ENCAPSULATION OF HORSERADISH PEROXIDASE INTO POLY(D,L-LACTIDE) BY THE MODIFIED PRECIPITATION METHOD

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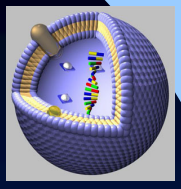
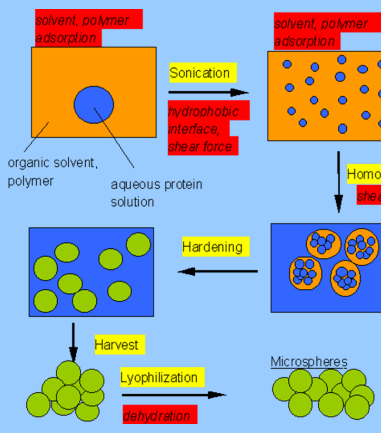
Introduction



Injectable poly-D,L-lactide (PDLLA) microspheres containing proteins or peptides as controlled release devices have been widely used for the treatment of human diseases and animal health. Fundamental understanding of the relationship among the size of microspheres, encapsulation efficiency and protein release capacity are essential for the design of microsphere delivery systems [1,2].

The modified precipitation method [3-6] is method of encapsulating hydrophilic drugs, especially protein and peptide ones, into microspheres. Since the release profiles of proteins dominantly depend on the nature and morphology of the polymer, drug distribution within microspheres and release temperature, the fabrication of microspheres with specific morphology and drug distribution is a challenge for chemical engineers [7]. PDLLA microspheres can protect proteins against biological inactivation and can ensure their release for long time frames, and at specified time. Finally, the size of the particles can be used to passively target the delivery vehicles for uptake by specific types of cells, such as professional antigen-presenting cells, or to target specific tissues [8].

Materials and Methods



Poly-D,L-lactide with an average molecular weight of 50,000 g/mol was purchased from Sigma (Sigma-Aldrich, Germany). Horseradish peroxidase (HRP) with molecular weight of 45 kDa was used as model protein. Microspheres were prepared by modified precipitation method [4]. The following parameters were varied: co-solvent was methanol or ethanol and the surfactant concentration was 1% w/v or 5% w/v. Individual preparations were repeated at least three times. Briefly, the method was performed as follows: 40 mg of commercial granules PDLLA (SIGMA-Aldrich, Germany) were dissolved in chloroprhorm, and 1 mg of HRP was dissolved in 1 ml of water. This mixture was added to methanol or ethanol to form a dispersion. This dispersion was added dropwise to 20 ml PVA solution containing 1 wt% or 5 wt% of PVA while the mixture was stirred at 1200 rpm using magnetic stirrer. After that, solution was homogenized 10 min on 25 000 rpm, centrifuged 2 hours on 4000 rpm, decanted and vacuum dried.

Results

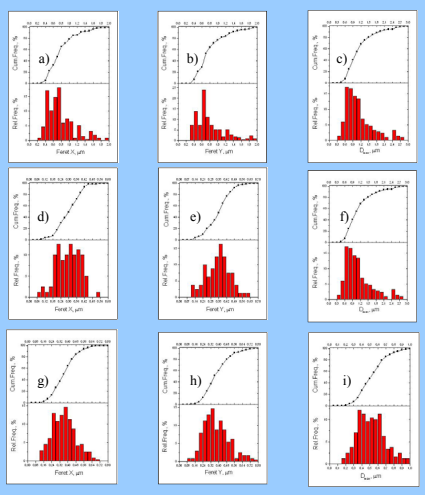


Fig. 2. Results of stereological analysis

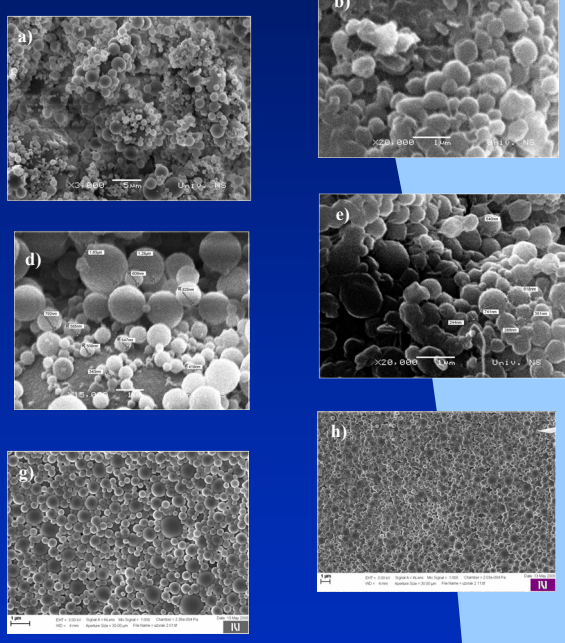


Fig. 1. *SEM images of HRP-loaded PDLLA spheres: a) batch 1. ((a) and (d)) (co-solvent ethanol and 1% w/v PVA), b) batch 2. ((b) and (e)) (co-solvent methanol and 5% w/v PVA, c) batch 3. ((c) and (f)) (co--solvent ethanol and 5% w/v PVA) and d) FESEM images of HRP-loaded PDLLA spheres-batch 3. ((g), (h) and (i)) (co-solvent ethanol and 5% w/vPVA)*

Table 1. Results of stereological analysis

PDLLA + HRP (Batch)	Feret X (nm)			Feret Y (nm)			Dmax (nm)		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
1.	240	1980	780 ±340	300	2230	800 ±400	390	2830	1130 ±520
2.	80	600	300 ±90	110	720	350 ±110	140	940	460 ±140
3.	120	820	360 ±120	100	810	360 ±130	180	1003	530 ±170

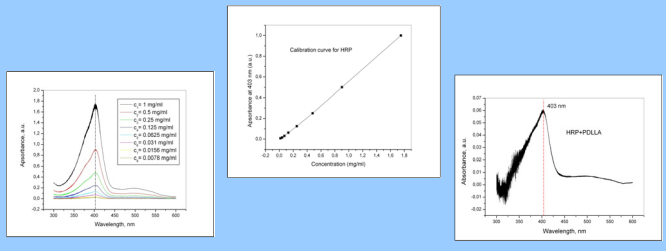


Fig. 3. *Spectrophotometric analysis of PDLLA-HRP powder prepared by precipitation method*

The aim of this study was to produce HRP loaded PDLLA spheres with the best properties for controlled and sustained delivery of HRP. The ability to control the size of PDLLA spheres with incorporated HRP, should facilitate the investigations of their scope for applicability in drug delivery [9]. We have investigated the key parameters to fabricate PDLLA spheres containing HRP as a model protein using the modified precipitation method. Various factors that influence the size and morphology of particles, encapsulation efficiency and initial release were varied. From the results obtained through the stereological examination we can see that particles from the first series are larger, having a size distribution with mean diameter of 1130 nm. (Fig. 2. (a-c) and Table 1.). The particles from the second and the third series are smaller, with mean diameter of 460 nm (Fig. 2. (d-f) and Table 1.) and 530 nm (Fig. 2. (g-i) and Table 1.). From the SEM images (Fig. 1. (a-f)) and FESEM images (Fig. 1. (g-i)) we can see that all PDLLA-HRP particles, prepared by modified precipitation method, have perfectly spherical shape, smooth surface and are non-agglomerated. The average diameter of the optimal PDLLA-HRP spheres, prepared by precipitation method is 530 nm (Table 1.). The UV spectroscopy has been used to estimate encapsulation efficiency of HRP loaded PDLLA particles (Fig. 3.). From the spectrophotometric analysis (Fig. 3.), the encapsulation efficiency of 46% is calculated. Differential scanning calorimetry (DSC) (Fig. 4.) and X-ray diffraction (XRD) (Fig. 5.) were used to characterize the particles.

Conclusions

HRP-loaded PDLLA particles were successfully obtained by precipitation method. PDLLA-HRP particles, prepared by modified precipitation method, have perfectly spherical shape, smooth surface and are non-agglomerated. In addition, the optimal particles were obtained with ethanol and 5% PVA. The mean diameter of the particles is 530 nm, and encapsulation efficiency is 46 %. The main advantage of this method is that it does not require an increase in temperature and, therefore, may be useful when the heat-sensitive drugs, like proteins, are used.

References

[1] Y. Ataman-Önal, S. Munier, A. Ganée, C. Terrat, P.-Y. Durand, N. Battail, F. Martinon, R. Le Grand, M.-H. Charles, T. Delair, B. Verrier, J. Control. Release 112 (2006) 175-185.
[2] Y.-Y. Yang, T.-S. Chung, X.-L. Bai, W.-K. Chan, Chem. Eng. Sci. 55 (2000) 2223-2236.
[3] M. Stevanović, J. Savić, B. Jordović, D. Uskoković, Colloid. Surface B 59 (2007) 215-223.
[4] M. Stevanović, N. Ignjatović, B. Jordović, D. Uskoković, J. Mater. Sci.-Mater.M. 18 (2007) 1339-1344.
[5] M. Stevanović, B. Jordović, D. P. Uskoković, J. Biomed. Biotechnol. (2007), in press (doi: 10.1155/2007/84965).
[6] I. Jovanović, B. Jordović, M. Petković, N. Ignjatović, D. Uskoković, Microsc. Res. and Tech. 71 (2008) 86-92.
[7] F. Mollica, M. Biondi, S. Muzzi, F. Ungaro, F. Quaglia, M. I. La Rotonda, P. A. Netti, J Mater Sci: Mater Med 19 (2008) 1587-1593.
[8] M. Liu, Z. Zhou, X. Wang, J. Xu, K. Yang, Q. Cui, X. Chen, M. Cao, J. Weng, Q. Zhang, Polymer 48 (2007) 5767-5779.
[9] N. Rizkalla, C. range, F.-X. Lacasse, P. Hildgen, J Microencapsul 23 (2006) 39-57.

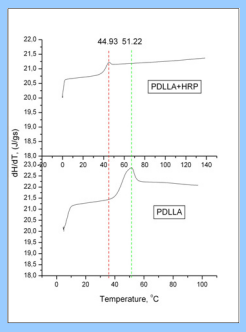


Fig. 4. *DSC diagrams of PDLLA powder and PDLLA-HRP powder*

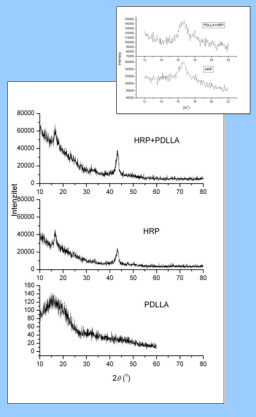


Fig. 5. *XRD diagrams of PDLLA, HRP and PDLLA-HRP powder*