

Mechanisms of Release from Multi-Responsive Polymer Magnetic SiO₂ Microgel

Yunli Hu ^{1, a}, Weijun Liu ^{2, b}, Fanhong Wu ^{1,2, c}

¹ School of Chemistry and Molecular Engineering, East China University of Science and Technology, Shanghai, 200237, China;

² School of Chemical and Environmental Engineering, Shanghai Institute of Technology, Shanghai, 201418, China.

^a hylpaipai@126.com, ^b liuwj@sit.edu.cn, ^c wfh@ecust.edu.cn

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 mcriegels 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 (FeCl₃•6H₂O), ferrous sulfate (FeSO₄•7H₂O) 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

According to a previously reported method, magnetite core-shell Fe₃O₄@SiO₂ microspheres were synthesized by a sol-gel process to coat a thin layer of dense amorphous silica on Fe₃O₄ particles. The Fe₃O₄@SiO₂ microspheres were then modified with 3-methacryloxypropyltrimethoxysilane (MPS), a polymerizable silane coupling agent, and Fe₃O₄@SiO₂-MPS microspheres thus obtained.

2.3 Preparation of MP-SiO₂-PNAAEF Microgels

The thermosensitive polymer of carboxyl-ended poly(NIPAM) coated magnetite nanoparticles microgels (MP-SiO₂-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-SiO₂-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-SiO₂-PNAA (300 mg), DCC (124 mg) and NHS (69 mg) were dissolved in 20 mL DMSO containing 200 μ L 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-SiO₂-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-SiO₂-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-SiO₂-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-SiO₂-PNAAE microgels were investigated with DOX as a model molecule. The DOX-loaded MP-SiO₂-PNAAEF microgels were prepared as follows: 20 mg MP-SiO₂-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-SiO₂-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-SiO₂-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 °C; pH 4.5, and 37 °C; pH 7.4 and 45 °C; pH 4.5 and 45 °C were also measured.

3. Resules

For the purpose of determining the drug delivery of MP-SiO₂-PNAAEF and MP-SiO₂-PNAAEM microgels as delivery vehicles, doxorubicin hydrochloride (DOX), an anticancer drug, was used as model drug to be loaded into MP-SiO₂-PNAAE, MP-SiO₂-PNAAEF and MP-SiO₂-PNAAEM microgels. The loading efficiencies of DOX in MP-SiO₂-PNAAE, MP-SiO₂-PNAAEF and MP-SiO₂-PNAAEM microgels were 27.5%, 41.2% and 39.5%, respectively. The DOX loading amount in MP-SiO₂-PNAAE, MP-SiO₂-PNAAEF and MP-SiO₂-PNAAEM microgels is 55, 82 and 79 mg/g, respectively. It indicates that, compared with MP-SiO₂-PNAAE, FA or MTHF modified the MP-SiO₂-PNAAE microgels equally improved the amount of DOX loaded.

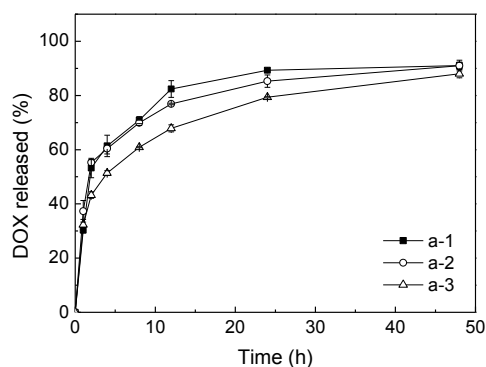


Figure 1 Time dependent DOX release at pH 4.5, T=45 °C as determined by UV spectroscopy (a-1): DOX-loaded MP-SiO₂-PNAAE; (a-2): DOX-loaded MP-SiO₂-PNAAEF; (a-3): DOX-loaded MP-SiO₂-PNAAEM

The release profiles of DOX from the DOX-loaded MP-SiO₂-PNAAE and MP-SiO₂-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. Moreover, it can be found that the release rate of three microgels is similar.

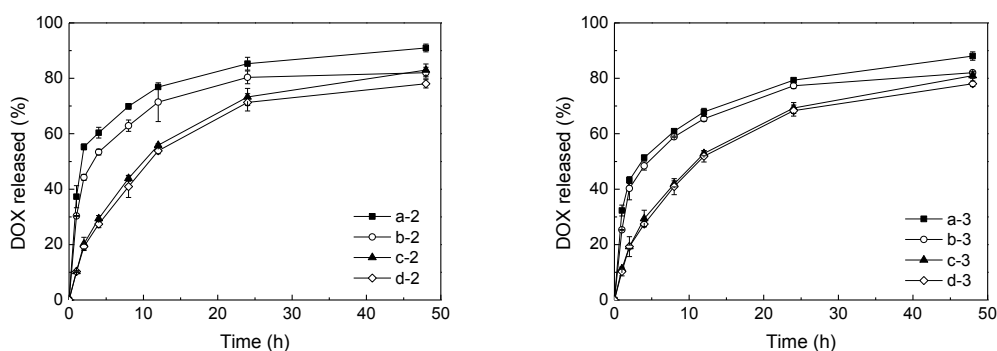


Figure 2 DOX release at the different pH and temperature DOX-loaded MP-SiO₂-PNAAEF:(a-2) pH 4.5, T=45 °C; (b-2) pH 4.5, T=37 °C; (c-2) pH 7.4, T=45 °C; (d-2) pH 7.4, T=37 °C. DOX-loaded MP-SiO₂-PNAAEM:(a-3) pH 4.5, T=45 °C; (b-3) pH 4.5, T=37 °C; (c-3) pH 7.4, T 45 °C; (d-3) pH 7.4, T=37 °C pH=4.5: 0.1M NaAc-HAc; pH=7.4: 0.1M PBS

Fig 2 shows the release curves of DOX-loaded MP-SiO₂-PNAAEF and MP-SiO₂-PNAAEM at different conditions. The release curves for MP-SiO₂-PNAAEF and MP-SiO₂-PNAAEM microgels are similar to each other. As case study of release using MP-SiO₂-PNAAEF, the difference is found for the release of DOX from the magnetic microgels at different temperatures. At 37oC (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 45oC (above LCST), the DOX release rate is faster than that at 37oC, However, the amount of DOX released from MP-SiO₂-PNAAEF microgels within 48h both over 80%. As a result, the loaded DOX was squeezed (together with water) out of the magnetic microgels.

In addition, the release of DOX is also pH-dependent. When the pH value decreases to 4.5, the DOX released from all MP-SiO₂-PNAAEF microgels is slightly higher than that at pH 7.4 both at 37 oC and 45 oC. 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 -NH₃⁺ state so that it cannot form hydrogen bond with magentic 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 drmatically 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 undersand the releasase 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: $\ln Q=KLnt+B$

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

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

Table 1 the result of fitting equation of three DOX-loaded microgels at pH 4.5,45°C

	MP-SiO ₂ -PNAAE	MP-SiO ₂ -PNAAEF	MP-SiO ₂ -PNAAEM
zero-order	$Q(\%)=0.985t+54.441$	$Q(\%)=0.9086t+55.143$	$Q(\%)=1.0371t+45.772$
	R=0.7583	R=0.8252	R=0.8856
first-order	$\ln(1-Q)= -0.0391t-0.8546$	$\ln(1-Q)= -0.0377t-0.7781$	$\ln(1-Q)= -0.0351t-0.5688$
	R=0.8945	R=0.9466	R=0.9716
Weibull	$\ln\{\ln[1/(1-Q)]\}=0.4158\ln t-0.6043$	$\ln\{\ln[1/(1-Q)]\}=0.4024\ln t-0.6394$	$\ln\{\ln[1/(1-Q)]\}=0.4288\ln t-0.9194$
	R=0.9882	R=0.9897	R=0.9987
Higuchi	$Q= 9.1468t^{1/2}+38.928$	$Q= 8.1654t^{1/2}+41.708$	$Q= 9.0797t^{1/2}+31.211$
	R=0.8762	R=0.9229	R=0.9648
Ritger-Peppas	$\ln Q= 0.3885\ln t + 3.5372$	$\ln Q= 0.2847\ln t+3.6987$	$\ln Q= 0.2994\ln t+3.5114$
	R=0.9340	R=0.9484	R=0.9899
Hixion-crowell	$(100-Q)^{1/3}=-0.0362t+3.4898$	$(100-Q)^{1/3}=-0.0354t+3.5609$	$(100-Q)^{1/3}=-0.0354t+3.8105$
	R=0.8660	R=0.9122	R=0.9482
Baker-Losdale	$3/2[1-(1-Q)^{2/3}]-Q=0.0053t+0.0799$	$3/2[1-(1-Q)^{2/3}]-Q=0.0051t+0.0696$	$3/2[1-(1-Q)^{2/3}]-Q=0.0049t+0.0403$
	R=0.8852	R=0.9407	R=0.9743

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

$$\frac{M_t}{M_\infty} = Ktn \quad (0 \leq \frac{M_t}{M_\infty} \leq 0.6)$$

Where M_t/M_∞ 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_∞ 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_∞) 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.

Table 2 the result of fitting equation of DOX-loaded MP-PNAAEF and DOX-loaded MP-PNAAEM microgels

	MP-PNAAEF		MP-PNAAEM	
pH=4.5 45°C	$\ln Q = 0.2847 \ln t + 3.6987$	$R = 0.9484$	$\ln Q = 0.2994 \ln t + 3.5114$	$R = 0.9899$
pH=4.5 37°C	$\ln Q = 0.2985 \ln t + 3.3145$	$R = 0.9743$	$\ln Q = 0.2918 \ln t + 3.403$	$R = 0.9670$
pH=7.4 37°C	$\ln Q = 0.5369 \ln t + 2.5552$	$R = 0.9790$	$\ln Q = 0.5131 \ln t + 2.5818$	$R = 0.9862$
pH=7.4 37°C	$\ln Q = 0.5303 \ln t + 2.5228$	$R = 0.9809$	$\ln Q = 0.5239 \ln t + 2.524$	$R = 0.9827$

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 pH 7.4, and faster at 45°C than at 37°C. The results suggest that the thermo- and pH-responsive magnetic microgels may be used as a suitable carrier for controlled drug delivery.

Acknowledgements

This work has been financially supported by the National Natural Science Foundation of China (NO. 21472126 and NO. 21672151).

References

- [1] X. Gu, J.J. Wang, Y.M. Wang, Y.N. Wang, H. Gao, G.L. Wu, Layer-by-layer assembled polyaspartamide nanocapsules for pH-responsive protein delivery, *Colloids and Surfaces B-Biointerfaces*, 108 (2013) 205-211.
- [2] Q.Q. Wang, M. Kong, Y. An, Y. Liu, J.J. Li, X. Zhou, C. Feng, J. Li, S.Y. Jiang, X.J. Cheng, X.G. Chen, Hydroxybutyl chitosan thermo-sensitive hydrogel: a potential drug delivery system, *Journal of Materials Science*, 48 (2013) 5614-5623.
- [3] H.J. Cha, J. Dai, J.C. Kim, Microgels composed of poly(ethylene imine) and carboxymethoxycoumarin: pH-dependent and photodependent integrity, *Journal of Applied Polymer Science*, 129 (2013) 644-651.
- [4] Y. Zhu, T. Ikoma, N. Hanagata, S. Kaskel, Rattle-type Fe₃O₄@SiO₂ hollow mesoporous spheres as carriers for drug delivery, *Journal of Materials Science*, 6 (2010) 471-478.

-
- [5] C. Rijcken, O. Soga, W. Hennink, C.v. Nostrum, Triggered destabilisation of polymeric micelles and vesicles by changing polymers polarity: an attractive tool for drug delivery, *Journal of controlled release*, 120 (2007) 131-148.
- [6] Y. Bae, W.-D. Jang, N. Nishiyama, S. Fukushima, K. Kataoka, Multifunctional polymeric micelles with folate-mediated cancer cell targeting and pH-triggered drug releasing properties for active intracellular drug delivery, *Molecular BioSystems*, 1 (2005) 242-250.
- [7] S.C. Alley, N.M. Okeley, P.D. Senter, Antibody–drug conjugates: targeted drug delivery for cancer, *Current opinion in chemical biology*, 14 (2010) 529-537.
- [8] N. Shamim, L. Hong, K. Hidajat, M.S. Uddin, Thermosensitive polymer (N-isopropylacrylamide) coated nanomagnetic particles: preparation and characterization, *Colloids and surfaces. B, Biointerfaces*, 55 (2007) 51-58.
- [9] M. Iiong, J. Lu, M. Kovoichich, T. Xia, S.G. Ruehm, A.E. Nel, F. Tamanoi, J.I. Zink, Multifunctional inorganic nanoparticles for imaging, targeting, and drug delivery, *ACS Nano*, 2 (2008) 889-896.
- [10] Y. Deng, W. Yang, C.C. Wang, S.K. Fu, A novel approach for preparation of thermoresponsive polymer magnetic microspheres with core–shell structure, *Advanced Materials*, 15 (2003) 1729-1732.
- [11] X. Yang, L. Chen, B. Han, X. Yang, H. Duan, Preparation of magnetite and tumor dual-targeting hollow polymer microspheres with pH-sensitivity for anticancer drug-carriers, *Polymer*, 51 (2010) 2533-2539.
- [12] X. Shuai, H. Ai, N. Nasongkla, S. Kim, J. Gao, Micellar carriers based on block copolymers of poly (ϵ -caprolactone) and poly (ethylene glycol) for doxorubicin delivery, *Journal of Controlled Release*, 98 (2004) 415-426.