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Microencapsulation of bovine hemoglobin: entrapment efficiency using W/O/W double emulsion technique

F. F. Lima[(1)*], C. T. Andrade[(1)], S. H. Wang[(2)], W. S. Drumond[(2)]

(1) IMA, Universidade Federal do Rio de Janeiro, felipelima@ima.ufrj.br
(2) PMT, Escola Politécnica, Universidade de São Paulo
* Corresponding author.

Abstract – The use of biopolymers for the development of oxygen carriers has been extensively investigated due to the wide variety of polymers available for biomedical applications. In this work, bovine hemoglobin (Hb) was encapsulated in a mixture of two bioresorbable copolymers, using a double emulsion technique. This technique is considered to be the best suited to encapsulate water-soluble drugs like proteins. For Hb encapsulation, spherical particles (Fig. 1) were produced. The effect of polymer composition on protein entrapment is shown in Fig. 2.

The use of biopolymers for the development of oxygen carriers has been extensively investigated due to the wide variety of polymers available for biomedical applications. Two different ABA triblock polymers were used to encapsulate bovine hemoglobin. Copolymer 1 (Co1) is a triblock copolymer, PLLA-PEG-PLLA, comprised by segments of poly(L,L-lactide) (PLLA) and poly(ethylene glycol) (PEG). Copolymer 2 (Co2) contains a central PEG segment (4,000 $g.mol^{-1}$) linked at both OH-end groups to a random copolymer of L,L-lactide and ε-caprolactone.

Microspheres containing Hb were prepared by a modified W/O/W double emulsion technique [1,2]. First, Hb aqueous solution was emulsified in organic solvent (methylene chloride) containing polymer by a high-speed homogenizer. Thereafter, the primary emulsion was poured into an aqueous solution containing 1% (w/v) PVA followed by a two-step re-emulsification for 25 s and 90 s, respectively. The double emulsion was subsequently added to 200 mL of a buffer solution (Tris.HCl 0.1 N pH 7.4). The microcapsules were recovered by partial evaporation, followed by centrifugation.

The amount of Hb entrapped into the microspheres was calculated by measuring the amount of Hb in the supernatant after centrifugation. Difference between the initial amount of hemoglobin (Hb_{total}) and the amount of Hb in the supernatant (Hb_{free}) allowed the determination of the entrapment efficiency (EE%) of hemoglobin in the microspheres. Results of EE% can be related to differences in viscosity and stability of W_1/O primary emulsions. The lowest EE% achieved by HbP-1 may be attributed to the high viscosity of W_1/O. The highest EE% achieved by HbP-3 may be attributed to the stability of its primary emulsion. Scanning electron microscopy (SEM) was used to evaluate the morphology of microspheres (Fig. 1).

Table 1: Mixture composition

Sample	Co1:Co2
HbP-1	0:100
HbP-2	25:75
HbP-3	50:50
HbP-4	75:25
HbP-5	100:0

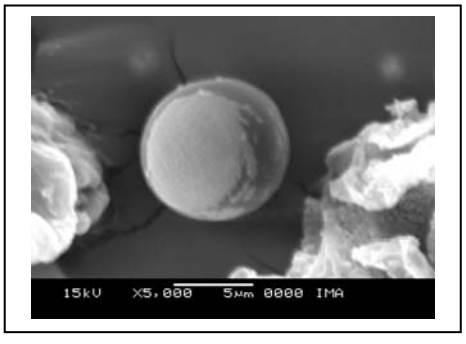

Figure 1: SEM image of sample P-4, without Hb.

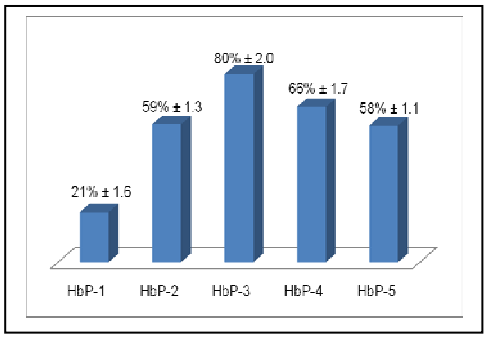

Figure 2: Effect of polymer blend composition on the efficiency of Hb encapsulation.

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[1] F.T. Meng *et al*. Colloids and Surfaces B: Biointerfaces (2004) 177 – 183.
[2] J. Zhao *et al.* Biomaterials (2007) 1414 – 1422.