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Temperature and pH Dual Responsive 2–(Dimethylamino) Ethanethiol Modified Starch Derivatives via a Thiol–yne Reaction for Drug Delivery

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Summary

In this study, a novel type of temperature/pH dual responsive polymer PyHES– DMAET ((2–hydroxy–3–(2–propynyloxy) propyl hydroxyethyl starch (PyHES))–2–(dimethylamino) ethanethiol (DMAET)) was synthesized. First, the temperature–responsive polymer PyHES was prepared via hydrophobic modification of hydroxyl groups in hydroxyethyl starch (HES) with propynylglycidyl ether (PGE) subsequently, pH–responsive tertiary amine group was connected to the propynyl group via a thiol–yne click reaction. Because PyHES–DMAET has pH–responsive amino groups and hydrophobic thioether groups, its aqueous solution exhibits excellent temperature/pH dual sensitivity, i.e. a good transference between the hydrophobic (or self–assembly) and hydrophilic (or swelling) state along as a result of changing temperature/pH values, these properties can be exploited, for hydrophobic drug release. The drug release reached 96% at 37°C and a pH of 6.5. The drug loading capacity of PyHES–DMAET was increased by increasing the degree of substitution (DS) of the hydrophobic propynyl groups in the PyHES. The highest drug loading capacity for doxorubicin (DOX) achieved in this study was 33wt%.

Keywords: hydroxyethyl starch; (2–dimethylamino) ethanethiol; temperature/pH dual responsive; drug release; thiolyne reaction

1. Introduction

In recent decades, temperature/pH dual responsive amphiphilic polymers have attracted the interest of researchers because they can affect certain functions by changing the hydrophilic–hydrophobic balance due to changes in the external environment. Therefore, these types of amphiphilic polymers are very useful, especially for drug delivery, tissue engineering, biotechnology, and related fields [1].

Among them, tertiary amine–containing polymers represent the most extensively studied group of temperature/pH dual responsive polymers [2]. However, most of the temperature/pH dual responsive polymers are prepared using petroleum chemical monomers as the main raw material and their use has biocompatibility and toxicity problems [3].

In recent years, many researchers have focused on biomass derivatives to improve the themperature/pH dual responsiveness of the polymers [4].

Polysaccharides are an inexpensive, non–toxic, biodegradable, biocompatible, and renewable widely available in nature and suitable drug delivery.

To data, several kinds of polysaccharides temperature/pH dual responsive polymers have been investigated. One synthetic method consists of graft copolymerization with a petroleum – based monomer polymer [5]. Another synthetic method consists of preparing the dual responsive polysaccharide derivative by directly grafting hydrophobic groups (to change the hydrophilic–hydrophobic balance) and pH–responsive groups on the polysaccharide backbone. This method avoids the above–mentioned disadvantages, but few studies have investigated this topic.

Recent approaches have developed a versatile method for the modification of polysaccharides via a click reaction [6]. Click reactions are performed under mild conditions, are highly quantitative and inert to other chemical moieties, allowing an elegant design for diverse materials with varied functionality. Wang et al. [7] prepared CUE–DEAET with cellulose derivative nanoparticles by a thiol–ene reaction on the double bond.

A high degree of substitution (DS) is often desired for the preparation of stimuli–responsive polysaccharides. In contrast to traditional thiol–ene chemistry used to data, thiol–yne coupling leads to the addition of two thiols across the triple bond, resulting in polysaccharides with a high DS. To the best of our knowledge, there are no reports to date on the modification of polysaccharides via thio–yne reactions.

In this study, we prepared the temperature/pH responsive starch derivative polymer 2–hydroxy–3–propynyloxy propyl hydroxyethyl–starch–2–(dimethylamino) ethanethiol (PyHES–DMAET) via a click reaction. PyHES is a temperature–responsive polymer with hydrophobic propynyl groups grafted onto the backbone of hydroxyethyl starch (HES). The PyHES structure contains a propynyl group that enables the effective group nucleophilic addition to triple bond with thiol–yne via a click reaction. First, PyHES was prepared by an etherification reaction, in which the hydrophobic propargyl–containing PGE was grafted on to HES; subsequently DMAET was grafted onto PyHES via the thiol–yne click reactions between thiol and alkynyl to prepare

PyHES–DMAET. In this study we determined the temperature/pH responsiveness of PyHES– DMAET in aqueous solution and the changes in the particle size as a result of changes in the temperature and pH values. We also investigated the drug–loading and drug–release properties of the PyHES– DMAET micelles.

2. Materials and methods

2.1 Materials

HES was obtained from Sigma–Aldrich (USA, MSOH=2.5, nominal molecular weight of 2.5×105). PGE (propynyl glycidylether) (>99%) was purchased from Beijing Chemistry Factory (Beijing, PR China). 2–(dimethylamino) ethanethiol (DMAET) was purchased from Youde Co., Ltd. (Shanghai, China). Analytical grade sodium hydroxide, hydrochloric acid, N, N′–Dimethylformamide (DMF), 2, 2–bimethoxy–2–phenylacetophenone (DMPA) and doxorubicin (DOX) (anti–cancer drug) were purchased from Aladdin Industrial Corporation (China).

2.2 Synthesis of PyHES

Five grams of HES (0.03 mmol of anhydroglucose units), 20 mL deionized water, and 3.0 g NaOH aqueous solution (40wt%) were added to a 50mL three–necked flask; the mixture was placed in a 60°C water bath and stirred for 1.0 h. After 1.0h, a predetermined amount of PGE was added to the flask dropwise. The subsequent reaction was carried out for 5h in low–light conditions at a temperature of 70°C. At the end of the reaction, the mixture was cooled to room temperature and neutralized to a pH of 7.0 by the addition of 6M HCl. The products were purified by dialysis in deionized water for three days, followed by concentration by a rotary evaporator and lyophilization.

2.3 Synthesis of PyHES–DMAET

Table 1. Preparation and characterization of PyHES

Sample	PGE: AGU[a]	DS[b]	Mw (×10^5)[c]	PDI[c]	Efficiency, %
PyHES1	2.2	0.47	2.5932	1.74	21.36
PyHES2	2.4	0.85	2.2242	2.25	35.41
PyHES3	2.6	1.02	2.3584	2.72	38.89
PyHES4	2.8	1.14	2.6632	3.18	44.28
PyHES5	3.0	1.28	2.5221	3.87	52.17

Four grams of PyHES and 3.2g of DMAET were dissolved in 20mL of deionized water under stirring; and 0.1g of DMPA dissolved in 20mL of DMF was added to a 100mL three–necked flask. The subsequent click reaction was carried out for 12h under 325nm ultraviolet (UV) light conditions and stirring at room temperature. At the end of the reaction, the mixture was acidified to a pH of 5.0 by the addition of 1M HCl. The products were purified by dialysis in deionized water for three days, followed by concentration by a rotary evaporator and lyophilization.

2.4 Characterization

The chemical structure of the HES, PyHES and PyHES–DMAET were qualitatively analyzed using Fourier–transform (FT–IR) spectroscopy (FT/IR–430, JASCO, Japan). The proton unclear magnetic resonance (1H NMR) spectra were recorded at room temperature on a BrukerAMX400 spectrometer. The HES, PyHES and PyHES–DMAET were dissolved in D_2O.

Gel permeation chromatography (GPC) (Agilent Technologies 1200 series, USA) was conducted to determine the molecular weights and molecular weight distributions. Dynamic light scattering (DLS) (Malvern Nano–ZS90, Britain) was performed to determine the average diameters of the aggregates of PyHES–DMAET. The fluorescence spectra were recorded on a spectrofluorophotometer (JASCO FP–6500, Japan) using pyrene for a hydrophobic fluorescent probe.

2.5 Determination of the transition temperature (Tcp) and transition pH

The Tcp was measured using a Mettler Toledo T90 with a thermo–controlled LAUDA RP200 (Germany). The transmittance of the PyHES in an aqueous solution (10 g/L) was determined at 590 nm under a heating and cooling rate of 1°C/min. Similarly, the transition pH was measured by an additional titration step.

2.6 Drug loading and release

In a 25mL round bottom flask, 50mg of PyHES–DMAET was ultrasonically dispersed into the 10 mL at pH 8.5 and then 5mL of DOX (5mg mL–1) was added to the solution. The flask was covered with foil and the mixture was stirred for 24 h at room temperature. Afterward, the DOX–loaded PyHES–DMAET was magnetically separated and washed with phosphate–buffered (PBS) (3×5mL) until the supernatant solutions became colorless. Subsequently, the supernatant solutions were collected and held for the ultraviolet–visible (UV–Vis) spectrum analysis. The drug–loading capacity (DLC) (%) and the drug–loading efficiency (DLE) (%) were calculated using the following equations (1 and 2):

$$DLC(\%)=(M_I-M_R)/M_C \times 100 \quad (1)$$

$$DLE(\%)=(M_I-M_R)/M_I \times 100 \quad (2)$$

where M_I is the initial DOX mass, M_R is the residual DOX mass in the supernatant solutions and M_C is the carrier mass. The release studies were performed at 37°C at pH 7.4 and 6.5, respectively. Firstly, 10mg PyHES–DMAET was dispersed in a 3mL buffer solution and placed in a dialysis bag with a molecular weight cut–off of 3.5kDa. The dialysis bag was immersed in 25mL of the same buffer medium and stirred at 37°C. The samples (2mL) were periodically aspirated and re–filled to the same volume using a fresh buffer solution. The amount of released DOX was analyzed by UV–Vis spectroscopy at 490nm. The drug release studies were performed in triplicate.

3. Results and Discussion

3.1 Synthesis of PyHES and PyHES– DMAET

Table 1 and 2 summarizes the characteristics of the five PyHES samples and five PyHES–DMAET samples.

Table 2. Preparation and characterization of PyHES– DMAET

Sample	Structure	DMAET: PyHES[a]	Mw (×105)[c]	PDI	Loading capacity (wt. %)	Loading efficiency (wt. %)
PD–1	PyHES1–DMAET	2.5	2.5241	2.94	–	–
PD–2	PyHES2–DMAET	2.5	2.7243	3.38	24	48
PD–3	PyHES3–DMAET	2.5	2.7456	3.88	27	54
PD–4	PyHES4–DMAET	2.5	2.6672	4.27	33	66
PD–5	PyHES5–DMAET	2.5	2.8576	4.68	–	–

3.2 Temperature/pH–Responsive Behaviors of the Polymers

The temperature–responsiveness of the PyHES–DMAET was determined by measuring the turbidity.

The thermo–responsive behavior of the three polymer samples PD–2, PD–3 and PD–4 in aqueous solution (10mg/mL, pH 8.6) are shown in Fig.1.

The main reason for the lack of sensitivity of PD–5 and its insolubility in water at room temperature is the persence of too many hydrophobic groups. Conversely, PD–1 has no temperature sensitivity in the 0–100°C range because of too few hydrophobic groups. This shows that hydrophobicity plays a crucial role in the sensitivity of the product.

Fig. 1 shows that, as the temperature increases, the transmittance of samples decreases rapidly within a certain temperature range.

The TCP decreases with increasing DS of PyHES (or with the increase in the sample number of PyHES–DMAET).

The heating and cooling curves of the samples almost overlap (Fig. 1) and the transmittance decreases dramatically during the heating process, demonstrating that PyHES–DMAET possesses excellent great thermo–responsiveness.

Fig. 2 shows the changes in the TCP values with the change in the pH value. This pH–dependent behavior was induced by the ionizable tertiary amino groups because more tertiary amino groups are protonated under acidic conditions at a lower pH, which increases the hydrophilicity of the polymer and leads to the increase in the TCP value.

For different DS products, it was observed that a decrease in the pH value resulted in a larger change in the hydrophilic–hydrophobic balance of the TCP.

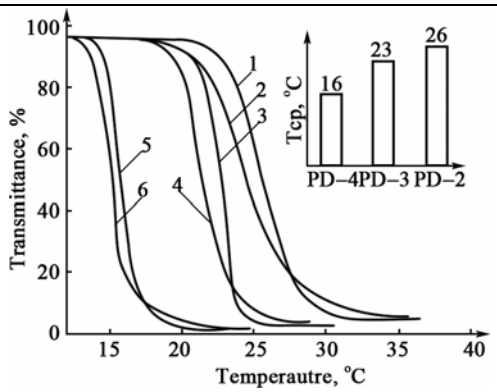

Figure 1. Temperature–responsive behaviors of PyHES–DMAET (10.0mg/mL) in pH 8.6 PBS solution
1–PD–2 heating, 2–PD–2 cooling, 3–PD–3 heating, 4–PD–3 cooling, 5–PD–4 heating, 6–PD–4 cooling

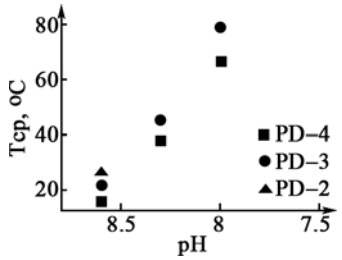

Figure 2. pH–dependent TCP of the samples

The pH responsiveness of the samples was determined by turbidimetry.

Fig. 3 a shows the transmittance–pH curves for the PyHES–DMAET polymer aqueous solution at 25°C.

When the pH value increases to a certain value, the transmittance decreases rapidly and a phase transition is observed, indicating the pH–responsive of the PyHES–DMAET polymer. The phase transition pH value of 8.8 is nearly identical for all samples.

The results were confirmed by a titration experiment. Fig. 3b shows the titration curve for the PD–4 samples in a 0.1g/L aqueous solution; where the x–axis represents the amount of added NaOH.

Two sharp changes in the pH value are observed with increased additions of 0.1M NaOH. The pKa value of the aqueous solution can be calculated based on the titration results [8] and the calculated pKa value of the PD–4 aqueous solution is 7.9.

The degree of ionization was calculated based on the pH and pKa values using the Henderson–Hasselbalch equation. Fig. 3 c shows the changes in the degree of ionization of the PD–4 sample as the solution pH changes.

The degree of ionization increases with increasing pH values. It shows that the increase in the pH drives the equilibrium to the right, decreasing the average charge per segment and increasing the hydrophobicity of the repeat unit.

The specific pH range of the deprotonate transition ionization was about pH 6.0–10.0 and its degree of ionization changed rapidly in the range of pH 6.5–8.8.

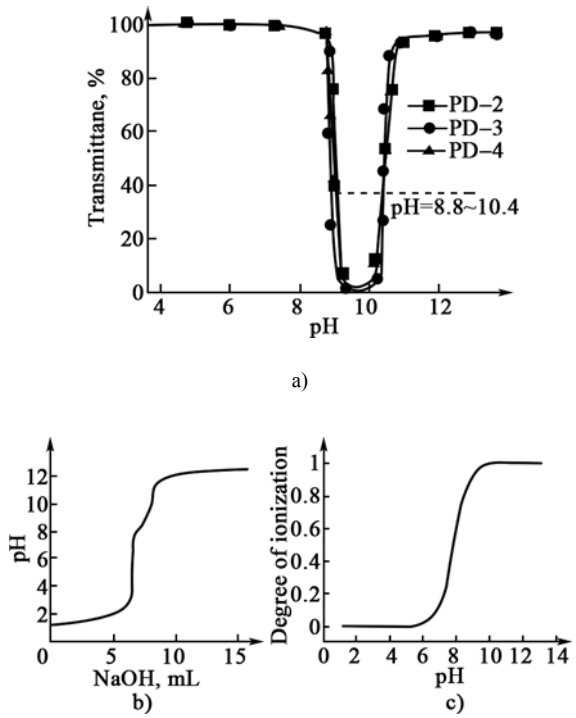

Figure 3. a)Transmittance of the samples in aqueous solution as a function of pH at 25°C, b) experimental titration curve obtained for a 0.1g/L aqueous solution of PD–4 using 0.1M NaOH, c) changes in the degree of ionization of the PD–4 (calculated).

The PyHES–DMAET polymer molecule contains two tertiary amino groups on one substituent. When the pH value is below neutral, the pH–dependent phase transition behavior of the PyHES– DMAET polymer aqueous solution was attributed to the ionizable tertiary amino groups; therefore, the hydrophilicity of the PyHES–DMAET polymer increases, and the water solubility is also high because it is dissolved completely in water. In contrast, when the solution pH is increased, the tertiary amino groups are deprotonated, leading to the generation of a hydrophobic domain and the formation of assemble. The turbidity is the result of the formation of a colloidal suspension of the PyHES– DMAET aggregates in water.

3.3 Behavior of drug loading and release from PyHES– DMAET

Fig. 4 a) shows the DCL of the samples at pH 8.0. As the DS of the PyHES increases, the DLC of PyHES–DMAET increases. It shown earlier that the increase in the DS of PyHES is equivalent to an increase in the number of hydrophobic blocks in the amphiphilic polymer structure which in turn results in increases in the amount of loaded hydrophobic drugs in the aqueous solution. The drug loading capacity was 24% (PD–2), 27% (PD–3), and 33% (PD–4) in our test range.

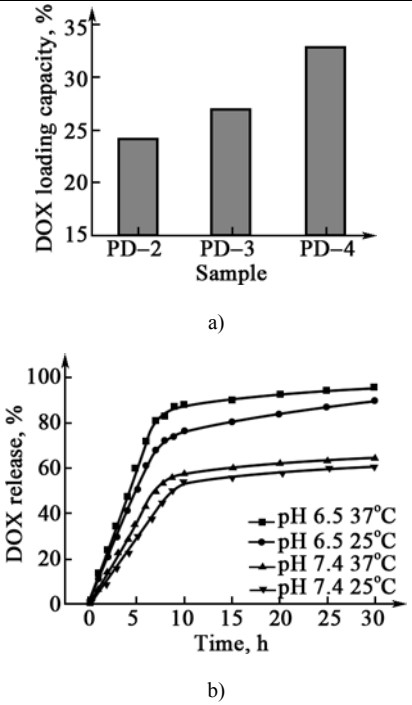

Figure 4. a) Drug loading capacity of the samples, b) temperature/pH–dependent DOX release from the PD–4 micelles in aqueous solution with different temperature and pH values.

The pH–dependent DOX release from the PyHES– DMAET polymeric micelles was investigated. The drug release of the DOX–loaded PD–4 aqueous solution at pH values 7.4 and 6.5 is shown in Fig. 4b. Within 30h at 25°C and pH 7.4, about 61% of the DOX is released. In contrast, at pH 6.5, the drug release is much higher at 89% DOX released in 30 h. On the other hand, the temperature also affects the drug release. At a temperature of 37°C, the drug release increases by 7% and 4% at pH values of 6.5 and 7.4 respectively.

The tertiary amino groups of the PyHES– DMAET micelles are protonated with the decrease in the pH value below neutral. From this value forward, the deformation of the PyHES–DMAET micelle structure resulting from the hydrophilicity of the protonated tertiary amino groups will promote the drug release. This is very important because tumor tissues have a more acidic extracellular environment and a lower cytosolic pH than the extracellular fluid. The drug can accumulate at the tumor sites and is rapidly released inside the cells after endocytosis internalization.

The results indicate that the PyHES– DMAET drug carrier can protect the drugs under alkaline conditions and is also able to achieve a large amount of drug release under below neutral conditions.

4. Conclusion

In summary, we have successfully developed a temperature/pH dual responsive PyHES–DMAET polymer with enhanced preparation methods and improved drug release capacities. This method can be extended to other polysaccharides. PyHES– DMAET is an amphiphilic starch derivative and achieves drug release by the conversion of a

hydrophilic to a hydrophobic state due to changes in the temperature and pH values in aqueous solution. The experimental results show that PyHES–DMAET is able to protect drugs under alkaline conditions and also shows good drug release capacity under below neutral conditions.

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