Preprint Title Nanofabrication method of amphiphilic spheres using linear hydrophobic polymer chains as templates

Authors Yan Hu, Zhen-Huan Wang, Ya-Lan Guan, Dan Zhao and Xin-Cai Xiao

Article Type Full Research Paper

ORCID® iDs Xin-Cai Xiao - https://orcid.org/0000-0002-7117-5305

Nanofabrication method of amphiphilic spheres using linear hydrophobic polymer chains as templates

Yan Hu[1,2#], Zhen-Huan Wang[1#],Ya-Lan Guan[1#], Dan Zhao[1,2] and Xin-Cai Xiao[1,2*]

[1]School of Pharmacy, South-Central University for Nationalities, Wuhan 430074, China

[2]National Demonstration Center for Experimental Ethnopharmacology Education, South-Central University for Nationalities, Wuhan 430074, China

Email: xcxiao@mail.scuec.edu.cn

* Corresponding author

[#]Yan Hu, Zhen-Huan Wang and Ya-Lan Guan contributed equally to this work.

Keywords:

nanofabrication, good solvent, poor solvent, reaction media, amphiphilic structure

Abstract

Investigation about nanocarriers has been a hot issue and nanofabrication method still plays an important role. In this work, a phenomenon that reacting linear polystyrene chains can collapse when a reaction media changed from tetrahydrofuran to water was utilized as templates to prepare nanospheres. Free radicals on the surface of the collapsed templates can initiate hydrophilic monomers *N*-isopropylacrylamide and *N,N'*-methylenebisacrylamide in water. Effect levels of some factors including stirring rate, *N*-isopropylacrylamide concentration and reaction time of styrene on size and monodispersity of the spheres were investigated. The results show the obtained spheres had polystyrene cores and crosslinked poly(*N*-isopropylacrylamide) shells. Monodispersed nanospheres with mean diameter 63nm can be obtained under optimized condition. The results also display that the first effect factor was the reaction time of styrene, and the second was the stirring rate. The proposed technology provides a mode to prepare monodisperse nanospheres or nanocapsules, which are highly attractive for targeting hydrophobic-drug delivery systems, oil-water separation, single molecule detect, colloid science and so on.

Introduction

Drug delivery system has been a strong interest in pharmaceutical field for a long time [1-3]. Especially in recent years, nanocarriers including nanospheres or nanocapsules have been hot issues on their design, characterization and fabrication because of their small size being able to traverse the smallest capillaries in the body, higher specific surface area [4], and multifunction. In the biomedical field [5-8], because of improving the stability and bioavailability of hydrophobic drugs, the nanocarriers have significance on science and technology research.

There have had some technologies to fabricate the micro- or nanocarriers. For example, miniemulsion technology [9] is used to prepare polyurethane nanocapsules with oily core, a mean diameter of 200 nm. Inverse emulsion microspheres polymerization technology [10] is utilized to obtain capsules with aqueous core and uniform polymeric shells with diameters ranging from 0.2 to 5μm. A one-pot inverse miniemulsion polymerization technology [11] is taken to gain thermosensitive poly(*N*-isopropylacrylamide) nanocapsules with diameter around 200 nm. Seed emulsion polymerization technology [12] is carried to prepare polymeric microspheres with yolk-shell structure. However, it is not nanosize in the strict sense. Up till now, some strategies for nanocarriers is still continuing [13-16].

In this project, we report firstly on a technology utilizing collapsed polymer chains as templates to prepare nanospheres. Styrene (St) and *N*-isopropylacrylamide (NIPAM) were used as reaction materials because they can be initiated by free radicals. Tetrahydrofuran (THF) and water were chosen as reaction media because hydrophobic St and PSt can dissolve in THF, and THF can dissolve in water. Both NIPAM and poly(*N*-isopropylacrylamide) (PNIPAM) were hydrophilic. The expanded PSt chains in THF grow with increasing reaction time and St dosage. Free radicals on end of the PSt chains are able to exist for some time under nitrogen environment and initiate other monomers polymerizing. When THF solution including polymerizing PSt chains is pumped to aqueous solution including NIPAM and *N, N'*-methylenebisacrylamide, the conformation of PSt chains changes from expanded to collapsed. The collapsed chains are used as templates and PNIPAM are initiated simultaneously by the free radicals, therefore amphiphilic nanospheres including PSt cores and crosslinked PNIPAM shells can be obtained. The concept of the proposed fabrication procedure of the nanospheres is schematically illustrated in Figure 1. To explore preparation conditions, effects of some factors such as stirring rate, NIPAM concentration and reaction time of St on the nanospheres were investigated. The objective of this study is to obtain some guidance for design and preparation of nanospheres or nanocapsules for different applications.

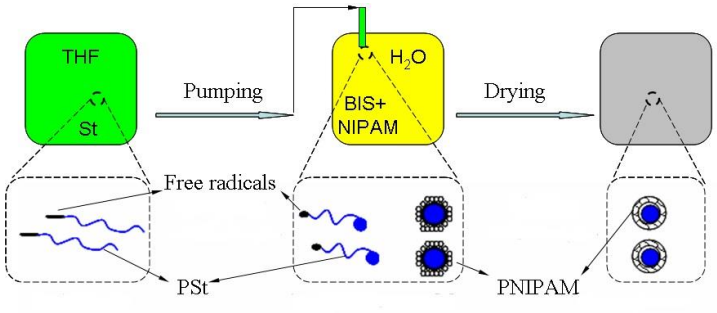


Figure 1. Synthetic route of nanospheres with polystyrene cores-poly(*N*-isopropylacrylamide) shells.

Results and Discussion

In this work, after St (4.0 ml) in THF (200 ml) solution being initiated at 70 ^{0}C for some time under stirring, 16 ml of THF solution including linear PSt chains with the free radicals was pumped into a series of NIPAM (1.25×10^{-3} g/ml, 1.04×10^{-3} g/ml, 0.75×10^{-3} g/ml and 0.11×10^{-3} g/ml) and BIS aqueous solution, respectively. Experimental parameters were listed in Table 1. The solubility parameter is a very important factor for the formation, size and distribution of the nanospheres. The polarity of the mixture changed simultaneously when THF was pumped into water. The polarity of the mixture, δ_m, can be calculated following as,

$$\delta_m = \varphi_1\delta_1 + \varphi_2\delta_2 = \sum\varphi_i\delta_i \quad (1)$$

where δ_i, φ_i are the solubility parameter and volume fraction of solvent *i*, respectively [15]. When 16 ml of THF solution including polymerizing PSt chains and monomer St was pumped into 175 ml of NIPAM and BIS aqueous solution. According to Equation 3, the solubility parameter of the mixture [22.23 (cal/cm^3) $^{1/2}$] was close to that of water [23.4 (cal/cm^3) $^{1/2}$], which meaned the addition of THF could not change the polarity of the aqueous solution (The solubility parameter as shown in Table 2). When THF was mixed with water, the conformation of the linear PSt chains changed from expanded to collapsed, meanwhile the unreaction monomer St separated from aqueous solution due to the hrdrophobic property of St and PSt. Because of little dosages and its polarity, St can't continue to polymerize when NIPAM and BIS were polymerizing in the aqueous solution. NIPAM and BIS polymerized under the free radicals on the surface of the collapsed PSt templates. These hydrophilic poly(NIPAM-*co*-BIS) shells helped the spheres to suspend in aqueous solution. Both the homogeneously stabilizing samples in aqueous solution and our previous work [16] can prove that the structure of the spheres was PSt cores and PNIPAM shells.

Table 1. Experimental parameters for preparation of the nanospheres

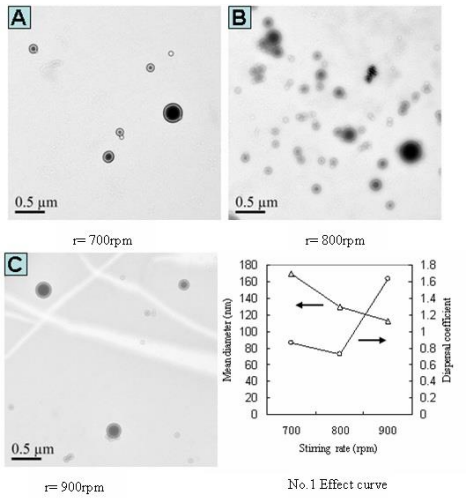
No.	Polymerization time and conversion of PSt in Step 1		Polymerization of PNIPAM shell in Step 2	
	Polymerization time (h)	Conversion (%)	C_{NIPAM} ($\times10^{-3}$ g/ml)	$R_{higher\ speed}$ (rpm)
A				700
B	8	21.45	1.25	800
C				900
D			0.75	
E	8	21.45	1.04	800
F			1.25	
G	4	13.45		
H	6	22.08	0.11	800
I	8	21.45		

Table 2. Solubility parameter of solvent under the different ratio

Solvent	Solubility parameter δ_m /(cal/cm^3)$^{1/2}$
Water	23.4
THF	9.5
Mixture(V_{water}/V_{THF}=175/16)	22.23

120

121 Figure 2 shows that TEM micrographs of the final structure prepared at different stirring rates and
122 effect curves of the stirring rate on the mean diameter and monodispersity. From TEM micrographs,
123 the obtained cores-shells structure was easily observed. Different stirring rates resulted in different
124 shear force. When the linear PSt chains with the free radicals were pumped into NIPAM aqueous
125 solution, the precursor spheres were formed by the shear force, and the free radicals on the surface of
126 the precursor spheres can initiate polymerization reaction. From the effect curves, the final spheres
127 diameter decreased with increasing the stirring rate. When the stirring rate was larger than 800 rpm,
128 the mean diameters became smaller because the shear force can generate smaller precursor spheres.
129 Meanwhile, because of the meeting chances of the precursor spheres increasing, the monodispersity
130 of the spheres decreased. When the stirring rate was lower than 800 rpm, the diameter of the
131 nanospheres decreased obviously. Therefore, the better stirring rate to prepare monodispersed
132 nanospheres was chosen to 800 rpm. In the following experiment, the stirring rate was adopted at 800
133 rpm.



134

135 **Figure 2.** TEM micrographs of the core-shell nanospheres prepared with different stirring rates (Scale bar = 0.5μm) and
136 effect curve of stirring rates on the mean diameter and monodispersity. The sample code (A, B, C) is defined in Table 1.

137 Figure 3 illustrates SEM micrographs of the spheres prepared at different NIPAM concentration. The
138 PNIPAM shells were fabricated by free radical polymerization method. By this method, the NIPAM
139 was grafted on the surfaces of the obtained PSt templates. The mean diameter increased with

increasing NIPAM concentration. The mean diameter of the spheres decreased slightly when the NIPAM concentration was more than 1.04×10^{-3}g/ml, which may result from falling out of larger PNIPAM chains. On the other hand, the monodispersity became worse with increasing NIPAM concentration because the aggregation of the nanospheres increased with increasing NIPAM concentration.

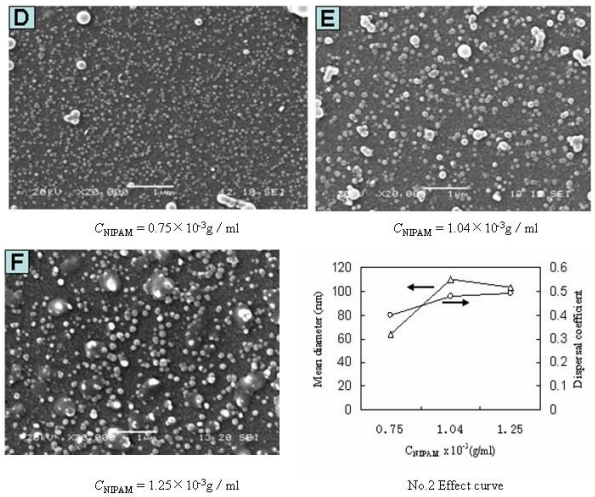


Figure 3. SEM micrographs of the core-shell nanospheres prepared with different NIPAM concentrations (Scale bar = 1μm) and effect curve of NIPAM concentrations on the mean diameter and monodispersity. The sample code (D, E, F) is defined in Table 1.

Figure 4 displays SEM photographs of the final samples prepared at different polymerization time. The polymerization of St follows chain propagation, thus different polymerization time results in different length of PSt chains. When the PSt were pumped into aqueous solution, the size of the collapsed precursor spheres should decide the size of the samples. In other word, polymerization time of styrene leaded to different size of nanospheres. The SEM images show that the prepared nanospheres had mean diameters between 40-70 nm. With increasing the reaction time from 4h to 6h, the mean diameters of the samples were almost at the same level, and the final morphology became better. However, when the polymerization time increased further, the mean diameter of the samples increased obviously, and the final monodispersity became worse. When the polymerization time was not very long (e.g., less than 6h), the length of linear PSt chains was relatively short and the small size of the precursor spheres was not very large. This was helpful for preparing smaller spheres with good monodispersity (δ<0.4) [16, 17]. However, the diameter of the precursor spheres increased so fast when the polymerization time was more than 6h.

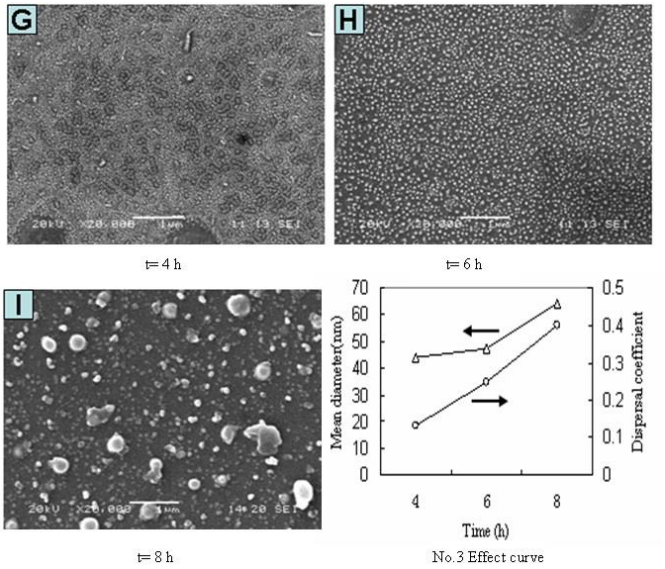


Figure 4. SEM micrographs of the core-shell nanospheres prepared with different polymerization times (Scale bar = 1μm) and effect curve of polymerization times on the mean diameter and monodispersity. The sample code (G, H, I) is defined in Table 1.

From the above results, to prepare PSt core and PNIPAM shell spheres by using this technology, the first effect factor was the polymerization time of St, and the second was the stirring rate. The optimal parameters should be selected at a stirring speed with 800 rpm, a relatively dilute NIPAM concentration such as 0.11×10^{-3} g/ml, and 6 h polymerization time of St. The preparation parameters of the sample H was close to the optimizing conditions. Figure 5 shows the hydrodynamic diameter of the sample H determined by temperature-programmed photon correlation spectroscopy. The result displays that the mean hydrodynamic diameter was about 63 nm, and the number percentage of the nanospheres with diameters from 59 to 69 nm was up to 95% which means the monodispersity of nanospheres.

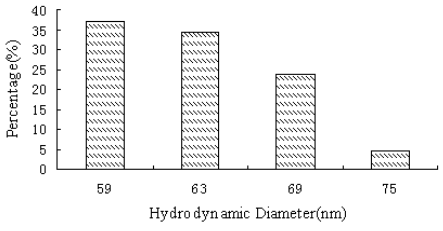


Figure 5. The hydrodynamic diameters of the sample H at 25 °C

Conclusion

In summary, we have prepared polystyrene cores-crosslinked poly(*N*-isopropylacrylamide) shells nanospheres by using collapsed polystyrene as templates. The collapsed polystyrene cores/templates

were resulted from changing polarity of the solvent. The poly(*N*-isopropylacrylamide) shells were fabricated under the free radicals on the surface of the templates. Reaction parameters including stirring rate, *N*-isopropylacrylamide concentration and reaction time of styrene on the size, monodispersity of the nanospheres were investigated. The results of TEM and SEM of the samples show that proposed technology of the nanospheres is practicable. The results also display that the first effect factor is the polymerization time of styrene, and the second is the stirring rate. This research provides a new technology to prepare monodisperse nanospheres, which are highly attractive for application in targeting hydrophobic-drug delivery systems, chemical separations, and sensors and so on.

Experimental

Materials

Monomer *N*-isopropylacrylamide (NIPAM) was kindly supplied by Kohjin Co. Ltd, Japan, and purified with a mixed solvent including hexane and acetone before use. Styrene (St) was used after treating with 10 wt% NaOH to remove an inhibitor. Tetrahydrofuran (THF) was purchased from Sinopharm Chemical Reagent Co.,Ltd., China, was Analytical Reagent grade and was stored in iced-box and used directly without any further treatment. Azodiisobutyronitrile (AIBN) was purchased by Shanghai Test Four Hewei Chemical Co. Ltd., China, was Analytical Reagent grade and was used directly without any further treatment. *N,N'*-methylenebisacrylamide (BIS) as a crosslinker was purchased by Changsha Oumay Biotech Co., Ltd., China, and used after purifying by recrystallization. The water used in all the experiment processes was distilled water.

Preparation of Nanospheres

The proposed fabrication of the nanospheres included two-step process according to that published previously [18]. The first step was to obtain linear chains of polystyrene (PSt) by free radical method. The process was as follows. A 200 ml of THF (2.47 mol) solution including 4.0 ml of St (3.5×10^{-2} mol) and 0.054 g of AIBN (3.29×10^{-4} mol) was added into 250 ml three-necked round-bottom flask equipped with a condenser, a nitrogen inlet and a pump outlet. The solution was bubbled by nitrogen to remove oxygen. The polymerization was performed under reflux to overcome the volatilization of THF at 70 ^{0}C for some time at 200 rpm.

The second step was to prepare poly(*N*-isopropylacrylamide) (PNIPAM) shells on the collapsed PSt templates. A series of NIPAM (1.9×10^{-3} mol, 1.6×10^{-3} mol, 1.2×10^{-3} mol, 1.7×10^{-4} mol) and BIS (1 wt % to NIPAM dosage. 1.42×10^{-5} mol, 1.18×10^{-5} mol, 8.5×10^{-6} mol, 1.25×10^{-6} mol, respectively) were dissolved in 175 ml of distilled water in 250 ml three-necked round-bottom flask including a nitrogen inlet, a stirrer and a pump inlet. The solution was bubbled by nitrogen for 30 min before reaction. When 16 ml of THF solution including linear PSt chains with the free radicals was pumped into NIPAM aqueous solution in droplets, monomers NIPAM were initiated by the free radicals on the surface of the collapsed PSt templates. Polymerization reaction was proceeded for 1h at 40 ^{0}C under stirring at higher speed, then continued for 3h under stirring at 200 rpm. The final products were vacuumized in order to remove unreacted styrene at room temperature.

Conversion of PSt in the First Step

After St polymerized at 70 ^{0}C for 4h, 6h, 8h, respectively, each of 10 ml THF solution was taken into a flask, then added toluene to terminate the reaction and added an excess of methanol to precipitate PSt. After centrifuging, drying and weighing, a conversion rate of St at different reaction time was calculated as follows,

$$\text{Conversion} = \frac{m_{PSt}}{m_{St} + m_{AIBN}} \times 100 \quad (2)$$

where m_{PSt}, m_{St} and m_{AIBN} denote the mass of PSt, St and AIBN, respectively. The conversions of PSt were listed in Table 1.

Characterizations of Morphology, Mean Diameter and Monodispersity

Scanning electron microscope (SEM, Hitachi S-450, Japan) and transmission electron microscope (TEM, TecnaiG2 20-type, Czech Republic) were used to observe the morphology of the samples. For SEM observations, all samples were mounted on a copper stub and coated in sputter style with gold. For TEM observations, all samples were dripped on the copper nets and dried under UV light.

The mean diameter and monodispersity were obtained by statistical method using a digital image analysis system based on the SEM and TEM photographs. During the analysis, the number in each photograph need be more than 300. The mean diameter was equal to sum of multiplication of the size and corresponding number percent of all spheres. To character quantitatively the monodispersity of the samples, an index named the size dispersal coefficient, δ, is defined as

$$\delta = \frac{D_{90} - D_{10}}{D_{50}} \quad (3)$$

where D_n (n = 10, 50, and 90) denotes the cumulative number percentage of spheres with a diameter up to D_n equal to n %. The δ is smaller, the size distribution means narrower [16, 17].

Characterization of Hydrodynamic Diameters of the Samples

The hydrodynamic diameters of the prepared samples at room temperature were determined by temperature-programmed photon correlation spectroscopy (TP-PCS, Brookhaven BI-9000AT, USA). The dispersed samples in water were allowed to equilibrate thermally for 10-15 min before measurements. The hydrodynamic diameters of spheres were calculated from diffusion coefficients by the Stokes-Einstein equation, and all correlogram analyses were performed by using the manufacturer-supplied software.

Acknowledgements

This research was funded by the National Natural Science Foundation of China (21276287, 20976202).

ORCID® iDs

Xin-Cai Xiao- https://orcid.org/0000-0002-7117-5305

References

1. Hou, X. *Adv. Mater*. **2016**, *28*, 7049–7064. doi: 10.1002/adma.201600797
2. Zhang, R.L.; Guan, Y.L.; Xiao, M.; Xiao, X.C. *ChemistrySelect* **2017**, *2*, 279-282. doi: 10.1002/slct.201601617.
3. Du, H.H.; Xiao, X.C. *RSC Advances* **2015**, *5*, 88021- 88026. doi: 10.1039/c5ra14491d
4. Yue, L.L.; Xie, R.; Wei, J.; Ju, X.J.; Wang, W.; Chu, L.Y. *J. Colloid Interface Sci.* **2012**, *377*, 137-144. doi: 10.1016/j.jcis.2012.04.009
5. Xiao, X.C.; Hong, Z.G. **2010**, *5*, 483-486. doi: 10.2147/IJN.S10907
6. Yan, G.P.; Zong, R.F.; Li, L.; Fu, T.; Liu, F.; Yu, X.H. *Pharm. Res*. **2010**, *27*, 2743-2752. doi: 10.1007/s11095-010-0275-7
7. Gao, J.H.; Liang, G.L.; Cheung, J.S.; Pan, Y.; Kuang, Y.; Zhao, F.; Zhang, B.; Zhang, X.X.; Wu, E.X.; Xu, B. *J. Am. Chem. Soc.* **2008**, *130*, 11828-11833. doi: 10.1021/ja803920b
8. Kim, J.; Kim, H.S.; Lee, N.; Kim, T.; Kim, H.; Yu, T.; Song, I.C.; Moon, W.K.; Hyeon, T. *Angew Chem. Int. Ed.* **2008**, *47*, 8438-8441. doi: 10.1002/anie.200802469
9. Torini, L.; Argillier, J.F.; Zydowicz, N. *Macromolecules* **2005**, *38*, 3225-3236. doi: 10.1021/ma047808e
10. Wu, D.; Scott, C.; Ho, C.C.; Co, C.C. *Macromolecules* **2006**, *39*, 5848-5853. doi: 10.1021/ma060951i
11. Cao, Z.H.; Ziener, U.; Landfester, K. *Macromolecules* **2010**, *43*, 6353-6360. doi: 10.1021/ma101115t
12. Zhang, M.C.; Lan, Y.; Wang, D.; Yan, R.; Wang, S.N.; Yang, L.; Zhang, W.Q. *Macromolecules* **2011**, *44*, 842-847. doi: 10.1021/ma102477u
13. Deveza, L.; Ashoken, J.; Castaneda, G.; Tong, X.M.; Keeney, M.; Han, L.H.; Yang, F. *ACS Biomater. Sci. Eng*. **2015**, *1*, 157−165. doi: 10.1021/ab500051v
14. Xiao, Y.; Wiesner, M.R. *J. Hazard Mater.* **2012**, *215-216*, 146−151. doi: 10.1016/j.jhazmat.2012.02.043
15. Xiao, X.C.; Lu, C. *J. Wuhan Univ. Technol.-Mater. Sci. Ed.* **2012**, *27*, 1048-1052. doi: 10.1007/s11595-012-0598-9
16. Xiao, X.C.; Chu, L.Y.; Chen, W.M.; Wang, S.; Xie, R. *Langmuir* **2004**, *20*, 5247-5253. doi: 10.1021/la036230j
17. Chu, L.Y., Xie, R., Zhu, J.H., Chen, W.M., Yamaguchi, T., Nakao S.I. *J. Colloid Interf. Sci.* 2003, 265, 187–196. doi:10.1016/S0021-9797(03)00350-3
18. Wang, Z.H.; Xiao, X.C. *Adv. Mater. Res.* **2013**, *634-638*, 2242-2245. doi: 10.4028/www.scientific.net/AMR.634-638.2242