Evaluation of Poly (D, L-lactide-co-glycolide) for Nanoencapsulation of Alpha 1-antitrypsin and *In Vitro* Release Study

Sareh Arjmand*, Elham Bidram*, Abbas Sahebghadam Lotfi, Hamid Mahdavi, Maryam Alavi

Abstract—**Alpha 1-antitrypsin is a member of serine protease inhibitors which mainly inactivates neutrophil elastase and prevents the destruction of the pulmonary extracellular matrix. Alpha 1-antitrypsin deficiency causes emphysema in adults and liver disease in children. The main goal of the present study was to investigate ability of poly (D, L-lactide-co-glycolide) nanoparticles to carry the alpha 1-antitrypsin protein as a potential therapeutic agent for the protection of lung tissue against free proteolytic activity. Poly (D, L-lactide-co-glycolide nanoparticles with an average size of 550 nm were produced by the "oil-in-oil emulsification solvent evaporation method" and successfully loaded with the alpha 1-antitrypsin protein during the production of the nanoparticles. The constructed nanoparticles were then characterized in terms of size, morphology and fourier transform infrared spectroscopy which was subsequently followed by studying the *in vitro* release of the protein. The resulting profile indicated that the protein was completely released from the polymeric nanoparticles after 36 days. In conclusion, our data suggests that poly (D, L-lactide-co-glycolide) nanoparticles could be an effective, potential carrier for delivery of alpha 1-antitrypsin protein.**

Index Terms— **Alpha 1-antitrypsin, Nanoencapsulation, Oil-in-oil emulsification solvent evaporation, Poly (D,L-lactide-co-glycolide)**

I. Introduction

Human alpha 1-antitrypsin (AAT) is a mid-sized diffusible glycoprotein which is secreted mainly by hepatocytes and alveolar macrophages and to a lesser extent by other tissues [1-2]. This molecule is present in high concentrations in most tissues and is a major protein component of the lung epithelial lining fluid [3]. Due to it inhibits a broad range of proteases, especially neutrophil elastase, plays an important role in maintaining protease-antiprotease homeostasis in lungs [4]. AAT deficiency predisposes patients to emphysema, juvenile cirrhosis and hepatocellular carcinoma [5-6]. Human plasma derived AAT is an FDA licensed product, used for replacement therapy in patients with hereditary AAT deficiency. However, the plasma source itself is limited, derived proteins are expensive [7-8] and because of AAT's short half-life in the bloodstream -approximately 6 days- patients need frequent administrations (60 mg kg- every 7 days) to maintain the therapeutic levels (~1.3 g/l) for all long life [9-10]. However, long-term administration of weekly doses is not well-accepted by patients [11].

Recently, therapeutic agents like pharmaceutical proteins, nanoencapsulated in a polymeric matrix or absorbed or conjugated onto the surface, and that are administered orally or injected locally, have been used [12-15]. These nanoencapsulated particles are useful for drug delivery in a convenient and controlled manner with smaller quantities of drugs and longer intervals when compared with non-encapsulated ones [16-18]. Nanoparticles (NPs) can release the embedded, absorbed or conjugated protein in a sustainable manner [19].

One of the most common types of NPs is made of poly lactic acid (PLA), poly glycolic acid (PGA), and their copolymer poly (D, L-lactide-co-glycolide) (PLGA) (Fig. 1). This copolymer has been approved by the USA Food and Drug Administration (FDA) and has notable characteristics like excellent biocompatibility, controllable biodegradability and high safety besides its nontoxic nature [20-22]. The emulsification solvent evaporation method is used routinely for nano/microencapsulation [23-26]. In the present work, we have explored the potential of a modified version of this method for the preparation of AAT-loaded PLGA NPs and subsequent characterization of the fabricated NPs.

Manuscript received March 30, 2011. This work was supported in part by the ICGEB. * They have made equal contribution.

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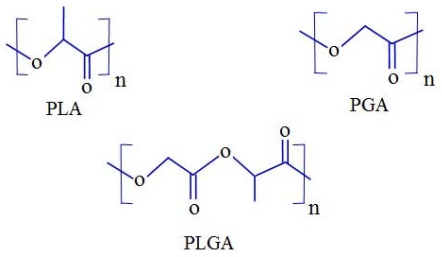

Figure1: The chemical structure of PLA, PGA, PLGA

II. Materials and Methods

A. Preparation of AAT

In this study we used recombinant AAT which is produced by the methylotrophic yeast *Pichia pastoris.* This recombinant protein is expressed under the control of the inducible alcohol oxidase 1 (AOX1) promoter, and is produced in a secretary manner. Measured AAT in the media was 60 mg/l which was purified from the other components of the medium by nickel-affinity chromatography and subsequently lyophilized. Quality and authenticity of heterologous AAT were confirmed by western blot and elastase inhibitory capacity (EIC) assays (unpublished data).

B. Preparation of PLGA NPs

AAT-loaded NPs were prepared by the oil in oil (o/o) emulsification solvent evaporation technique. For this purpose 3 ml of polymer solution was prepared by dissolving 5 mg of PLGA copolymer (RG 504H® with a monomer ratio of 50:50, Boehringer Ingelheim, Germany) in acetonitrile. One miligram of purified AAT was then dissolved in 250 µl of deionized water and slowly added in a drop-wise manner to the polymer solution. The resulting mixture was added to 40 mL of viscous liquid paraffin (Merck, Germany) containing Span 80 (Fluka, Switzerland) and stirred for two hours to ensure complete evaporation of acetonitrile. Nanospheres were then collected by centrifugation at 20000 g for 30 minutes at 15 ºC and washed twice with n-hexane to remove mineral oil. Particles were filtered, dried and stored under refrigeration in desiccators until further used. Blank NPs were prepared according to the same procedure omitting protein.

C. Study of the surface morphology and particle size of NPs by scanning electron microscopy (SEM)

Microscopic characteristics of AAT-loaded PLGA NPs were investigated by SEM (Philips, XL30, Netherland). The samples were placed on a double stick tape over aluminum stubs to get a uniform layer of particles. They were then gold-coated using a sputter gold coater at a current of 40 mA and 10-3 Torr pressure for 400 seconds at a thickness of 400 Å. Gold-coated particles were subsequently cooled over dry ice to avoid being melted under high magnification due to exposure by electron beam.

D. Encapsulation yield and efficiency

The yield of nanoencapsulation was calculated as follows:

$$\text{Yield (\%)} = [(W2-W1)/W1] \times 100 \quad (1)$$

where W1 is the initial weight of polymer and W2 is the weight of particle collected on the filter surface.

The amount of protein loaded onto the PLGA NPs was determined directly by recovering the protein from the NPs. A ten milligram sample of dried NPs was accurately weighed, transferred to a separator funnel and dissolved in 2 mL of acetone. After adding 4 mL of phosphate buffer to the funnel, it was capped with a stopper and the resulting mixture was agitated in a shaker for two days. The funnel was then left hanging on a metal ring. After complete separation of the two aqueous and organic phases, the aqueous layer was collected for protein assay. A commercial human AAT enzyme-linked immunosorbent assay (ELISA) quantitation kit (GenWay, USA) was used to determine protein concentration in the aqueous extract using phosphate buffer as a mobile phase. The experiments were performed in triplicate. The encapsulation efficiency of the nanosphere was calculated as below:

$$\text{Encapsulation efficiency (\% w/w)} = (\text{actual drug content}/\text{nominal drug content}) \times 100 \quad (2)$$

E. Study of protein-NP interaction through fourier transform infrared spectroscopy (FTIR)

In order to investigate possible molecular interaction between protein and the polymer, FTIRspectroscopy (Perkin-Elmer, USA) of the pure protein, control PLGA NPs and AAT-loaded PLGA NPs was carried out. The FTIR spectra were collected in the range of 4000–400 cm^{-1} at room temperature and 2 cm^{-1} resolution, with a scan number of 128 and an optimized gain for all samples.

F. Enzyme-Linked ImmunoSorbent Assay (ELISA)

ELISA was used for quantitative determination of released AAT levels in the medium using human alpha 1-antitrypsin ELISA Quantitation Kit (GenWay Biotech, Inc) according to the manufacturer's recommendations. For all experiments the samples were diluted as 1:100 in sample/conjugate diluents (50 mM Tris, 0.14 M NaCl, 1% (w/v) BSA, 0.05% (v/v) Tween 20, pH 8.0). Different concentrations of commercial human AAT (Sigma) were used to draw standard curve.

G. Study of in vitro protein release

Two samples of PLGA NPs (10 and 15 mg) were weighed and each immersed in one milliliter of phosphate buffered saline (PBS) (pH 7.2) in 2-mL microtubes. The suspensions were then incubated at 37 ºC with constant shaking (120 rpm). At appropriate time intervals, one mL of the medium was removed from each tube and replaced by the same quantity of fresh PBS solution. The withdrawn media were analyzed using ELISA for quantitative determination of the released protein and its integrity. For all experiments, the samples were diluted as 1:100 in sample/conjugate diluent buffer (50 mM Tris, 0.14 M NaCl, 1% (w/v) bovine serum albumin (BSA), 0.05% (v/v) Tween 20, pH 8.0). Different concentrations of commercial human AAT (Sigma, USA) were used to construct a standard curve. The corresponding protein release-profiles were established by plotting of the cumulative percentage of the released protein (Eq. (3)) versus the time.

Cumulative amount of released protein (%) = (Σ M_t/M_{actual}) ×100 (3)

where M_t is the amount of AAT released at time t, and M_{actual} is the actual amount of AAT- loaded onto the NPs.

H. Elastase Inhibitory Activity (EIC) assay

Elastase activity was measured by EnzChek® Elastase Assay Kit (Molecular Probes, Inc.) according to the manufacturer's recommendations. 2 ml of samples were concentrated 5 times into empty tubes (speedvac).The presence of an inhibitor such as AAT blocks the substrate digestion and therefore subsequent fluorescent emission. The resulting change in fluorescence level was monitored using a standard fluorometer (Hitachi F-3010) with a maximum absorption at 505 nm and a maximum fluorescence emission at 515 nm. Commercial human AAT and was used as a positive control and the kit elution buffer was applied as negative control. The study were done in duplicate.

III. Results

A. Characterization of AAT-loaded NPs

The SEM micrographs of PLGA NPs and AAT-loaded PLGA NPs indicated that both types of particle have spherical shapes and relatively smooth surfaces (Fig. 2A & 2B). NPs size was measured using nanostructure measurement software. The results showed in comparison to the control NPs, AAT-loaded NPs were of a smaller size (with an average size of 550 nm) and lower yield with about 50% efficiency. The size and yield of NPs are depicted in table1.

TABLE 1. PHYSICOCHEMICAL CHARACTERIZATION OF PLGA NPS, WITHOUT (BATCH NO.1) AND WITH AAT (BATCH NO.2)

Batch No.	Polymer Concentration (w/v%)	Stirring Speed (rpm)	Surfactant Conc. (v/v%)	Temp (□C)	Size (µm)	Yield (%)	Encapsulation Efficiency
1	1.25	5500	0.50	25	770	73.5	w/o AAT
2	1.25	5500	0.50	25	550	40.2	~ 50

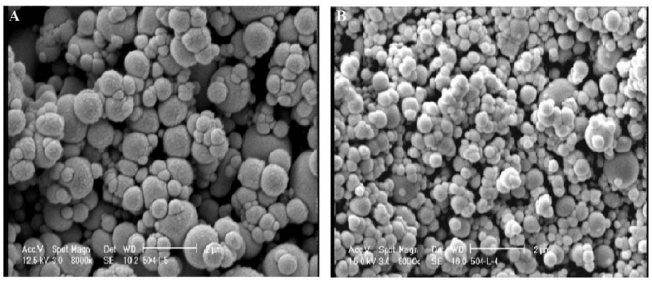

Figure 2. A) SEM image of control NPs without AAT , B) SEM image of NPs containing AAT

B. Protein-polymer interaction

The FTIR spectroscopy method was used to investigate the interaction between the functional group of the protein under study and the carrier NPs. The spectra of the PLGA NPs and AAT-loaded PLGA NPs showed two distinct peaks at wave numbers 1300-1450 cm^{-1} and 1720-1800 cm^{-1}, which were assigned to δCH and V(C=O), however, there were no changes in the position of peaks (Fig. 3).

C. In vitro release study

An *in vitro* release study was performed to determine the protein release profile and assess the state of the encapsulated AAT. The resulting AAT release profiles indicated three phases; the first was an initial burst phase (day 1 to 3) with 60% of the AAT released. The second phase was an intermediate one (day 4 to 9) with a low and continuous release including approximately a 36% of release and finally a third phase (till day 36) was detected with a moderate burst (Fig. 4).

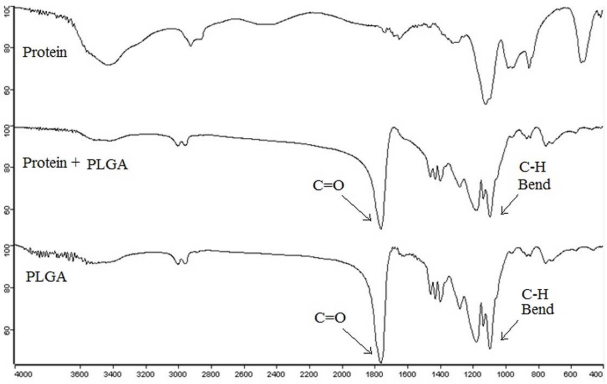

Figure 3. The FTIR spectra of AAT, PLGA+ AAT and PLGA NPs.

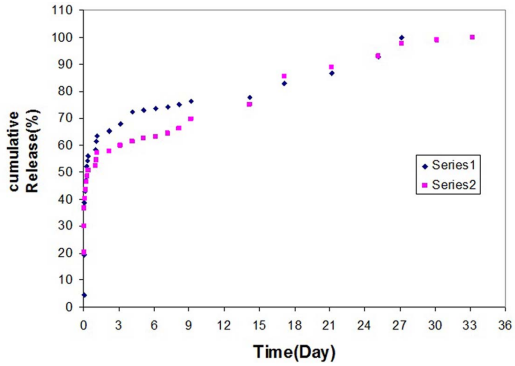

Figure 4. Release profiles of AAT from PLGA NPs (♦ 15 mg NPs, ■ 10 mg NPs).

D. AAT activity assay

The results of inhibitory activity of released AAT against elastase for four selected samples were shown in Fig. 5. The activity assayed in three interval times (0, 10 and 20 min).

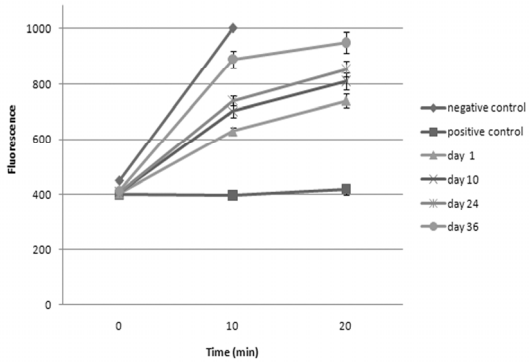

Figure 5. Diagram of inhibitory activity against elastase. Positive control completely inhibited elastase and prevented fluorescent substrate digestion while in negative control elastase digested the substrate and the fluorescence reached to the maximum of measurable level. The samples showed a decreasing activity over times. Error bar represents standard errors.

IV. Discussion

Pharmaceutical AAT protein is a blood-derived high-cost product and puts financial pressures on many patients in need. Isolating the products of this kind from blood is associated with main obstacles like source limitation and virus contamination [27-28]. Although recombinant technology can overcome these problems, but it is still not enough and there are difficulties regarding short biological half-life of some proteins like AAT and subsequent need for repeated injections which is undesirable [29-32]. Improving the manner of delivery could be an effective strategy in this case which makes the products more economical and affordable [33-35]. Meanwhile controlled release can provide predictable delivery of pharmaceutical agents from polymeric matrixes including biodegradable synthetic polymers like poly(D,L-lactide-co-glycolide) (PLGA) and natural polymers such as collagen, chitosan and alginate to overcome mentioned difficulties [36-37]. Furthermore because of the sustained release properties, sub cellular size, and biocompatibility with tissues and cells, nanoparticles are one of the suitable solution for drug administration [38]. In present study, the PLGA copolymer which has excellent biocompatibility and biodegradability properties was used for this purpose. The AAT protein was encapsulated in this copolymer by an o/o emulsification solvent evaporation technique with significant efficiency. A drug-excipient interactions study employing FTIR spectroscopy was performed to determine if there was any interaction between the recombinant AAT protein and the PLGA polymer. FTIR spectroscopy is a powerful technique that can indicate interactions between the various functional groups of protein and excipient molecules. The other important feature of AAT-polymer formulation is protein release behavior. The profiles obtained during the release study show three different phases. The first fast release in 3 first days may be due to the AAT protein sitting on or embedded in the PLGA NPs' surface or it may have arisen from the lack of any chemical interaction between the protein and polymer. The second phase, showing a continuous slow release can be attributed to the solubilization of the nanocapsules. Finally, the third phase from day nine to the end demonstrating a faster release could be the result of polymer degradation. In fact during 36 days of the *in vitro* protein release study, over 95% of the loaded protein was found to be released. According to results of activity assay AAT can maintain its activity over a long time when it is nanoecapsulated in PLGA nanoparticles. Lower activity which was observed in comparison to positive control is due to smaller amounts of AAT in samples. The decreasing trend of EIC can be attributed in part to diluting the samples in every sampling (section G in materials and methods). Hence, the retardation of the burst phase, the slow release characteristics of the PLGA NPs and maintaining protein activity during long time, proposed that this new biodegradable system can be a noteable carrier for prolonged protein drug delivery.

V. Conclusion

The present work has demonstrated the fabrication of the AAT protein- loaded PLGA NPs via a simple o/o emulsification solvent evaporation technique. Evaluation of these loaded NPs indicated that they have spherical morphology, high protein incorporation efficiency, and good stability. The protein was effectively protected by the starch environment when entrapped in PLGA NPs, demonstrating sustained release from its encapsulated polymer *in vitro*. According to our results, this newly designed system can eventually be considered as a potent AAT protein delivery system *in vivo*.

Acknowledgment

The authors would like to thanks Dr. Mehdi Shamsara for his kindly collaboration.

References

[1] M. Brantly, *et al.*, "Repair of the secretion defect in the Z form of alpha 1-antitrypsin by addition of a second mutation," *Science,* vol. 242, pp. 1700-2, Dec 23 1988.
[2] H. Parfrey, *et al.*, "Alpha(1)-antitrypsin deficiency, liver disease and emphysema," *Int J Biochem Cell Biol,* vol. 35, pp. 1009-14, Jul 2003.
[3] J. K. Stoller and L. S. Aboussouan, "Alpha1-antitrypsin deficiency," *Lancet,* vol. 365, pp. 2225-36, Jun 25-Jul 1 2005.
[4] J. E. Gadek and E. R. Pacht, "The protease-antiprotease balance within the human lung: implications for the pathogenesis of emphysema," *Lung,* vol. 168 Suppl, pp. 552-64, 1990.
[5] B. Gooptu and D. A. Lomas, "Conformational pathology of the serpins: themes, variations, and therapeutic strategies," *Annu Rev Biochem,* vol. 78, pp. 147-76, 2009.
[6] B. Gooptu, *et al.*, "Crystallographic and cellular characterisation of two mechanisms stabilising the native fold of alpha1-antitrypsin: implications for disease and drug design," *J Mol Biol,* vol. 387, pp. 857-68, Apr 10 2009.
[7] F. Kueppers, "The role of augmentation therapy in alpha-1 antitrypsin deficiency," *Curr Med Res Opin,* vol. 27, pp. 579-88, Mar 2011.
[8] P. C. Gotzsche and H. K. Johansen, "Intravenous alpha-1 antitrypsin augmentation therapy: systematic review," *Dan Med Bull,* vol. 57, p. A4175, Sep 2010.
[9] J. K. Stoller, *et al.*, "Biochemical efficacy and safety of a new pooled human plasma alpha(1)-antitrypsin, Respitin," *Chest,* vol. 122, pp. 66-74, Jul 2002.
[10] I. Petrache, *et al.*, "Safety and efficacy of alpha-1-antitrypsin augmentation therapy in the treatment of patients with alpha-1-antitrypsin deficiency," *Biologics,* vol. 3, pp. 193-204, 2009.
[11] N. P. Zamora, *et al.*, "Intravenous human plasma-derived augmentation therapy in alpha 1-antitrypsin deficiency: from pharmacokinetic analysis to individualizing therapy," *Ann Pharmacother,* vol. 42, pp. 640-6, May 2008.
[12] D. J. Bharali, *et al.*, "Novel nanoparticles for the delivery of recombinant hepatitis B vaccine," *Nanomedicine,* vol. 4, pp. 311-7, Dec 2008.
[13] M. C. Fontana, *et al.*, "Nanocapsules prepared from amorphous polyesters: effect on the physicochemical characteristics, drug release, and photostability," *J Nanosci Nanotechnol,* vol. 10, pp. 3091-9, May 2010.
[14] C. N. Murthy, *et al.*, "Nanoencapsulation of [60] fullerene by a novel sugar-based polymer," *J Nanosci Nanotechnol,* vol. 2, pp. 129-32, Apr 2002.
[15] M. Aboubakar, *et al.*, "Study of the mechanism of insulin encapsulation in poly(isobutylcyanoacrylate) nanocapsules obtained by interfacial polymerization," *J Biomed Mater Res,* vol. 47, pp. 568-76, Dec 15 1999.
[16] W. H. De Jong and P. J. Borm, "Drug delivery and nanoparticles:applications and hazards," *Int J Nanomedicine,* vol. 3, pp. 133-49, 2008.
[17] C. P. Reis, *et al.*, "Nanoparticulate delivery system for insulin: design, characterization and in vitro/in vivo bioactivity," *Eur J Pharm Sci,* vol. 30, pp. 392-7, Apr 2007.
[18] A. Kumari, *et al.*, "Biodegradable polymeric nanoparticles based drug delivery systems," *Colloids Surf B Biointerfaces,* vol. 75, pp. 1-18, Jan 1 2010.

[19] B. Shekunov, "Nanoparticle technology for drug delivery: from nanoparticles to cutting-edge delivery strategies - part II," *IDrugs,* vol. 8, pp. 402-3, May 2005.
[20] H. Hamishehkar*, et al.*, "The effect of formulation variables on the characteristics of insulin-loaded poly(lactic-co-glycolic acid) microspheres prepared by a single phase oil in oil solvent evaporation method," *Colloids Surf B Biointerfaces,* vol. 74, pp. 340-9, Nov 1 2009.
[21] J. M. Lu*, et al.*, "Current advances in research and clinical applications of PLGA-based nanotechnology," *Expert Rev Mol Diagn,* vol. 9, pp. 325-41, May 2009.
[22] R. A. Jain, "The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices," *Biomaterials,* vol. 21, pp. 2475-90, Dec 2000.
[23] J. Emami*, et al.*, "Particle size design of PLGA microspheres for potential pulmonary drug delivery using response surface methodology," *J Microencapsul,* vol. 26, pp. 1-8, Feb 2009.
[24] V. Karavelidis*, et al.*, "Nanoencapsulation of a water soluble drug in biocompatible polyesters. Effect of polyesters melting point and glass transition temperature on drug release behavior," *Eur J Pharm Sci,* vol. 41, pp. 636-43, Dec 23 2010.
[25] N. Duran*, et al.*, "Microencapsulation of antibiotic rifampicin in poly(3-hydroxybutyrate-co-3-hydroxyvalerate)," *Arch Pharm Res,* vol. 31, pp. 1509-16, Nov 2008.
[26] P. K. Choudhury and M. Kar, "Controlled release metformin hydrochloride microspheres of ethyl cellulose prepared by different methods and study on the polymer affected parameters," *J Microencapsul,* vol. 26, pp. 46-53, Feb 2009.
[27] M. Farshid*, et al.*, "The clearance of viruses and transmissible spongiform encephalopathy agents from biologicals," *Curr Opin Biotechnol,* vol. 16, pp. 561-7, Oct 2005.
[28] W. K. Hoots, "History of plasma-product safety," *Transfus Med Rev,* vol. 15, pp. 3-10, Apr 2001.
[29] S. S. Pai*, et al.*, "Poly(ethylene glycol)-modified proteins: implications for poly(lactide-co-glycolide)-based microsphere delivery," *AAPS J,* vol. 11, pp. 88-98, Mar 2009.
[30] F. Kratz, "Albumin as a drug carrier: design of prodrugs, drug conjugates and nanoparticles," *J Control Release,* vol. 132, pp. 171-83, Dec 18 2008.
[31] F. Xu*, et al.*, "Optimizing drug delivery for enhancing therapeutic efficacy of recombinant human endostatin in cancer treatment," *Crit Rev Ther Drug Carrier Syst,* vol. 24, pp. 445-92, 2007.
[32] C. Alves Costa and C. Santos, "[Alpha-1 antitrypsin deficiency. The experience of Pulido Valente Hospital with augmentation therapy]," *Rev Port Pneumol,* vol. 15, pp. 473-82, May-Jun 2009.
[33] M. M. Cheng*, et al.*, "The economic value of reducing medication dosing frequency with drug delivery technologies: an evidence assessment," *J Med Econ,* vol. 13, pp. 193-202, 2010.
[34] O. Salata, "Applications of nanoparticles in biology and medicine," *J Nanobiotechnology,* vol. 2, p. 3, Apr 30 2004.
[35] S. Saraf, "Process optimization for the production of nanoparticles for drug delivery applications," *Expert Opin Drug Deliv,* vol. 6, pp. 187-96, Feb 2009.
[36] X. Wang*, et al.*, "Silk microspheres for encapsulation and controlled release," *J Control Release,* vol. 117, pp. 360-70, Feb 26 2007.
[37] F. Shahidi and R. Abuzaytoun, "Chitin, chitosan, and co-products: chemistry, production, applications, and health effects," *Adv* Food *Nutr Res,* vol. 49, pp. 93-135, 2005.
[38] C. Pinto Reis*, et al.*, "Nanoencapsulation II. Biomedical applications and current status of peptide and protein nanoparticulate delivery systems," *Nanomedicine,* vol. 2, pp. 53-65, Jun 2006.

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