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Development of enzyme-loaded nanoparticles: effect of pH

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Enzyme superoxide dismutase (SOD) incorporation parameters were evaluated after immobilization in polyisobutylcyanoaclylate (PIBCA) nanoparticles. After initialization of the anionic mechanism of polymerization, pH was increased and its effect on the characteristics of PIBCA nanoparticles analysed. Our goal included optimization of enzyme activity during incorporation into nanoparticles and the influence on size distribution. Unloaded nanoparticles were not significantly affected by the pH increase. At pH 3 the size distribution indicates a bimodal distribution: 58 nm (63%) and 146 nm (37%). When pH was increased to 5 after 1 h of polymerization the size distribution is: 57 nm (70%) and 125 nm (30%). When pH was increased to 5, after 2 h of polymerization, the size distribution is 67 nm (56%) and 160 nm (44%). Meanwhile, the retention of activity of SOD in polymerization medium is 49% at pH 3, and 98% at pH 5. The effect of pH increase from 3 to 5, after 1 h of polymerization, on the characteristics of loaded nanoparticles is an increase of retention of enzyme activity (18 to 30%); and the evidence of a pH-dependent smaller size population of loaded nanoparticles. In fact at pH 3 the size distribution is 83 nm (15%), 195 nm (15%), 440 nm (70%) and when pH is increased from 3 to 5 the size distribution becomes 55 nm (30%); 170 nm (30%); 430 nm (40%).

1. Introduction

Nanoparticles of polyalkylcyanoacrylate (PACA) [1], biodegradable polymeric colloidal drug carriers, have been extensively studied for the incorporation of several molecules used in human and veterinary medicine [2]. Meanwhile only a few results are present in literature concerning the incorporation of proteins and enzymes in nanoparticles [3]. Due to the physicochemical properties of these macromolecules inactivation can occur during their incorporation into polymeric nanoparticles. Several parameters should be studied in order to better incorporate enzymes (e.g. the anionic mechanism of polymerization of alkylcyanoacrylates, requiring polymerization mediums of pH 3, can compromise the activity of most enzymes).

In this work the effect of pH increase, during polymerization, on the characteristics of unloaded and enzyme loaded polyisobutylcyanoaclylate (PIBCA) nanoparticles was evaluated. The effect of the pH increase after initialization of the anionic mechanism of polymerization on the characteristics of nanoparticles of polyisobutylcyanoaclylate (PIBCA) and the effect of this approach to optimize the reduction of activity of an enzyme during incorporation into nanoparticles was evaluated.

2. Materials and methods

2.1. Materials

Isobutylcyanoacrylate (IBCA) was a gift from Loctite International, Dublin, Ireland. Superoxide Dismutase (SOD) (EC 1.15.1.1.) was from Sigma. Other reagents were analytical grade.

2.2. Methods

2.2.1. Preparation of nanoparticles

Nanoparticles were prepared by emulsion polymerization of IBCA, with 4 h under magnetic stirring. In brief the monomer (25 µl) was added under stirring to the polymerization medium: 2475 µl of citric acid (1 mM) containing glucose (5%), dextran 40 (0.5%), Symperonic F68 (1%). When enzyme-loaded nanoparticles were prepared, the enzyme (2 mg) was incorporated directly into the polymerization medium. The polymerization was considered complete 4 h after initiating the dispersion of the monomer, under constant stirring.

When pH modification was performed, sodium hydroxide was added to the polymerization medium, and the preparation maintained under stirring until final polymerization.

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2.2.2. Particle size analysis

The particle size distribution of nanoparticles was measured by photon correlation spectroscopy (PCS) using a laser light scattering equipment ZetaSizer 3, from Malvern Instruments, UK.

2.2.3. Quantification of enzyme loaded into nanoparticles

The quantification of the enzyme loaded into nanoparticles was performed by SE-HPLC, using a Licrospher 300 Diol column, from Merck, associated with a pre-column Licrospher 100 Diol, and using an HPLC from Beckman, System Gold, equipped with a spectrophotometric detector.

The incorporation efficacy was obtained by the ratio: $(E_f/E_i) \times 100$, E_i being the enzyme quantified in the polymerization medium before addition of the monomer and E_f the enzyme quantified in the polymerization medium after completion and separation of the colloidal carriers by ultracentrifugation (100 000 g, 2 h).

2.2.4. Activity of incorporated enzyme

The catalytic activity of the incorporated enzyme was quantified after destruction of nanoparticles with esterase.

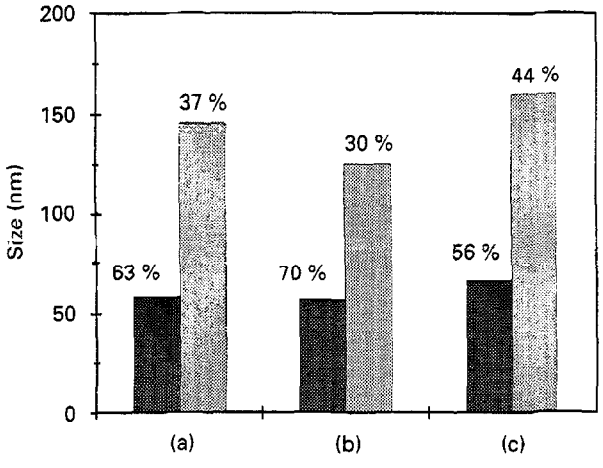

Figure 1 Size distribution of PIBCA unloaded nanoparticles: (a) without alteration of pH, pH = 3; (b) with alteration of pH from 3 to 5 after 1 h of polymerization; (c) with alteration of pH from 3 to 5 after 2 h of polymerization.

TABLE I Stability of SOD in polymerization medium as a function of pH

pH	Retention of activity (%)
3	49
5	98

TABLE II Characteristics of enzyme-loaded nanoparticles as a function of pH

pH	Size (nm)	Efficacy of entrapment (%)	Retention of activity (%)[a]
3	83 (15%); 195 (15%); 440 (70%)	> 95	18
3 and 5	55 (30%); 170 (30%); 430 (40%)	98	30

[a] After disruption of nanoparticles

3. Results

The effect of the increase of pH (from 3 to 5) after 1 and 2 h of polymerization on the size of nanoparticles was evaluated (Fig. 1).

The stability of SOD in the polymerization medium, pH = 3 and in polymerization medium with pH corrected to pH = 5, was compared (Table I).

The characteristics of enzyme-loaded nanoparticles prepared at pH 3 and pH increased from 3 to pH 5 after the first hour of polymerization, were compared (Table II).

All the results are averages of three independent experiments.

4. Conclusions

The size of unloaded PIBCA nanoparticles is not significantly affected by pH increase, and the efficacy of entrapment of the enzyme is independent of pH increase. The smaller size population of loaded nanoparticles is pH-dependent, which implies a pH dependency of the interaction between enzyme and polymer. An increase in retention of activity was observed with an increase of pH after 1h. This approach seems useful in maximizing the retention of activity of enzymes incorporated into PIBCA nanoparticles. Complementary studies need to be followed in order to elucidate more appropriately the mechanism of enzyme incorporation (conformational changes, potentiation of hydrophobic interactions, etc.).

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References

1. P. COUVREUR, *Crit. Rev. Ther. Drug Carrier Syst.* **5** (1988) 1.
2. P. COUVREUR, E. FATTAL and A. ANDREMONT, *Pharmaceutical Res.* **8** (1991) 1079.
3. J. GAUTIER, J. GRANGIER, A. BARBIER, P. DUPONT, D. DUSSOSSOY, G. PASTOR and P. COUVREUR, *J. Controlled Release* **20** (1992) 67.