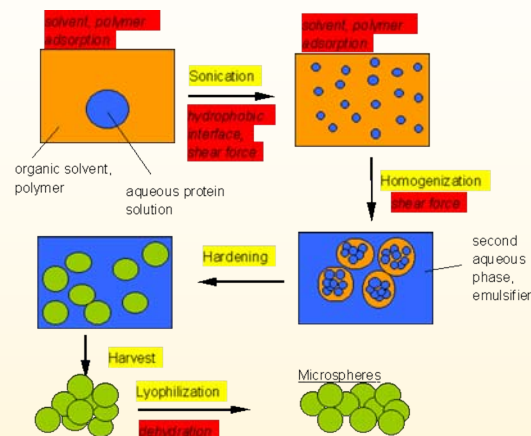


## Introduction

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 [3,4] 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 [5]. 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 [6].

## 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]. Briefly, the method was performed as follows: 40 mg of commercial granules PDLLA (SIGMA-Aldrich, Germany) were dissolved in chloroform, and 1 mg of HRP was dissolved in 1 ml of water. This mixture was added to ethanol to form a dispersion. This dispersion was added dropwise to 20 ml PVA solution containing 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

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 [7]. The sphere preparation technique is so complex that it is very difficult to predict optimal conditions for a specific polymer. In practice, optimal conditions have to be empirically found for each polymer drug system. 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 FESEM images (Fig. 1.) 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 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. 2.). From the spectrophotometric analysis (Fig. 2.), the encapsulation efficiency of 46% is calculated. Differential scanning calorimetry (DSC) (Fig. 3.) and X-ray diffraction (XRD) (Fig. 4.) were used to characterize the particles.

Table 1. Results of stereological analysis

	Min	Max	Mean
Feret X (nm)	120	820	360±120
Feret Y (nm)	100	810	360±130
Dmax (nm)	180	1003	530±170

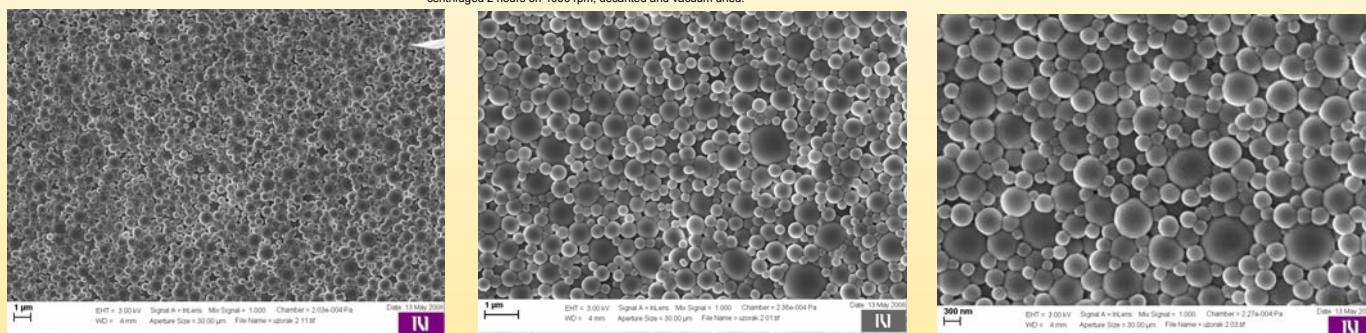


Fig. 1. FESEM images of HRP-loaded PDLLA spheres

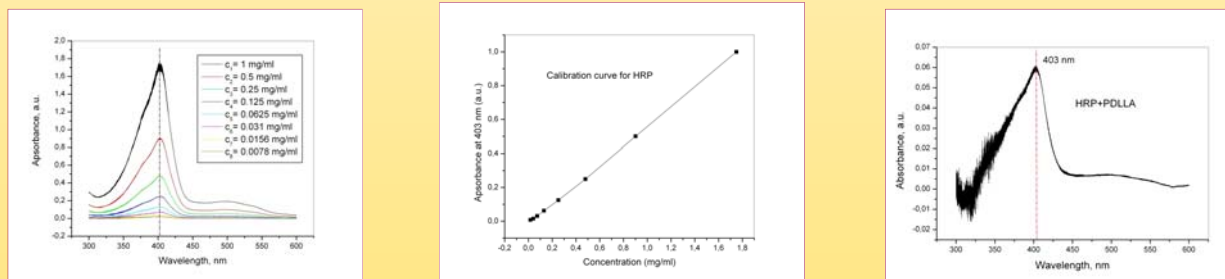


Fig. 2. Spectrophotometric analysis of PDLLA-HRP powder prepared by precipitation method

## 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. 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.

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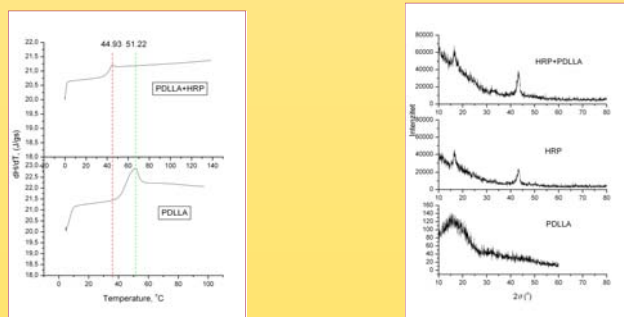


Fig. 3. DSC diagrams of PDLLA powder and PDLLA-HRP powder

Fig. 4. XRD diagrams of PDLLA, HRP and PDLLA-HRP powder

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