

## Mechanisms of Solute Release from Multi-Responsive Polymer Magnetic Microgel

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### Abstract

In the present work, a targeted anticancer drug delivery system have been developed. DOX was used as a model drug for the study of release kinetics. It is found that DOX entrapped in the microgels was released with a rate regulated and the drug loading amount. Moreover, the release of DOX from the loaded microgels was much faster at pH 4.5 than at pH7.4, and faster at 45 °C than at 37 °C. The results suggest that the thermo- and pH-responsive magnetic mcriogels may be used as a suitable carrier for controlled drug delivery.

### Keywords

Microgels, Thermo- and pH- responsive, Drug release.

### 1. Introduction

A successful nanocarrier for drug may be engineered to present high biocompatibility, colloidal stability, protection of active therapeutic drugs, improved pharmacokinetics, and evade the reticuloendothelial system. Furthermore, surface-modification of nanocarriers, which have the ability to control the release in response to different external stimuli such as pH[1], temperature[2], light[3], magnetic fields[4], redox reactions[5], enzymes[6] and even antibodies[7], will greatly improve pharmacokinetics. Recently, dualstimuli-responsive or ternarystimuli-responstive nanocarriers were prepared and applied in extended fields, especially in control-released drug delivery system (DDS)[8,9].

### 2. Experimental

#### 2.1 Materials

N-isopropylacrylamide (NIPAM) and N,N'-methylene bisacrylamide (MBA) were purchased from Tokyo Chemical Industry Co., Ltd (Japan). Branched polyethyleneimine (PEI, MW: 1800) was purchased from Shanghai Meryer Chemical Reagents Company (China). Doxorubicin hydrochloride salt (DOX) was purchased from Taizhou Shenxin Unite Co., Ltd (China). Acrylic acid (AA), folic acid(FA), L-5-Methyltetrahydrofolate (MTHF), ammonium persulfate (APS), sodium dodecyl sulfate(SDS), dicyclohexylcarbodiimide (DCC), N-hydroxysuccinimide (NHS), ferric chloride (FeCl<sub>3</sub>•6H<sub>2</sub>O), ferrous sulfate (FeSO<sub>4</sub>•7H<sub>2</sub>O) and ammonium citrate tribasic (Citric) were obtained from Shanghai Chemical Reagents Company (Shanghai, China). NIPAM was recrystallized from hexane solution and dried in vacuum prior to use. All other reagents and solvents used in the study were analytical grade and obtained from commercial sources.

#### 2.2 Preparation of Magnetite Nanoparticles

Magnetite nanoparticles (Fe<sub>3</sub>O<sub>4</sub> magnetic fluid) were prepared by coprecipitation method according to the reports[10] with minor modifications. Briefly, 8.3 g FeSO<sub>4</sub>•7H<sub>2</sub>O and 16.2 g FeCl<sub>3</sub>•6H<sub>2</sub>O were dissolved into 700 mL distilled H<sub>2</sub>O under a mechanical stirrer with nitrogen atmosphere for 0.5 h. Then, 60 mL NH<sub>3</sub>•H<sub>2</sub>O were dropped into the above Fe<sup>3+</sup>/Fe<sup>2+</sup> solution. After stirring for 1 h, the

temperature was heated to 90 °C and maintained for 2 h. The resulting magnetite nanoparticles were collected with a magnet and washed with 2 M HNO<sub>3</sub>, then washed with deionized water for several times until to neutral. The obtained magnetite nanoparticles were redispersed in 200 mL of ammonium citrate tribasic solution (0.5 M), and stirred for 1 h at 90°C, then collected with a magnet and washed with deionized water and acetone each for three times. The magnetite nanoparticles (abbreviated as MP) were then dried under vacuum, and stored under vacuum until use.

### 2.3 Preparation of MP-PNAAEF microgels

The thermosensitive polymer of carboxyl-ended poly(NIPAM) coated magnetite nanoparticles microgels (MP-PNAA) was synthesized by free-radical copolymerization of NIPAM and AA in water using APS and MBA as an initiator and a cross-linking agent according to the reports[11] with a few modifications.

In the next step, the carboxyl-ended MP-PNAA was copolymerized with the amine groups of PEI by the reaction of the carboxylic acid groups activated with dicyclohexylcarbodiimide. In a typical reaction, the above resultant MP-PNAA (300 mg), DCC (124 mg) and NHS (69 mg) were dissolved in 20 mL DMSO containing 200 µL triethylamine and the mixture was stirred for 5 h at room temperature. The solution was added to a large excess of PEI (450 mg) in 10 mL DMSO with vigorous stirring. After 24 h, the magnetic microgels with grafted PEI to MP-PNAA (designated as MP-PNAAE), were precipitated in water. The precipitated sample was isolated by centrifugation, and then purified by dialysis against distilled water (MWCO 8000-14000) to remove the unreacted 1800 Da PEI and other residues. The products were then dried under vacuum, and stored under vacuum until use.

Finally, FA (100 mg) was activated by DCC (31 mg) and NHS (26 mg), dissolved in DMF/DMSO (9 mL 3:1) solution with stirring for 4 h. Subsequently, the MP-PNAAE microgels (300 mg suspended in 20 mL DMSO) were added to the activated FA solution and allowed to react with stirring under anhydrous conditions overnight at room temperature. After the reaction, the mixture was centrifuged and washed with DMSO, water and ethanol several times to obtain FA modified polymer magnetic microgels (designated as MP-PNAAEF).

Similarly, MTHF (100 mg) was activated by DCC (31 mg) and NHS (26 mg), dissolved in DMF/DMSO (9 mL 3:1) solution with stirring for 4 h, and then, MP-PNAAEM microgels were prepared successfully by the same way.

### 2.4 Loading of Doxorubicin (DOX)

The drug loading and release behavior under different temperature and pH environments of the resultant MP-PNAAE microgels were investigated with DOX as a model molecule. The DOX-loaded MP-PNAAEF microgels were prepared as follows: 20 mg MP-PNAAEF microgels were mixed with 2 mL DOX solution in deionized water (2 mg/mL). The mixture was stirred in an orbital shaker at 220 rpm and 25 °C for 72 h. The DOX-loaded magnetic microgels were separated by centrifuging at 14,000 rpm for 20 min, and then washed with 23 mL deionized water. The resulting DOX-loaded MP-PNAAEF magnetic microgels were frozen and lyophilized to obtain the dried product. To evaluate the amount of DOX loaded, the residual DOX content (RDOX) was measured using a UV spectrophotometer at a wavelength of 480 nm. The DOX-loading content and encapsulation efficiency of the magnetic microgels are calculated using the following equations:

$$\text{DOX loading content (LC\%)} = ((\text{ODOX}-\text{RDOX})/\text{MNPs}) \times 100\%$$

$$\text{DOX encapsulation efficiency (LE\%)} = ((\text{ODOX}-\text{RDOX})/\text{ODOX}) \times 100\%$$

Here, ODOX is the original DOX content, MNPs is the amount of lyophilized magnetic microgels.

### 2.5 In Vitro Release of DOX

A 10 mg sample of the above prepared DOX-loaded MP-PNAAEF microgels was immersed in 5 mL of PBS at 37 °C and shaken at 200 rpm. At certain time intervals, 3 mL of solution was taken out by centrifugation to determine the concentration of DOX released and 3 mL of fresh PBS was added to

the tube containing the DOX-loaded MP-PNAAEF microgels. The concentration of DOX in the above solution collected was determined using a UV spectrophotometer at 480 nm. In order to investigate the influence factor of DOX release, different conditions were applied, such as pH 7.4 and 37 oC; pH 4.5, and 37 oC; pH 7.4 and 45 oC; pH 4.5 and 45 oC were also measured.

### 3. Resules

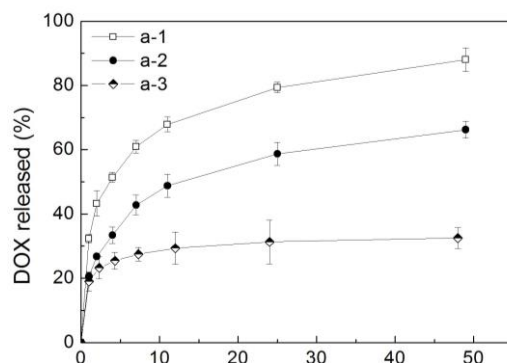


Fig. 1 Time dependent DOX release at pH 4.5, T=45°C as determined by UV spectroscopy (a-1): DOX-loaded MP-PNAAE; (a-2): DOX-loaded MP-PNAAEF; (a-3): DOX-loaded MP-PNAAEM

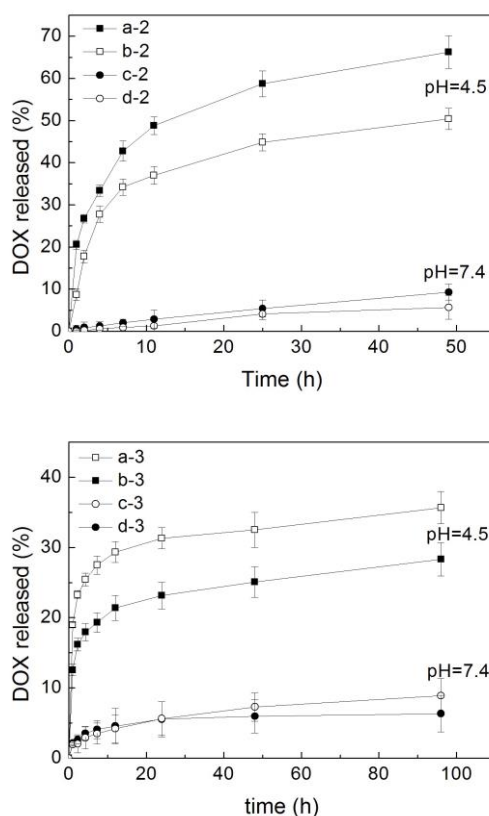


Fig. 2 DOX release at the different pH and temperature DOX-loaded MP-PNAAEF:(a-2) pH 4.5, T=45°C; (b-2) pH 4.5, T=37°C; (c-2) pH 7.4, T=45°C; (d-2) pH 7.4, T=37°C. DOX-loaded MP-PNAAEM:(a-3) pH 4.5, T=45°C; (b-3) pH 4.5, T=37°C; (c-3) pH 7.4, T=45°C; (d-3) pH 7.4, T=37°C pH=4.5: 0.1M NaAc-HAc; pH=7.4: 0.1M PBS

For the purpose of determining the drug delivery of MP-PNAAEF and MP-PNAAEM microgels as delivery vehicles, doxorubicin hydrochloride (DOX), an anticancer drug, was used as model drug to be loaded into MP-PNAAE, MP-PNAAEF and MP-PNAAEM microgels. The loading efficiencies of DOX in MP-PNAAE, MP-PNAAEF and MP-PNAAEM microgels were 47.6%, 65.4% and 54.2%,

respectively. The DOX loading amount in MP-PNAAE, MP-PNAAEF and MP-PNAAEM microgels is 78, 130 and 134 mg/g, respectively. It indicates that, compared with MP-PNAAE, FA or MTHF modified the MP-PNAAE microgels equally improved the amount of DOX loaded.

The release profiles of DOX from the DOX-loaded MP-PNAAE and MP-PNAAEF microgels in NaAc-HAc (pH 4.5) at 45°C are shown in Fig. 1. Three microgels exhibited typical sustained release behaviors. A fast release happened within 10 h, followed by a relatively slow release rate until the end of assay testing. However, it can be found that the release rate of both MP-PNAAEF and MP-PNAAEM microgels is slower than that of MP-PNAAE microgels. The volume phase transition property of magnetic microgels may be appreciably impacted by the incorporated FA or MTHF. By contrast, the release rate of MP-PNAAEM microgels is slower than that of MP the deswelling degree of MP-PNAAE microgels is larger than the later, thus leading to faster release rate and greater cumulative release amount. -PNAAEF microgels. This is attributed to the characteristic difference between FA and MTHF. As seen from Fig. 1, the MP-PNAAEF microgels are in the deswelling state completely, but the MP-PNAAEM microgels are not, also demonstrating the cause of faster release rate and greater release amount.

Fig 2 shows the release curves of DOX-loaded MP-PNAAEF and MP-PNAAEM at different conditions. The release curves for MP-PNAAEF and MP-PNAAEM microgels are similar to each other. As case study of release using MP-PNAAEF, the difference is found for the release of DOX from the magnetic microgels at different temperatures. At 37°C (below LCST), the magnetic microgels are still in a swollen state and the DOX release mainly depends on the diffusion, and thus the drug release is relatively slow. At 45°C (above LCST), the DOX release rate is faster than that at 37°C, and the amount of the DOX released from MP-PNAAEF microgels within 48 h increased slightly from 50% to 66%. The increased drug release at 45°C is attributed to the collapse of the microgel. As a result, the loaded DOX was squeezed (together with water) out of the magnetic microgels.

In addition, as exhibited in Fig. 4, the release of DOX is also pH-dependent. When the pH value decreases to 4.5, the DOX released from all MP-PNAAEF microgels (above 50%) is much higher than that at pH 7.4 both at 37 °C and 45 °C. The reasons are as follows: (1) The microgels have a smaller size at pH 4.5, and some of the hydrogen bonds are broken which would make the loaded DOX squeezed out quickly; (2) The amino group of DOX is protonated as  $-NH_3^+$  state so that it cannot form hydrogen bond with magnetic microgels in acid medium[11]. Meanwhile, the protonated DOX has a higher solubility[12]. When the pH value decreased from 7.0 to 5.0, the solubility of DOX dramatically increased from 0.41 g/L to 18 g/L. Consequently, at pH 4.5, the amount of drug released from MP-PNAAEF increases rapidly.

To better understand the release mechanisms of the loaded DOX from the microgels, the following semiempirical equation, which has been successfully utilized to study many other drug delivery systems:

zero-order equation:  $Q=Kt+B$

first-order equation:  $\ln(1-Q)=Kt+B$

Weibull equation:  $\ln\ln(1/(1-Q))=KLnt+B$

Higuchi equation:  $Q=Kt^{1/2}+B$

Ritger-Peppas equation:  $\ln Q=KLnt+B$

Hixson-crowell equation:  $(100-Q)^{1/3}=Kt+B$

Baker-lonsdale equation:  $3/2[1-(1-Q)^{2/3}]-Q=Kt+B$

Tab. 1 The result of fitting equation of three DOX-loaded microgels at pH 4.5, 45°C

	MP-PNAAE	MP-PNAAEF	MP-PNAAEM
zero-order	$Q(\%)=0.9967t+46.343$	$Q(\%)=0.8549t+30.354$	$Q(\%)=0.2259t+23.712$
	$R=0.8776$	$R=0.8896$	$R=0.8019$
first-order	$\ln(1-Q)=-0.0339t-0.5856$	$\ln(1-Q)=-0.0167t-0.3552$	$\ln(1-Q)=-0.0031t-0.2712$
	$R=0.9678$	$R=0.9368$	$R=0.8160$
Weibull	$\ln\{\ln[1/(1-Q)]\}=0.4275\ln t-0.9071$	$\ln\{\ln[1/(1-Q)]\}=0.407\ln t-1.4391$	$\ln\{\ln[1/(1-Q)]\}=0.159\ln t-1.4878$
	$R=0.9991$	$R=0.9964$	$R=0.9766$
Higuchi	$Q=8.8447t^{1/2}+32.166$	$Q=7.5447t^{1/2}+18.328$	$Q=2.0697t^{1/2}+20.226$
	$R=0.9576$	$R=0.9653$	$R=0.9080$
Ritger-Peppas	$\ln Q=0.2532\ln t+3.5619$	$\ln Q=0.3068\ln t+3.0819$	$\ln Q=0.1364\ln t+3.0086$
	$R=0.9869$	$R=0.9901$	$R=0.9735$
Hixion-crowell	$(100-Q)^{1/3}=-0.0341t+3.7927$	$(100-Q)^{1/3}=-0.0206t+4.119$	$(100-Q)^{1/3}=-0.0043t+4.2406$
	$R=0.9431$	$R=0.9222$	$R=0.8113$
Baker-Losdale	$3/2[1-(1-Q)^{2/3}]-Q=0.0047t-0.0425$	$3/2[1-(1-Q)^{2/3}]-Q=0.0021t-0.0169$	$3/2[1-(1-Q)^{2/3}]-Q=0.0003t-0.0107$
	$R=0.9712$	$R=0.9636$	$R=0.8480$

Then, we used to fit the in vitro release profiles of microgels:

$$\frac{M_t}{M_\infty} = Kt^n \quad (0 \leq \frac{M_t}{M_\infty} \leq 0.6)$$

Where  $M_t/M_\infty$  is the fractional DOX release (the percentage of the total DOX amount incorporated into the scaffold),  $M_t$  is the concentration of DOX released at time  $t$ ,  $M_\infty$  is the concentration of DOX released at equilibrium,  $k$  is a constant relating to the properties of the matrix and the drug, and  $n$  is the release exponent that depends on the transport mechanism and the geometry of the device. According to this classification, there are four distinguishable modes of dissolution: (1) the value of  $n=0.5$  suggests Fickian or Case I transport behavior in which the relaxation coefficient is negligible during transient sorption; (2) the value of  $n=1$  refers to a non-Fickian or Case II mode of transport where the morphological changes are abrupt; (3) if  $0.5 < n < 1$ , the transport process is anomalous, corresponding to Case III, and the structural relaxation is comparable to diffusion; (4) a value of  $n < 0.5$  indicates a pseudo-Fickian behavior of diffusion where sorption curves resemble Fickian curves, but the approach to final equilibrium is very slow. By plotting  $(M_t/M_\infty)$  versus  $\log(t)$ , the  $n$  and  $k$  values as well as corresponding determination coefficients ( $R$ ) were obtained, as listed in Table 2. For the blend microgels, the  $n$  values were found to be in the range of 0.1-0.5 at pH=4.5, showing the pseudo-Fickian diffusion release mechanism. The  $n$  values were found to be in the range of 0.6-0.8 at pH=7.4, showing anomalous transport process, and the structural relaxation is comparable to diffusion.

Tab. 2 The result of fitting equation of DOX-loaded MP-PNAAEF and DOX-loaded MP-PNAAEM microgels

条件	MP-PNAAEF		MP-PNAAEM	
pH=4.5 45°C	$\ln Q=0.3068\ln t+3.0819$	$R=0.9901$	$\ln Q=0.1364\ln t+3.0086$	$R=0.9735$
pH=4.5 37°C	$\ln Q=0.4381\ln t+2.5101$	$R=0.9347$	$\ln Q=0.4721\ln t+2.6014$	$R=0.9827$
pH=7.4 45°C	$\ln Q=0.7429\ln t-0.7072$	$R=0.9987$	$\ln Q=0.6626\ln t+0.5518$	$R=0.9876$
pH=7.4 37°C	$\ln Q=1.5784\ln t-3.432$	$R=0.9141$	$\ln Q=0.9238\ln t-1.3583$	$R=0.9567$

#### 4. Conclusion

In the present work, a targeted anticancer drug delivery system have been developed. DOX was used as a model drug for the study of release kinetics. It is found that DOX entrapped in the microgels was released with a rate regulated and the drug loading amount. Moreover, the release of DOX from the loaded microgels was much faster at pH 4.5 than at pH7.4, and faster at 45°C than at 37°C. The results suggest that the thermo- and pH-responsive magnetic mcriogels may be used as a suitable carrier for controlled drug delivery.

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