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A novel thermo-responsive drug delivery system with positive controlled release

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Abstract

The model drug, 5-fluorouracil (5-FU) was loaded into the poly(*N*-isopropylacrylamide) (PNIPA) hydrogel at 25 °C, then the drug-loaded, swollen hydrogel sample was carefully enveloped in the dialysis bag to form a novel thermo-responsive drug delivery system (DDS). The concentration of released 5-FU was monitored at 266 nm on the UV spectrophotometer. We found that this novel DDS provides a positive drug release pattern and the drug, 5-FU, was released faster at the increased temperature (37 °C, >25 °C) than the one at the decreased temperature (10 °C, <25 °C). This was attributed to the double control of the thermo-sensitivity of the hydrogel matrix and the dialysis membrane. By employing the fast response PNIPA hydrogel instead of the conventional hydrogel in this novel DDS, we can further control the drug release rate and/or drug release amount etc., without changing the positive, thermo-responsive drug release pattern.

Keywords: Thermo-responsive; Poly(*N*-isopropylacrylamide); Hydrogel; Controlled release; Drug delivery system

1. Introduction

Recently, there has been increased interest in responsive hydrogels for utilisation as the smart drug delivery system (smart-DDS) in the field of controlled drug release, to meet the need for prolonged and better control of drug administration (Hoffman et al., 1986; Bae et al., 1987; Hoffman, 1991; Bromberg and Ron, 1998; Park, 1999; Ranjha and Doelker, 1999; Sershen et al., 2000; Kost and Langer, 2001). As we know, in conventional drug delivery, the drug concentration in the blood increases to a toxic level as the drug is taken, then the drug concentration decreases to an ineffective level and the patients have to take the drug frequently. In order to eliminate or reduce the above disadvantages, drug delivery system (DDS) for control release was designed to maintain the drug release with the predetermined dose and prolong the curing-time in the targeted body compartment. Compared to the conventional DDS, the advantages of the

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smart-DDS are self-evident because the drug amount can be auto-controlled by external changes, such as temperature, electric fields, pH and photo fields etc.

Thermo-responsive hydrogel is a most extensively studied, responsive and polymeric material, including various polymers, such as the *N*-substituted polyacrylamide, polymethylacrylamide and poly(ethylene oxide) etc. Poly(*N*-isopropylacrylamide) (PNIPA) hydrogel is a typically thermosensitive material, which exhibits a phase transition temperature (T_{tr}) or lower critical solution temperature (LCST) at ≈ 33 °C (Hirokawa and Tanaka, 1984; Hoffman, 1991; Takei et al., 1994; Liang et al., 1998; Zhang and Zhuo, 2001). As the external temperature cycles around this phase transition temperature, the polymer chains undergo a coil-globule transition (Wang et al., 1998; Wang and Wu, 1999). Correspondingly, the three-dimensional PNIPA hydrogel returns to a shrunken state and displays phase separation, i.e. abrupt collapse in volume as the temperature is increased above LCST. The abrupt shrinking in the volume of the PNIPA hydrogel to the increased temperature has produced extensive research interest directed at applications to the controlled release of drugs.

Normally, the selected drug is physically loaded in the swollen thermo-responsive hydrogel and the drug release is controlled by the external temperature changes due to the thermo-reversible properties of the PNIPA hydrogel. Generally regarded, the drug exhibits a Fickian release (Cussler et al., 1984), which depends on the swelling ratio of the hydrogel. As the temperature is increased above the LCST, PNIPA hydrogel may shrink and quickly form a dense, thick skin layer (Matsuo and Tanaka, 1988; Kaneko et al., 1995), which leads to the burst release initially and then the release of the drug in the network matrix is stopped. A typical release pattern was reported by Kim's research group (Bae et al., 1989) and an on–off release pattern of the model drug, indomethacin, was achieved by regulating the temperature between 20 and 30 °C. A series of investigations based on the thermo-responsive hydrogels was carried out and much useful data were obtained (Hoffman, 1987; Bae et al., 1989; Okano et al., 1991; Kim et al., 1992; Schild, 1992; Kim et al., 2000). In these cases, the thermo-responsive hydrogels provided a negative temperature-responsibility to the drug release, i.e. slow drug release at increased temperature and rapid drug release at decreased temperature.

In some cases, a positive controlled release pattern, i.e. rapid drug release at increased temperature and slow drug release at decreased temperature, is urgently needed when the DDS is specially designed to respond to an increase in the body temperature resulting from diseases, such as inflammation or cancers etc. In this paper, we proposed a novel thermo-responsive DDS to give a positive controlled release pattern. 5-Fluorouracil (5-FU) is an antineoplastic drug, used in the palliative treatment of cancers of the gastrointestinal tract, breast and respiratory tract etc. Here, 5-FU was chosen as a model drug which was loaded into the PNIPA hydrogel at room temperature (25 °C), then the drug incorporated hydrogel was carefully enveloped in the dialysis bag to form a novel DDS with double controlled release layers (the gel network and the dialysis membrane). At different predetermined temperature (10 and 37 °C), the concentration of released 5-FU was monitored at 266 nm on an UV spectrophotometer. We found that 5-FU was released more rapidly at 37 °C (>25 °C) than at 10 °C (<25 °C). Furthermore, a fast responsive PNIPA hydrogel was used in this paper to demonstrate that the drug release rate and the release amount of this DDS can be modulated without reversing the positive controlled release pattern. This novel, thermo-responsive DDS may be useful in cases where the positive drug controlled release is needed.

2. Materials and methods

2.1. Materials

N-isopropylacrylamide (NIPA) was synthesised and purified according to Liu et al. (1993)). *N*,*N*′-methylenebisacrylamide (MBAAm), ammonium persulfate (APS) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) were all of analytical grade and used without further purification. 5-Fluorouracil (5-FU, $M_w = 130.08$,

Wuhan drug plant, Wuhan, People's Republic of China) was purified by recrystallisation in distilled water three times. The dialysis bag, with a molecular weight cut-off of 2000 (Sigma, St. Louis), was used as provided.

2.2. Preparation of PNIPA hydrogels

The polymerisation of the conventional PNIPA hydrogel (designated as Gel-con) was carried out in distilled water solution (NIPA concentration 5 wt.%) at room temperature (25 °C) for 6 h, using APS and TEMED as a pair of redox initiator. MBAAm was used as the crosslinker (1.5 wt.% based on monomer). Here, the polymerisation of the fast responsive PNIPA hydrogel (designated as Gel-fast) was carried out at −18 °C for 24 h, according to the method we reported previously (Zhang and Zhuo, 1999a) with the same chemical composition as that of Gel-con. After the polymerisation, the produced hydrogels were cut into discs. These samples were immersed in distilled water at room temperature for 48 h and the water was refreshed every few hours in order to leach out the chemical residue during this period.

2.3. Drug loading and DDS preparation

The swollen PNIPA sample was dried in vacuum overnight until its weight remained unchanged. The vacuum dried PNIPA xerogels were immersed in the 0.5 wt.% 5-FU aqueous solution at 25 °C for at least 24 h to reach the equilibrated state. During this period, the drug diffused into the hydrogel network with the water. Then, the drug loaded hydrogel system was carefully enveloped in the dialysis bag, ensuring the removal of the air bubbles in the dialysis bag before sealing and thus, the drug-loaded hydrogel and the dialysis bag coating combined to form a novel DDS. Here, the DDS containing the Gel-con was designated as DDS-con, while the DDS containing the Gel-fast was designated as DDS-fast.

2.4. Standard absorbance curve

The standard calibration curve of the absorbance as a function of the 5-FU concentration was studied at 266 nm on the UV spectrophotometer.

2.5. In vitro drug release study

5-FU release experiment was conducted by immersing the above drug loaded DDS into the distilled water, equipped with an external stirrer (100 *r*/min) at 10 and 37 °C, respectively. During the drug release experiment, 2 ml aliquots of the release media was taken out with reconstitution of 2 ml fresh distilled water at every predetermined time interval and the concentration of the 5-FU released from this DDS was monitored at 266 nm using the UV spectrophotometer.

3. Results and discussion

3.1. Drug delivery system design

The thermo-responsive mechanism of the PNIPA hydrogel has been intensively studied (Bae et al., 1990; Inomato et al., 1990; Tokuhiro et al., 1991; Feil et al., 1993). Fig. 1 shows the chemical structure of the monomer (NIPA), crosslinker (MBAAm) and the synthesis representation of the PNIPA hydrogel. From this figure, we can find a hydrophilic/hydrophobic balance in the side chains of PNIPA, which brings about the hydrogen bonds between the water molecules and hydrophilic groups, as the hydrogel contacts with water. These hydrogen bonds act cooperatively to form a stable shell around the hydrophobic groups, which lead to the good solubility of the PNIPA hydrogel at low temperature. As the temperature is increased, these interactions become weak and the entrapped water molecules is released out slowly. When the temperature reaches the LCST or above, these interactions are destroyed and the hydrophobic interactions among the hydrophobic groups are strengthened dramatically and abruptly, which leads to the collapse of the polymer chains and the phase transition of the network of hydrogel. Here, we point out that at the temperature range below the LCST, the volume of the PNIPA hydrogel also decreases with the increase of temperature, although the decrease in volume is tender and small.

Fig. 2 schematically illustrates the novel, smart-DDS, which was designed to demonstrate a positive, thermo-responsive drug release. In this DDS, the key is try to envelope the drug loaded PNIPA hydrogel at a suitable temperature with the dialysis bag. During the release process, the loaded drug need diffuse/transit two release layers (one is the gel matrix and the other is the dialysis membrane) to reach the release media. That is to say that the drug release is double controlled by the gel matrix and the dialysis membrane sequentially. Due to the thermo-sensitivity of the PNIPA hydrogel and the non-responsive nature of the dialysis membrane, the positive, thermo-responsive drug release pattern of this novel DDS is possible.

As the above smart-DDS is immersed in the release media at the decreased temperature (below the drug loading temperature), the PNIPA hydrogel in this system swells and the volume of hydrogel becomes enlarged, which leads to the block of the pores in the dialysis membrane enveloped on the gel's surface. As a result, the loaded drug in the hydrogel network is blocked from diffusing out from the pores of the dialysis membrane. On the other hand, when the smart-DDS conducts the drug release at an increased temperature (above the drug loading temperature), the PNIPA hydrogel will shrink and the volume of hydrogel is decreased, that leads to many voids between the gel matrix and the dialysis membrane. Thus, the drug will diffuse out through the voids quickly. Of course, at this stage the drug release rate is controlled by the suitable dialysis membrane. Obviously, during the shrinking process, some parts of the loaded drug will be entrapped in the shrunken gel and the amount of entrapped drug depends on the thermo-response rate and decrease in volume etc.

3.2. Standard calibration curve

The calibration curve of the absorbance as a function of the 5-FU concentration at 266 nm, shown in Fig. 3, has a linear relationship with a correlation coefficient (r) of 0.9999. This linear relationship can be quantificationally described as the following equation: $A = (55.834c - 10.93) \times 10^{-3}$, where A is the absorbance and c is the concentration (ug/ml) of the drug (5-FU).

3.3. In vitro drug release from the DDS-con

Fig. 4 shows the cumulative drug release from the DDS-con in distilled water at two temperatures (10 and 37 °C). This figure clearly shows

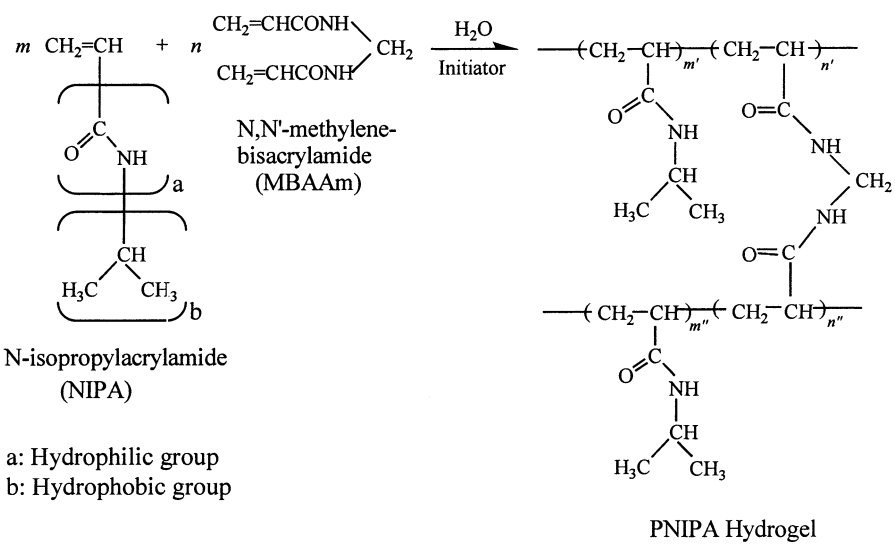

Fig. 1. Chemical structure of the monomer (NIPA), crosslinker (MBAAm) and the synthesis representation of the PNIPA hydrogel.

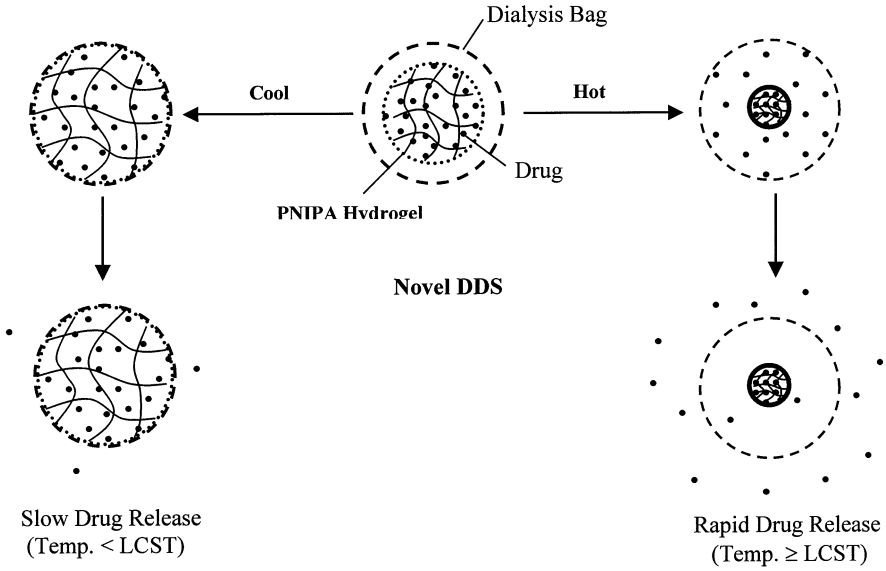

Fig. 2. Schematic illustrations of the novel, thermo-responsive drug delivery system (DDS) to give a positive drug release by modulating the external temperature.

that the drug is released faster in a hot environment (37 °C) than in a cooler environment (10 °C). This is attributed to the thermo-response in volume of the conventional PNIPA hydrogel in the DDS, as discussed above. The release curves in this figure directly support that our above considerations and DDS design is effective in giving a positive drug controlled release, i.e. drug release rate is accelerated at an increased temperature.

3.4. In vitro drug release from the DDS-fast

In the DDS-fast, the fast response PNIPA hydrogel (Gel-fast) was used instead of the conventional PNIPA hydrogel. In some applications of the thermo-responsive PNIPA hydrogels, such as the on–off switches (Bae et al., 1987), the response rate and the volume changes of the conventional PNIPA hydrogel is unsatisfying and there is a clear need for the thermo-responsive gel with an improved response rate and enlarged volume changes to the external temperature changes. Several attempts (Kabra and Gehrke, 1991; Wu et al., 1992; Yoshida et al., 1995; Kaneko et al., 1998) have been proposed to achieve fast thermo-responsive PNIPAAm gel and we also proposed several strategies in this regard (Zhang and Zhuo, 1999a,b,c, 2000, 2001). Here, we chose the cold-synthesized PNIPA hydrogel, which exhibits rapid deswelling rate, as well as the rapid reswelling rate (Zhang and Zhuo, 1999a). On the other hand, the volume changes to the temperature of this hydrogel are also larger than that of the conventional hydrogel. Obviously, the enlarged changes in volume of the Gel-fast in the

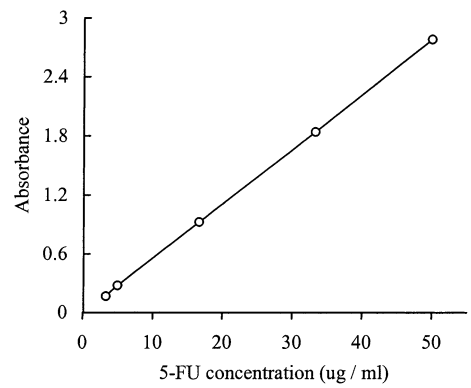

Fig. 3. The standard calibration curve of the absorbance as a function of the 5-FU concentration at 266 nm on the UV spectrophotometer.

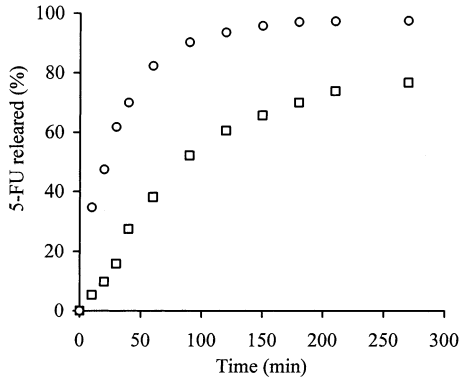

Fig. 4. Cumulative 5-FU release as a function of the time from DDS-con (10 °C: □; 37 °C: ○).

DDS-fast at the different temperature will be more notable, compared with volume changes of the Gel-con. As a result, the pores in dialysis membrane are seriously blocked and the drug release from these blocked pores is also seriously prevented when the DDS-fast conducts the drug release in the cool release media (10 °C). On the other hand, when the DDS-fast conducts the drug release in the hot release media (37 °C), the drug can be released more rapidly because much more voids are produced due to the dramatically fast shrinking rate of the Gel-fast at 37 °C.

Fig. 5 shows the cumulative drug release from the DDS-fast in distilled water at two temperatures (10 and 37 °C). This figure also shows an accelerated drug release in a hot environment (37 °C). However, as the experiment continued,

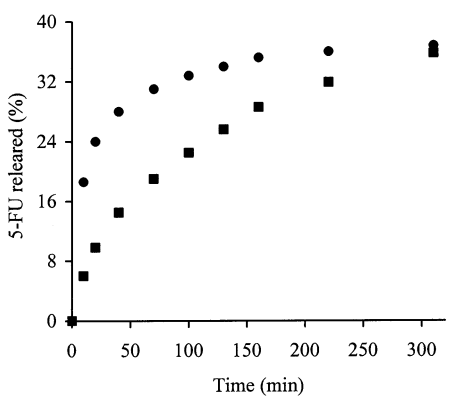

Fig. 5. Cumulative 5-FU release as a function of the time from DDS-fast (10 °C: ■; 37 °C: ●).

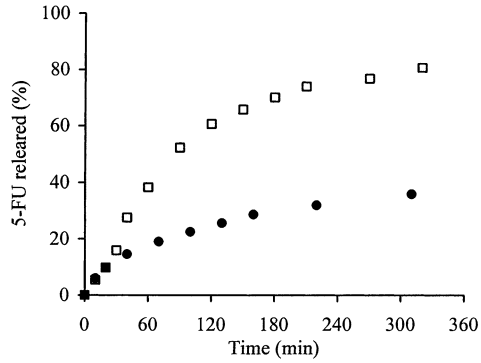

Fig. 6. Cumulative 5-FU release as a function of the time from different DDSs (DDS-con: □; DDS-fast: ●) at 10 °C.

the drug release rate from the DDS-fast slowed down and finally only $\approx 40\%$ drug was released out. We suggested that due to the collapse of the polymer chains and volume shrinkage of the gel, some drugs were entrapped in the shrunken matrix, which leads to the slowing down of the drug release. That is to say, the slowing down of the release rate at 37 °C is due to the shortage of the free drug in the DDS-fast. As to the case at 10 °C, due to the fast increased volume, the seriously blocked pores critically impeded the drug release. But, as the time goes on, the cumulative drug release would exceed the cumulative drug release at 37 °C, although the release rate is very slow.

3.5. In vitro drug release from different DDSs

We further compared the drug release rate from different DDSs at the same temperature (Figs. 6 and 7). At the decreased temperature (10 °C, Fig. 6), drug release rate from the DDS-con is much more rapid than that from the DDS-fast. This is understandable because the much increased volume of the Gel-fast at 10 °C seriously blocked the pores in the dialysis membrane, as discussed above. While at the increased temperature (37 °C, Fig. 7), due to the shortage of the free drug in the dialysis bag, the drug released from the DDS-fast is also much less than that from the DDS-con. So, by modulating the properties, such as the response rate, swelling extent etc. of the PNIPA

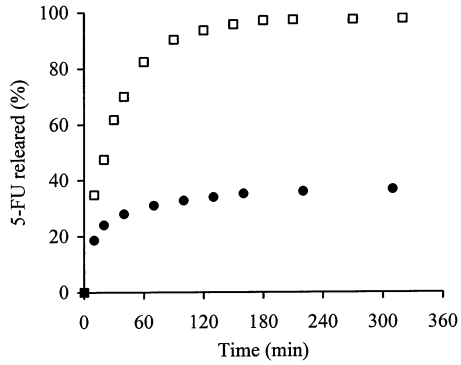

Fig. 7. Cumulative 5-FU release as a function of the time from different DDSs (DDS-con: □; DDS-fast: ●) at 37 °C.

hydrogel used in this novel system, we can further control the drug release rate and/or drug release amount etc. without changing the positive, thermo-responsive drug release pattern.

4. Conclusion

In this paper, we proposed a concept to design a novel smart drug delivery system (smart-DDS) by using the dialysis membrane as the coating layer to the naked, drug-loaded hydrogel system. Compared with the negative drug release pattern of the conventional PNIPA DDS, this novel smart-DDS can give a positive drug release pattern, i.e. rapid drug release rate at an increased temperature and slow drug release rate at a decreased temperature. We also employed the fast response PNIPA hydrogel instead of the conventional PNIPA hydrogel to further control the drug release rate and release amount without reversing the positive drug controlled release pattern. The DDS reported here has to be improved or optimised and further studies are underway. This novel DDS would have potential and promising applications in cases where positive drug controlled release is need.

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