. The solution was then injected between two glass plates of a gel casting system (Hoefer SE 600, Pharmacia Biotech, USA) and polymerization was allowed to proceed (10). The membranes were then cut into discs by the use of a steel punch with internal diameter of 50 mm. The transition temperature of hydrogel membranes were determined by DSC (Mettler TA 4000 System, Switzerland).

Drug permeation determination

A two chamber diffusion cell was used to determine the drug permeation through hydrogel membrane. The volume of each chamber was 75 ml. The membrane discs were assembled between the chambers. The diffusion media for HU and Er were Sörensen buffer (pH 7.4) and alcoholic buffer solution (pH 8.0) respectively. The donor chamber was filled with drug solution and the receiver chamber with buffer solution.

Both chambers were stirred by the use of a glass stirring rotator at 50 rpm. The cell was placed in a thermostated water bath (Memmert, Italy) to control temperature. The drug flux through hydrogel membrane at temperatures below and above the phase transition temperature of hydrogel was recorded as a function of temperature.

In this experiment, membranes were pre-equilibrated in water at the same temperature range (25 °C, 31 °C, 43 °C, and 45 °C) at which the diffusion studies were performed. The release of HU and Er from the thermoresponsive membrane was investigated at different temperatures of
25°C, 31°C (below LCST), 43 °C, and 45 °C (above LCST).

Samples of HU and Er from the release media were collected at pre-selected time intervals and. HU was measured according to BP 98 method and Er was measured by UV-Vis spectrophotometer at 486 nm (Shimadzu 2100, Japan). The permeability coefficients (P) were calculated according to the following equation: $M=PSC_d t$, Where M is the amount of drug permeated to the receiver at time t, P is the permeability coefficient, C_d is the concentration of permeate in the donor compartment (which is considered constant during the time of permeation study); S is the effective surface area for permeation. The slope of the straight line when plotting M versus t yields PSC_d, of which S and C_d are known, and P can be easily calculated.

RESULTS AND DISCUSSION

As copoly NIPAAm/AAm exhibits temperature sensitivity, it was chosen for the development of a thermoresponsive drug delivery system. The phase transition temperature of the hydrogel, as determined by DSC was 37°C. HU and Er were selected as model drugs to investigate the permeability of a low and high molecular weight substance through the thermosensitive hydrogel membranes. The results for HU and Er release through the thermoresponsive hydrogel membrane are shown in figures 1-3 and 4-6 respectively.

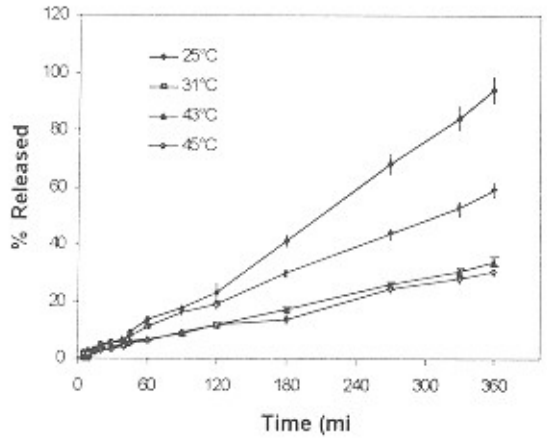

Fig. 1. Hydroxy urea release from thermo-responsive hydrogel membrane at different temperatures±SD (n=3).

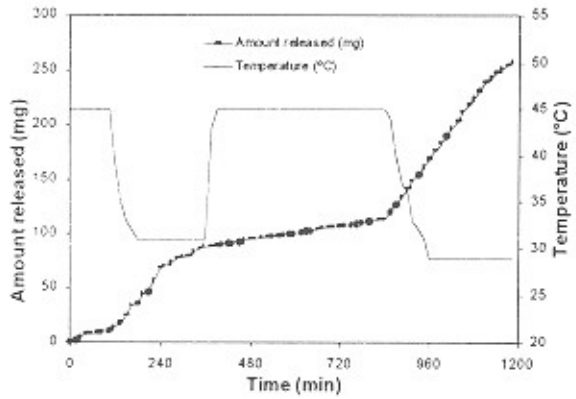

Fig. 2. On-off release of hydroxy urea at temperatures fluctuated below and above phase transition temperature of membrane.

Figures of 1 and 4 show the release of HU and Er through thermoresponsive membrane at temperatures below and above the phase transition temperature of the hydrogel. These results indicate that the release rate from membrane varies with temperature considerably. The rate of HU and Er release at temperatures below the phase transition temperature (25°C and 31°C) are similar and are much higher than temperatures above the phase transition temperature of the hydrogel membrane.

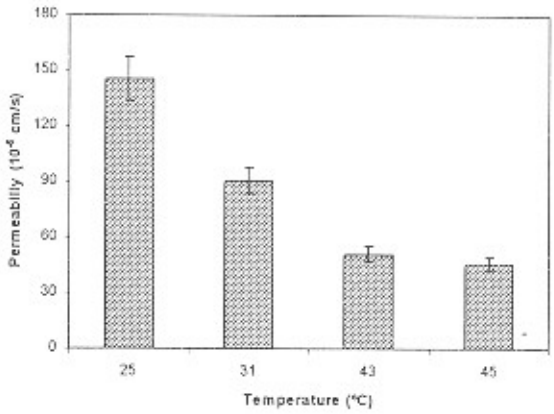

Fig. 3. Permeability of hydroxy urea through thermo-responsive hydrogel membranes at different temperatures ± SD (n=3).

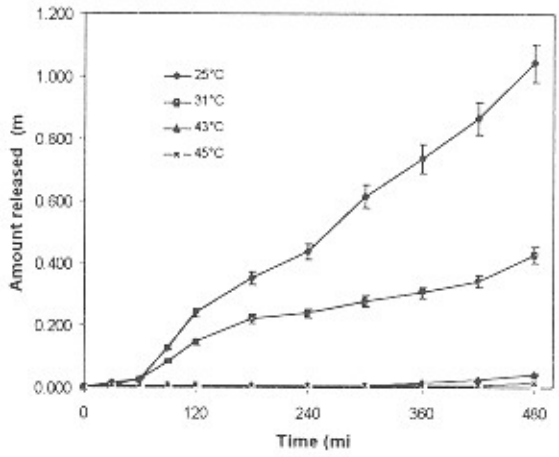

Fig. 4. Erythromycin release from thermo-responsive hydrogel membrane at different temperatures±SD (n=3).

The release of Hydroxy urea through thermoresponsive hydrogel membrane at temperatures below and above the phase transition temperature of hydrogel followed zero-order kinetics (table 1). However, erythromycin release through thermoresponsive hydrogel membrane did not follow the same pattern. At temperatures above the phase transition temperature of hydrogel, no or very small drug release was observed. At temperatures below phase transition temperature of hydrogel, drug release followed zero order kinetics

(table 1). HU release through hydrogel membrane at temperatures above the phase transition temperature was reduced markedly, but did not completely cease. This may be due to the fact that hydrogels are not completely dehydrated at temperatures above the phase transition temperature and the permeation of the low molecular weight HU is still possible. Figures 2 and 5 show the effect of temperature fluctuation of the drug release medium between 31°C (below the phase transition temperature of the hydrogel) and 45°C (above the phase transition temperature of the hydrogel) from thermoresponsive membranes. As can be seen, on-off drug release occurs and drug release is markedly reduced at temperatures above the hydrogel phase transition temperature.

Table 1. Effect of temperature on R^2 and slope of zero order curve fitted release of hydroxy urea through thermoresponsive hydrogel membranes

Temperature	25°C	31°C	43°C	45°C
R^2	0.990	0.999	0.995	0.990
Slope	0.250	0.162	0.094	0.085

The results of HU and Er permeability through a thermosensitive hydrogel membrane are summarized in figure 3 and 6.

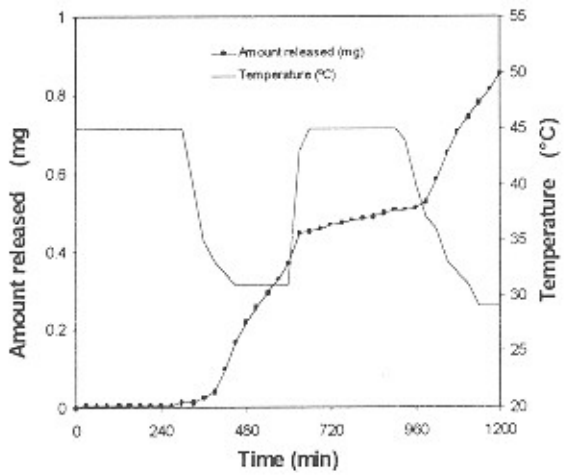

Fig. 5. On-off release of erythromycin at temperatures fluctuated below and above phase transition temperature of hydrogel membrane.

For a normal polymer depending on the magnitude of its activation energy, which should be overcome by thermal energy, the solute diffusion is expected to increase with increase in temperature. However, as can be seen in figures 3 and 6, for thermo-responsive polymers, the permeability of HU and Er decreases with increase in the temperature above the phase transition temperature of the hydrogel. This may be attributed to a direct enthalpic effect as well as a swelling effect. The direct effect of temperature depends on the thermosensitivity of hydrogels swelling.

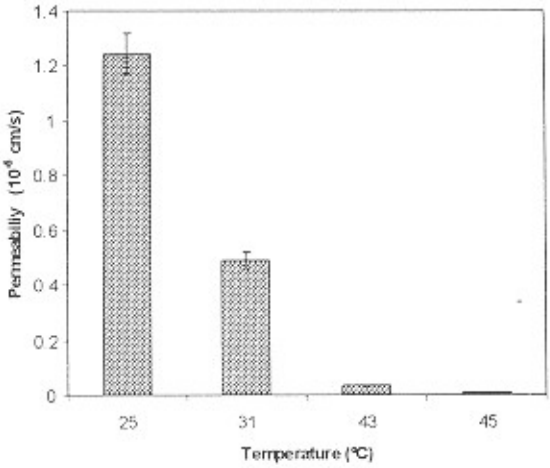

Fig. 6. Permeability of erythromycin through thermoresponsive hydrogel membranes at different temperatures ± SD (n=3).

The role of water content and temperature in the diffusion of hydrophilic solutes through swollen membranes has been described by the free volume theory by Yasuda et al. (24) and more recently, in a modified theory by Peppas et al. (25). These theories include several parameters, such as diffusional activation energy and diffusional jump length. In the case of thermoresponsive hydrogels, all parameters may be mutually affected. Therefore, difficulties may be encountered in applying the free volume approach to solute permeation through thermosensitive hydrogels in studying each factor independently. The water content, however, is still a strong factor which affects solute permeation. These results indicate that the major factor affecting solute permeation through thermosensitive polymers is the hydration of polymers. Here, the role of temperature is an external stimulus, which induces a swelling change for a given polymer. Figure 3 and 6 represent the changes in HU and Er permeability, by variation of the temperature during permeation experiments. In these experiments, the membranes were pre-equilibrated in diffusion medium at each temperature. The stepwise temperature change during permeation was achieved by changing the

setting of temperature to each desirable degree. It was noticed that no significant lag times were observed in each experiment, which may be attributed to relatively rapid swelling changes of the membrane in response to temperature fluctuation. Complete reversible change of the drug permeability was also observed in response to a temperature change between 31°C and 45°C. Results showed increased permeation rate at lower temperatures. The increase in the rate of amount diffused at 31°C, is slightly higher than expected from the individual permeation study. Increased hydration, as well as an increased effective surface area may cause this phenomenon. The increase in surface area may be due to an overall increase in dimensions of the membrane at lower temperature. However, the membrane was fixed within the diffusion cell, and membrane expansion was not controlled.

CONCLUSION

In this study the use of thermoresponsive hydrogel membranes for controlled release of model drugs has been demonstrated. The drug release from thermoresponsive membrane could be significantly reduced when temperature was above the phase transition temperature of the polymer and significantly increased when the temperature was lowered below the phase transition temperature of the hydrogel. However this on-off drug release is more efficient when higher molecular weight entities are used. In this study, it is shown that the release of small molecular weight entities such as hydroxy urea at temperatures above the phase transition temperature of thermoresponsive hydrogel membranes could not be ceased completely which is a requirement for an ideal on-off controlled drug delivery system.

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