Journal of Microencapsulation, November 2005; 22(7): 705–714

Taylor & Francis
Taylor & Francis Group

Effects of formulation factors on encapsulation efficiency and release behaviour *in vitro* of huperzine A-PLGA microspheres

X. FU[1], Q. PING[1], & Y. GAO[2]

[1]*School of Pharmaceutical Sciences, China Pharmaceutical University, Nanjing, PR China and*
[2]*Beijing Institute of Pharmacology and Toxicology, Beijing, PR China*

(Received 7 June 2004; accepted 10 December 2004)

Abstract
To develop a long-acting injectable huperzine A-PLGA microsphere for the chronic therapy of Alzheimer's disease, the microsphere was prepared by using an o/w emulsion solvent extraction evaporation method based on a series of formulation design of the emulsion. The dialysis method was used for release analysis. The encapsulation efficiency and release amount of the microspheres were determined by a UV/VIS spectrophotometer. The morphology of the microspheres was observed by scanning electron microscopy. The distribution of the drug within microspheres was observed by a confocal laser scanning microscope. The results indicated that the PLGA 15 000 microspheres possessed a smooth and round appearance with average particle size of 50 μm or so. The encapsulation percentages of microspheres prepared from PLGA 15 000, 20 000 and 30 000 were 62.75%, 27.52% and 16.63%, respectively. The drug release percentage during the first day decreased from 22.52% of PLGA 30 000 microspheres to 3.97% of PLGA 15 000 microspheres, the complete release could be prolonged to 3 weeks. The initial burst release of microspheres with higher molecular weight PLGA could be explained by the inhomogeneous distribution of drug within microspheres. The encapsulation efficiency of the microspheres improved as the polymer concentration increased in the oil phase and PVA concentration decreased in the aqueous phase. The burst release could be controlled by reducing the polymer concentration. Evaporation temperature had a large effect on the drug release profiles. It had better be controlled under 30°C. Within a certain range of particle size, encapsulation efficiency decreased and drug release rate increased with the reducing of the particle size.

Keywords: *Huperzine A, poly(lactic-co-glycolic acid), microspheres, encapsulation efficiency, burst release*

Introduction

Biodegradation injectable microspheres have been studied widely in the last 30 years. They can prolong the duration of drug effect significantly and improve the compliance of patients. The total dose and some adverse reactions may be reduced because it can sustain a steady plasma concentration. For example, the effective dose of leuprorelin depot formulation

Correspondence: Qineng Ping, School of Pharmaceutical Sciences, China Pharmaceutical University, Nanjing 210009, PR China. Tel: +86 25 83271321. E-mail: pinqn@cpu.edu.cn

ISSN 0265-2048 print/ISSN 1464-5246 online
DOI: 10.1080/02652040500162196

is reduced to 1/4 ~ 1/8 of that needed in the repeated parenteral administration of a plain solution (Okada 1997). There is also no need to be embedded by surgical operation or be removed after the drug is released completely. Among many biodegradable polymers recently investigated, PLA and PLGA have received much attention due to their good biodegradable and biocompatible properties. Drug release rate can be controlled by selection of their molecular weight and copolymer compositions.

Huperzine A, an alkaloid extracted from Chinese herb Huperzia serrata (Thunb) Trev, is a potent and selective acetycholinesterase inhibitor. It is mainly used for the treatment of Alzheimer's disease patients (Tang 1996). At present, huperzine A is administrated daily either orally or by injection. In order to improve the compliance of patients and relieve its side effects, a huperzine A-biodegradable PLGA microsphere was developed in the paper.

There are several methods to prepare PLGA microspheres, such as the emulsion solvent evaporation method (O'Donnell and McGinity 1997), phase-separation method (Leelarasamee et al. 1988) and spray-drying method (Bain et al. 1999). The success of the microencapsulation method depends on many factors, including drug solubility, partition coefficient, polymer composition, molecular weight, etc. Huperzine A is a poorly water soluble substance, its solubility in water at 25°C is ~0.9 mg ml^{-1}. O/W emulsion was chosen to fabricate huperzine A-PLGA microspheres in this work and the effects of a series of formulation factors of O/W emulsion on the microencapsulation were investigated.

Materials

PLGA (W_η 15 000, 20 000, 30 000; lactide/glycolide ratio, 75/25) was purchased from Chengdu Institute of Organic Chemistry, Chinese Academy of Sciences; huperzine A was obtained from Beijing Institute of Pharmacology and Toxicology; polyvinyl alcohol (PVA-124) was obtained from Beijing Chemical Reagents Company. All other materials or solvent were of reagent or analytical grade.

Experiment methods

Preparation of microspheres

O/W emulsion solvent evaporation method was applied to fabricate huperzine A-PLGA microspheres. An amount of PLGA and huperzine A were added to 1 ml of DCM. After completely dissolved, it was poured into 3.5% w/v PVA-124 aqueous solution and then the mixture was emulsified by constant stirring at 600 rpm for 10 min by using a propeller stirrer (SXJQ-1, Zhengzhou, China) at 28°C. Then stirring at 300 rpm at 28°C was continued for 4 h to evaporate the organic solvent. The hardened microspheres were filtered, rinsed with distilled water and dried under vacuum.

Determination of huperzine A content

Huperzine A was extracted with 25 ml of a mixture of DCM and ethanol (1:1 v/v) from the microspheres. The solution was directly measured at 313 nm by using a UV/VIS spectrophotometer (UV-160, SHIMADAZU, Japan). The polymers did not interfere with absorbance of the drug at the specified wavelength. The encapsulation efficiency is expressed as the ratio of detected and added drug amount.

Release studies of huperzine A microspheres in vitro

The dialysis method was utilized for the study of the drug release *in vitro*. In brief, ~25 mg of microspheres were weighted and added to the dialysis bag with a cut-off molecular weight of 1 kDa, 1 ml phosphate buffered saline (PBS, 0.01 M, pH 7.4) was then added. The dialysis bag containing microsphere suspension was kept in beaker flasks filling 50 ml PBS as the release medium and shaken at a rate of 72 rpm at 37°C. Five millilitres of medium was drawn out at the pre-determined day intervals and the same volume of fresh PBS was replenished. Huperzine A concentration was determined in triplicate at 307 nm by spectrophotometer.

Microscopic observations

The microspheres were mounted onto metal stubs using double-sided adhesive tape. After vacuum-coating with a layer of gold, the surface of huperzine A microsphere was observed by scanning electron microscopy (Hitachi S-450, Japan).

Drug distribution within the microspheres

A Bio-Rad Radiance 2100^{TM} laser scanning confocal system in conjunction with a Nikon TE300 microscope was used to observe the drug distribution within the microspheres. An excitation wavelength of 488 nm was used. The microspheres were suspended in an anti-fading agent solution and the drug fluorescence was viewed through a 100× Nikon Plan Fluor oil immersion lens.

Results and discussion

Determination method of drug content in microspheres and drug concentration in release medium

The calibration curves of huperzine in DCM and ethanol (1:1 v/v) and release medium was linear within the range of $0.5 \sim 35\,\mu g\,ml^{-1}$ and $0.25 \sim 25\,\mu g\,ml^{-1}$. The average recovery was $99.16 \pm 1.51\%$ and $99.57 \pm 0.15\%$, respectively.

Encapsulation efficiency

It was reported that the encapsulation efficiency of microspheres using an O/W method was mainly dependent on drug partition coefficient in internal and external phases (Bodmeier and McGinity 1987, Al-Maaieh and Flanagan 2001). The acceleration of microsphere solidification may reduce the drug partitioning into the external aqueous phase and increase the encapsulation percentage (Zhu et al. 2001). The extracted rate of organic solvent from oil phase and its evaporation rate from aqueous phase were proven to be important factors.

As shown in Table I, the encapsulation efficiency was highly dependent on the molecular weight of PLGA. The encapsulation efficiency reduced significantly from 62.5% with PLGA 15 000 microspheres to 20% with PLGA 30 000 microspheres. However, the encapsulation efficiency of PLGA 20 000 and PLGA 30 000 microspheres had no significant difference. An attempt was made to add some water miscible solvents such as acetone and ethanol to DCM in order to speed up the solidification of microspheres, but the improvement of payload was very limited, especially in PLGA 20 000 and PLGA 30 000 microspheres.

Table I. Effect of the molecular weight and concentration of PLGA and PVA on encapsulation efficiency.[a]

Molecular weight	PLGA conc %(w/v)	PVA conc %(w/v)	Encapsulation efficiency (%)
15 000	40	3.5	62.75
	50	3.5	68.59
	60	3.5	71.09
	40	4.5	52.16
20 000:15 000[b]			
1:0	30	3.5	22.34
1:0	40	3.5	35.21
1:0	40	4.5	27.52
3:1	40	4.5	25.31
1:1	40	4.5	40.25
1:3	40	4.5	49.79
30 000:15 000[b]			
1:0	40	3.5	16.63
1:0	20	3.5	8.60
3:1	20	3.5	22.40

[a]Theoretical drug loading: 5%; [b]ratio of weight.

Table II. Effect of particle size on encapsulation efficiency of PLGA 15 000 microspheres.

Particle size (mesh)	Encapsulation efficiency (%)
125–200	56.63
200–400	52.48
400–700	52.16
Below 700	44.42

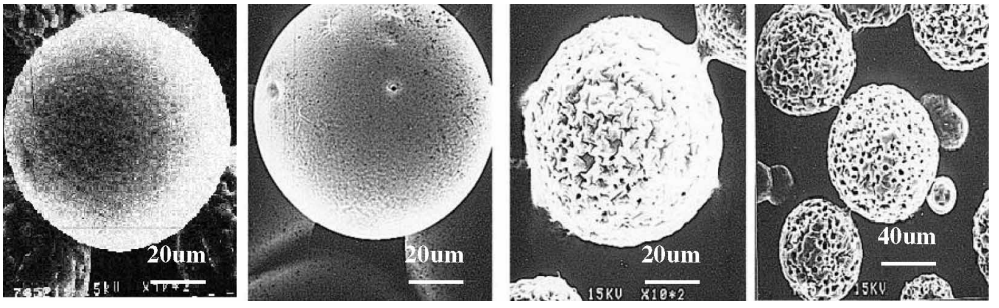
Mixing PLGA 15 000 with PLGA 20 000 or PLGA 30 000 facilitated the drug payload compared with the results of using the later two separately. This may contribute to the weaker compatibility of huperzine A with the higher molecular weight PLGA. Similar results were found by other authors in which the solubility of the tested chemicals in PLA polymer was decreased with increasing molecular weight (Bodmeier et al. 1989, Boury et al. 1997).

The results also indicated that the payload of three kinds of PLGA microspheres, especially that of higher molecular weight PLGA microspheres, was found to be enhanced by increasing the polymer concentration in the oil phase, because the rate of polymer precipitation from the oil phase speeded up in the solidification process at the same temperature and shear strength. In contrast, the increase of PVA-124 concentration in the continuous phase resulted in the decrease of the encapsulation efficiency. This may be explained by the fact that the solubility of huperzine A in the external aqueous phase was increased from $0.99 \pm 0.11\,\mathrm{mg\,ml^{-1}}$ in 3.5% PVA solution to $1.15 \pm 0.13\,\mathrm{mg\,ml^{-1}}$ in 4.5% PVA solution.

Table II indicates the influence of particle size on the encapsulation efficiency of microspheres. It was shown that particle size had little influences on encapsulation efficiency when microspheres were in the size range of $125 \sim 700$ mesh, but when the particle size was below 700 mesh the encapsulation efficiency tended to decrease. This was mainly because it took more time to form smaller emulsion droplets than larger ones in the emulsion process,

Table III. Effect of evaporation temperature on encapsulation efficiency.

Evaporation temperature (°C)	Encapsulation efficiency (%)
25	78.03
30	73.05
35	78.03
40	59.27



25°C 30°C 35°C 40°C

Figure 1. SEM pictures of microspheres fabricated at different evaporation temperature.

thus drug in the smaller microspheres had more chances to diffuse to the aqueous phase before polymer precipitation from the oil phase.

Table III indicates the influence of solvent evaporation temperature on the encapsulation efficiency of microspheres. It was found that the encapsulation efficiency of microspheres prepared at 40°C showed a significant decrease. This could be explained by the fact that microspheres fabricated at 40°C had much smaller pores on the surface than those microspheres fabricated at lower temperature (Figure 1). Those pores were produced by the fast evaporation of the organic solvent, through which drug could easily diffuse to the aqueous phase.

Huperzine A in vitro *release of PLGA microspheres*

There are many factors that influence the drug release of PLGA microspheres, such as the ratio of the copolymer, molecular weight, preparation process, etc. (O'Donnell and McGinity 1997). The burst effect of the microspheres attracted much attention because it may result in the severe adverse action or economical waste. In many cases, the burst release was attributed to the release of the drug on the surface of microspheres or underneath the surface of microspheres. So the burst release was connected closely with those factors that affected the drug dispersion in microspheres and the porosity and curvature caused by the organic solvent evaporation (Corre et al. 1997, Matsumoto et al. 1997).

Figure 2 demonstrates the release profiles of huperzine A-PLGA 15 000 microspheres prepared at different polymer concentrations. It suggested that the PLGA 15 000 concentrations in the internal phase had less effect on the drug release rate.

Figure 3 shows the release profiles of three molecular weight huperzine A-PLGA microspheres prepared at the same polymer concentration of 400 mg ml^{-1}. The much higher released amount of huperzine A during the first day was found in the microspheres

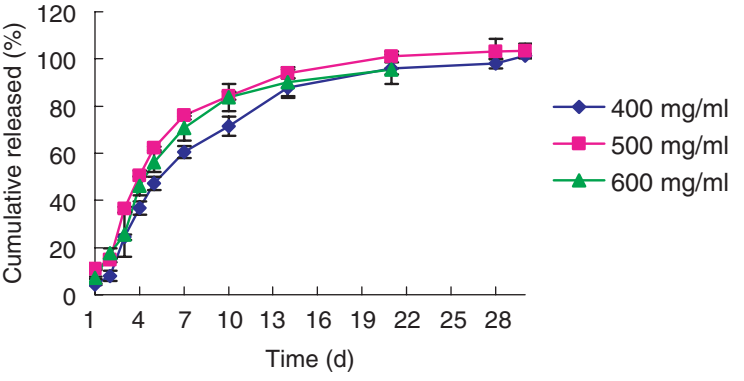


Figure 2. Effect of the polymer concentration in the oil phase on release profiles of huperzine A-PLGA 15 000 microspheres.

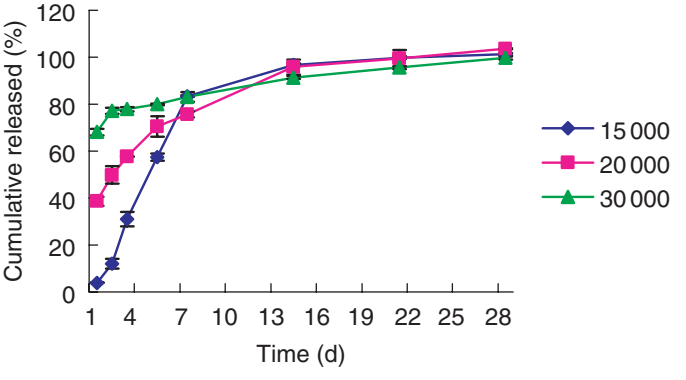


Figure 3. Effect of the molecular weight on huperzine A release from PLGA microspheres prepared at the same concentration (400 mg ml^{-1}) in the oil phase.

of PLGA 20 000 and PLGA 30 000. The initial releases of PLGA 15 000, PLGA 20 000 and PLGA 30 000 microspheres were ~3.5%, 45%, 78%, respectively. It can be seen in Figure 4 that the PLGA 30 000 microspheres had a more porous and rougher surface than PLGA 15 000 and 20 000 microspheres. The solution of PLGA 30 000 was more viscous than the solution of PLGA 15 000 or PLGA 20 000 at the same concentration and PLGA 30 000 was precipitated more rapidly from the oil phase to form the microspheres in which the comparative amount of solvent was embedded and resulted in high porosity and coarse surface as the solvent evaporated continuously. Similar results were observed in others' studies (Bodmeier et al. 1989). Drug release following the first day of PLGA 15 000 microspheres was significantly faster than PLGA 20 000 and 30 000 microspheres in which the degradation (hydrolysis) of PLGA became the limiting factor for the high molecular weight polymer.

To further understand the effect of the molecular weight of PLGA, PLGA concentration in the oil phase on the drug release behaviour, different polymer concentrations were used for the preparation of huperzine A microspheres of three kinds of PLGA. As shown in Figure 5, no significant difference of the surface roughness and the particle size of these microspheres were observed, the results indicated that with the higher molecular weight

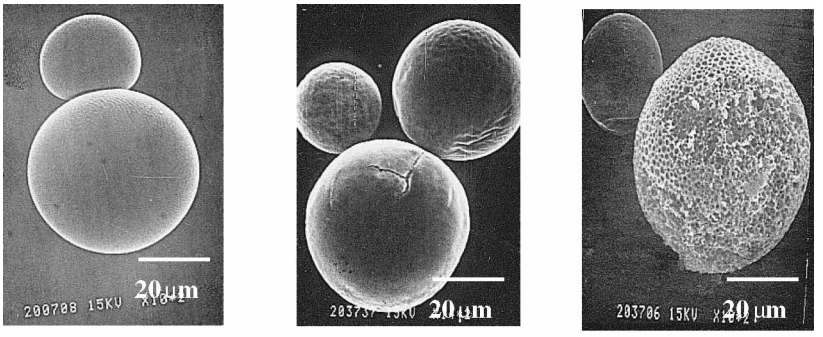


Figure 4. SEM photographs of the PLGA microspheres fabricated at the same concentration (400 mg ml^{-1}) in the organic solvent: (a) PLGA 15 000, (b) PLGA 20 000, (c) PLGA 30 000.

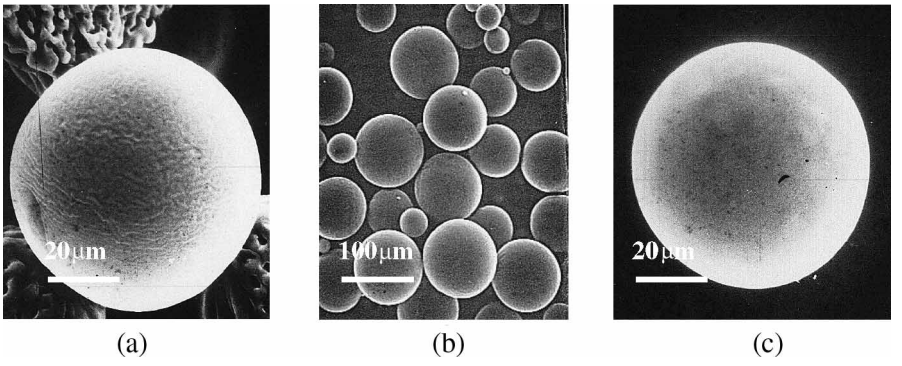


Figure 5. SEM pictures of microspheres using PLGA of different molecular weight fabricated at the different polymer concentration: (a) 15 000 polymer conc = 400 mg ml^{-1}, (b) 20 000 polymer conc = 300 mg ml^{-1}, (c) 30 000 polymer conc = 200 mg ml^{-1}.

polymers, the lower concentration in internal solvent could improve the formation of the microsphere. Figure 6 shows the release profiles of PLGA 15 000, PLGA 20 000, PLGA 30 000 microspheres fabricated separately at concentrations of 400, 300 and 200 mg ml^{-1}. Compared with the microspheres prepared with the higher polymer concentration of 400 mg ml^{-1}, the initial release on first day of PLGA 20 000 and PLGA 30 000 microspheres decreased significantly but were still high (23.51% and 22.52%, respectively). For PLGA 15 000 microspheres, the lowest initial release (3.92%) was observed at the highest polymer concentration of 400 mg ml^{-1}. The drug was hardly released from PLGA 30 000 microspheres following the first day because the drug loading was too low (1.03%) and the degradation rate of PLGA 30 000 was very slow.

The drug loading could not be used to explain the burst effect of PLGA 20 000 and PLGA 30 000 microspheres prepared in the lower concentrations, the actual drug loadings of PLGA 20 000 and PLGA 30 000 microspheres were 1.03% and 0.41%, respectively, much lower than that of PLGA 15 000 (3.53%). Although the burst release of PLGA 20 000 or PLGA 30 000 microspheres may be decreased to some extent as the polymer concentration decreased further, drug loading in this case was too low to be accepted.

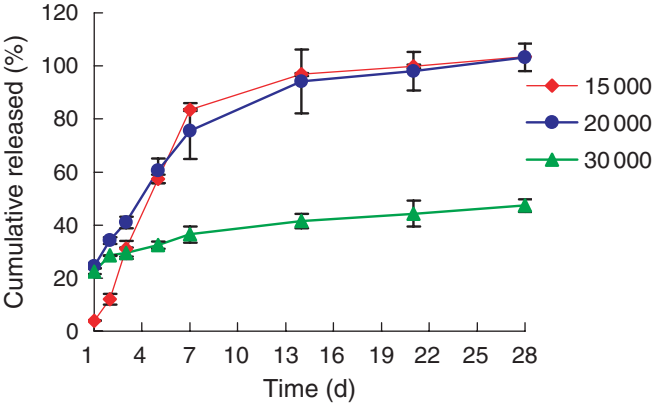


Figure 6. Effect of the molecular weight on huperzine A release from the microspheres prepared at different PLGA concentrations.

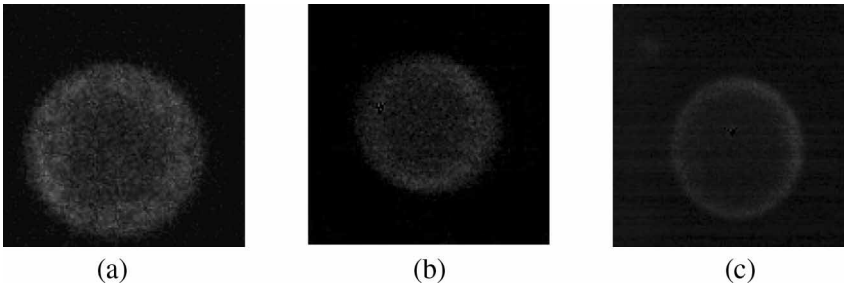


Figure 7. CLSM pictures of microspheres using PLGA of different molecular weight: (a) 15 000, (a) 20 000, (c) 30 000.

Initial burst release of microspheres might be related to the drug distribution within microspheres. As shown in CLSM photography (Figure 7), drug was distributed more uniformly in PLGA 15 000 microspheres compared with PLGA 20 000 and 30 000 microspheres, more drug was distributed on or near the surface of the later two microspheres. This resulted in the initial burst release of PLGA 20 000 and 30 000 microspheres. The poor compatibility of huperzine A with the higher molecular weight polymers resulted in the drug in the internal phase diffusing more easily to the aqueous phase during the process of microsphere solidification, thus more drug distributed on or near the surface of microspheres. This was consistent with the reports of other authors (Iwata et al. 1999, Yang et al. 2001).

Figure 8 shows the release profiles of microspheres with four different size ranges (125 ~ 200 mesh, 200 ~ 400 mesh, 400 ~ 700 mesh, below 700 mesh). It shows a significant rapid release when the particle size is below 700 mesh.

Figure 9 shows the release profiles of PLGA 15 000 microspheres prepared at 25, 30, 35 and 40°C. The drug release rate was improved markedly when evaporation temperature was very close to the DCM boil point such as 35 and 40°C. This could be explained by the surface morphology of the microspheres (Figure 1). With the improvement of the temperature, the evaporation rate of DCM was increased, more and more small pores could be observed

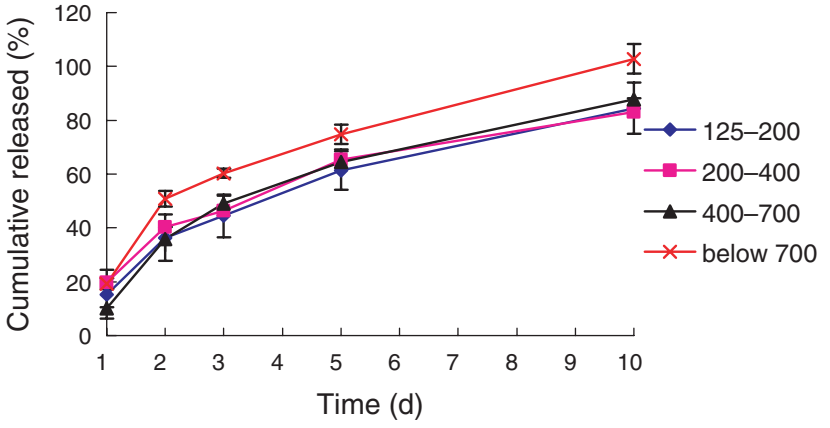


Figure 8. Effect of the particle size on huperzine A release from the microspheres.

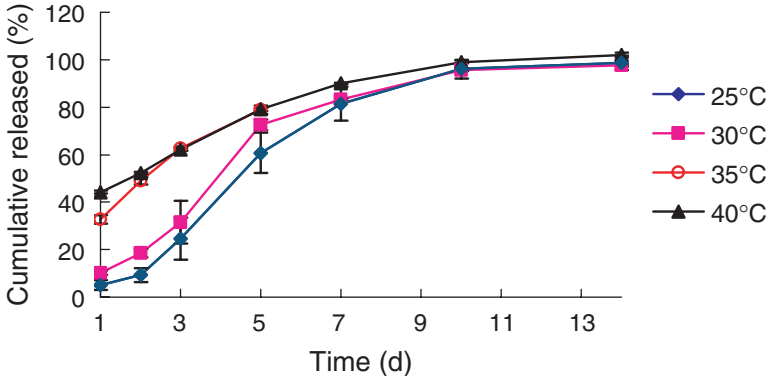


Figure 9. Effect of evaporation temperature on huperzine A release from the microspheres.

on the surface of microspheres, through which *in vitro* release medium could easily penetrate and the effective surface area available for drug release was increased. In order to obtain microspheres with smooth appearance and slow release profiles, evaporation temperature had better be controlled not too close to the organic solvent's boiling point.

Conclusions

O/W emulsion solvent evaporation method was applied successfully to fabricate huperzine A-PLGA 15 000 microspheres which possessed a smooth and round appearance with an average particle size of 45 μm or so, the complete release could be prolonged to 3 weeks. The polymer concentration in the oil phase has important effects on the encapsulation efficiency and initial burst release of microspheres, especially on those prepared with higher molecular weights of 20 000 and 30 000. The initial burst release of PLGA 20 000 and 30 000 microspheres can be reduced markedly by decreasing the polymer concentration in the oil phase. However, the initial burst release was still greater than that of PLGA 15 000 microspheres, which could be explained by the inhomogenous

distribution of drug within microspheres. The molecular weight of polymer and polymer concentration in organic solvent might affect the distribution and diffusion character of drug between the oil and aqueous phases. The evaporation temperature was one of the important factors to influence the encapsulation efficiency and *in vitro* release behaviour. The temperature had better be controlled below and not too close to the organic solvent's boiling point. Within a certain range of particle size, encapsulation efficiency decreased and drug release rate increased with the reducing of the particle size.

References

Al-Maaieh A, Flanagan DR. 2001. Salt and cosolvents effects on ionic drug loading into microspheres using an O/W method. Journal of Controlled Release 70:169–181.

Bain DF, Munday DL, Smith A. 1999. Solvent influence on spray-dried biodegradable microspheres. Journal of Microencapsulation 16:453–474.

Bodmeier R, McGinity JW. 1987. Polylactic acid microspheres containing quinidine base and quinidine sulphate prepared by the solvent evaporation technique. I. Method and morphology. Journal of Microencapsulation 4:279–288.

Bodmeier R, Oh KH, Chen H. 1989. The effect of the addition of low weight poly(D,L-lactide) on drug release from biodegradable poly(D,L-lactide) drug release from biodegradable poly(D,L-lactide) drug delivery systems. International Journal of Pharmaceutics 51:1–8.

Boury F, Marchais H, Proust JE, Benoit JP. 1997. Bovine serum albumin release from poly(α-hydroxy acid) microspheres: Effect of polymer molecular weight and surface properties. Journal of Controlled Release 45:75–86.

Corre PL, Rytting JH, Gajan V, Chevanne F, Verge RL. 1997. *In vitro* controlled release kinetics of local anaesthetics from poly(D,L-lactide) and poly(lactide-co-glycolide) microspheres. Journal of Microencapsulation 14:243–255.

Iwata M, Nakamura Y, McGinity JW. 1999. *In vitro* and *in vivo* release properties of brilliant blue and tumour necrosis factor-alpha(TNF-α) from poly(D,L-lactic-co-glycolic acid) multiphase microspheres. Journal of Microencapsulation 16:777–792.

Leelarasamee N, Howard SA, Malanga CJ, Ma JKH. 1988. A method for the preparation of poly(lactic acid) microencapsules of controlled particle size and drug loading. Journal of Microencapsulation 52:147–157.

Matsumoto A, Matsukawa Y, Suzuki T, Yoshino H, Kobayashi M. 1997. The polymer-alloys method as a new preparation method of biodegradable microspheres: Principle and application to cisplatin-loaded microspheres. Journal of Controlled Release 48:19–27.

O'Donnell PB, McGinity JW. 1997. Preparation of microspheres by the solvent evaporation technique. Advance Drug Delivery Reviews 28:25–42.

Okada H. 1997. One- and three-month release injectable microspheres of the LH-RH superagonist leuprorelin acetate. Advance Drug Delivery Reviews 28:43–70.

Tang XC. 1996. Huperzine A (Shuangyiping): A promising drug for Alzheimer's disease. Acta Pharmacologica Sinica 17:481–484.

Yang Y-Y, Chung T-S, Ng NP. 2001. Morphology, drug distribution, and *in vitro* release profiles of biodegradable polymeric microspheres containing protein fabricated by double-emulsion solvent extraction/evaporation method. Biomaterials 22:231–241.

Zhu KJ, Jiang HL, Du XY. 2001. Preparation and characterization of hcG-loaded polylactide or poly(lactide-co-glycolide) microspheres using a modified W/O/W emulsion solvent evaporation technique. Journal of Microencapsulation 18:247–260.