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Human Serum Albumin (HSA) Nanoparticles as Drug Delivery System: Preparation, Optimization and Characterization Study

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ABSTRACT

Nanoparticles (NPs) have been developed as an important strategy to deliver low molecular-weight drugs, as well as biomacromolecules such as proteins or DNA. The body distribution of colloidal drug delivery systems was mainly influenced by two physicochemical properties namely particle size and surface characteristics. Particle size is a crucial parameter, in particular for the *in vivo* behavior of NPs after intravenous injection. The objective of the present study was the preparation of human serum albumin (HSA) nanoparticle (NP) by desolvation method and optimization of NP by applying the Taguchi method together with characterization of the NP bioproducts for drug delivery application. Several process parameters were examined to achieve a suitable size of NP such as pH, HSA concentration, organic solvent adding rate and the ratio of organic solvent/HSA solution. Taguchi method with L_{16} orthogonal array robust design was implemented to optimize experimental conditions of the purpose. This approach facilitates the study of interaction of a large number of variables spanned by factors and their settings with a small number of experiments leading to considerable saving in time and cost for the process optimization. As a result of Taguchi analysis in this study, pH and ratio of organic solvent/HSA solution were the most influencing parameters on the particle size. The minimum size of NPs (53 nm) were obtained at pH 9, 75 $mg.ml^{-1}$ HSA concentration, ratio of organic solvent/HSA solution of 4 and organic solvent adding rate of 1.5 $ml.min^{-1}$. The mechanistic of the optimum conditions for preparing protein NPs and their characterization as drug delivery vehicles are discussed.

Keywords: desolvation method, drug carrier, drug loading, human serum albumin, nanoparticles, Taguchi method
Abbreviations: HSA, human serum albumin; **NP**, nanoparticle

INTRODUCTION

Nanotechnology, or systems/devices manufactured at the molecular level, is a multidisciplinary scientific field undergoing explosive development. A part of this field is the development of nanoscaled drug delivery devices (Kayser *et al*. 2005). The definition of 'nanotechnology' which is most commonly adhered to is 'materials considered to have dimensions of between 1 and 100 nm, with those materials having unique or unusual properties different from those of bulk materials of the same composition because of their size and surface phenomena.' However, in many cases, NPs are considered to be any sub-micron sized particles. Let us first consider the key advantages that we wish nanotechnology in general and NPs in particular to bring to drug delivery. Of course, these must include the holy grails of, i) improving drug bioavailability through enhancing aqueous solubility, ii) increasing the residence time in the body (increasing the half-life for clearance/increasing specificity for its cognate receptor), and iii) targeting the drug to a specific location in the body (its site of action) with a concomitant reduction in the quantity of drug required and dosage toxicity, enabling the safe delivery of toxic therapeutic drugs, and protection of non-target tissues and cells from severe side effects (Irving 2007).

NPs can be prepared from a variety of materials such as proteins, polysaccharides and synthetic polymers. The selection of matrix materials is dependent on many factors including: (a) size of NPs required; (b) inherent properties of the drug, e.g., aqueous solubility and stability; (c) surface characteristics such as charge and permeability; (d) degree of biodegradability, biocompatibility and toxicity; (e) drug release profile desired; and (f) antigenicity of the final product (Jahanshahi and Babaei 2008).

New delivery strategies, intended to enhance the efficacy of these macromolecules, have been extensively described (Malik *et al*. 2007). Polymeric NPs have shown a certain degree of success for the delivery of proteins and vaccines to the systemic circulation and to the immune system. Since size and surface properties, i.e. surface charge and hydrophobicity, of NPs have been recognized as crucial characteristics, especially, surface modified colloidal carriers such as NPs were demonstrated to be a promising and useful tool in the development of drug carrier systems with the intention of administering biotechnology engineered products.

Polymeric NPs have attractive physicochemical properties such as size, surface potential, hydrophilic-hydrophobic balance, etc. and for this reason they have been recognizes as potential drug carrier for bioactive ingredient such as anticancer drugs, vaccines, oligonucleotides, peptides, etc. (Jahanshahi *et al*. 2008d). The major advantage of colloidal drug carrier systems is the possibility of drug targeting by a modified body distribution as well as the enhancement of the cellular uptake of a number of substances. They present several characteristics that make them suitable candidates to develop efficient mucosal administration forms, to achieve long circulation time after parental administration, to modify the body distribution, and to offer drug protection against *in vivo* acid and enzymatic degradation (Bravo-Osuna *et al*. 2007). NPs consisting of synthetic biodegradable polymers, natural biopolymers, lipids and polysaccharides have been developed and tested over the past decades.

Recently, the idea of using NPs made from natural biodegradable polymers to deliver drugs has provoked great interests (Li *et al*. 2008). Among these polymeric systems those based on proteins may be very promising. On the other hand, protein NPs offer some specific advantages over them. Protein NPs generally vary in size from 50-300 nm

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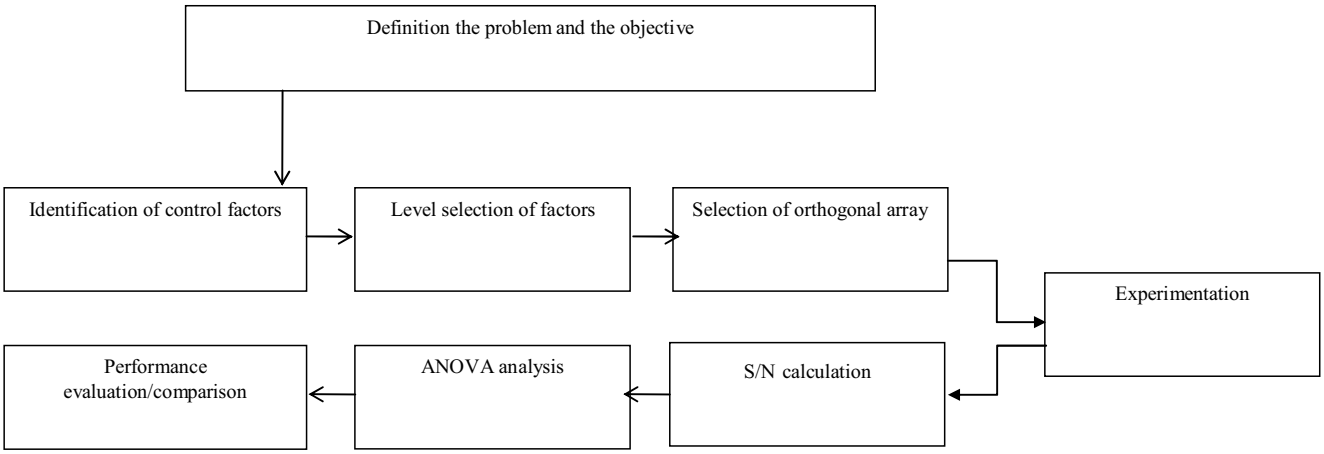

Fig. 1 Factor optimization using Taguchi method.

and they hold certain advantages over the other drug delivery systems such as greater stability during storage, stability *in vivo*, non-toxicity, non-antigen (Lin *et al*. 1994) and ease to scale up during manufacture (Jahanshahi *et al*. 2004). Nowadays active research is focused on the preparation of NPs using proteins like albumin, gelatin, gliadin and legumin. According to the literature, albumin NPs have been selected for many research topics (Muller *et al*. 1996; Weber *et al*. 2000; Arnedo *et al*. 2004; Jahanshahi *et al*. 2008a).

Albumin is an attractive macromolecular carrier and widely used to prepare microspheres and microcapsules, due to its bioavailability, nontoxicity and nonimmunogenicity (Kratz *et al*. 1997). Attractive features of biopolymer albumin include: its reported biodegradation into natural products; its lack of toxicity and antigenicity (Peppas 1995); maintenance of constant or nearly constant blood levels; improved patient compliance (Heller and Baker 1980) as well as ready availability. A number of studies have shown that albumin accumulates in solid tumors (Masumura and Maeda 1986; Takakura *et al*. 1990) making it a potential macromolecular carrier for the site-directed delivery of antitumor drugs.

Among these, human serum albumin (HSA) is a promising material and was used in a multitude of studies for particle preparation (Michaelis *et al*. 2006). HSA is the most abundant protein in human blood plasma. It is produced in the liver. Albumin comprises about half of the blood serum protein. Human serum albumin (molecular weight of 65 kDa) belongs to a multigene family of proteins (He and Carte 1992) and is the major soluble protein of the circulating system with a blood concentration about 50 $mg.ml^{-1}$. Human serum albumin consists of 585 amino acids containing 35 cystein residues which build 17 disulfide bridges (Langer *et al*. 2008).

In this work fabrication of human serum albumin (HSA) NPs by desolvation method and optimization of NPs by applying the Taguchi method are considered. The properties of HSA fabricated NPs are affected by various parameters such as initial protein concentration, pH value, glutaraldehyde concentration (crosslinker), agitation speed, ratio of organic solvent/ HSA solution and organic solvent adding rate which are discussed herein. In addition, AFM and SEM characterize the shape and morphology of the products. This study is intended to establish a rational basis for the controlled production and application of protein- based NPs as drug carrier system.

MATERIALS AND METHODS

Materials

HSA (fraction V, purity 96-99%) and glutaraldehyde solution were commercially supplied by Sigma Aldrich. Ethanol and other reagents were purchased from Merck (Germany).

Preparation of HSA nanoparticles

HSA NPs were prepared using desolvation technique. In principle, between 50 and 200 mg HSA was dissolved in 2 ml of purified water or 10 mM NaCl solution, then titrated to pH 7.5-9 and under constant stirring desolvation of HSA solution was achieved by drop-wise addition of ethanol. After the desolvation process, 8% glutaraldehyde in water (between 0.235 and 1.175 $\mu l.mg^{-1}$ HSA) was added to induce particle cross linking. Cross linking process was performed under stirring of the suspension over a time period of 24 hr. The resulting NPs were purified by five cycles of centrifugation (25000 × *g*, 10 min) and redispersion of the pellet to the original volume in 10 mM NaCl at pH values of 7.5-9. Each redispersion step was performed in an ultrasonication bath. The following parameters were changed to study their effect on the characteristics of the NPs: pH value, HSA concentration, agitation speed, glutaraldehyde concentration [%], organic solvent adding rate and the ratio of organic solvent/HSA solution. The pH value were set in the range of 7.5 to 9, concentration of HSA were set in the range of 25 to 100 $mg.ml^{-1}$, ratio of organic solvent/HSA solution has been used between 1 and 4 and organic solvent adding rate has been adjusted between 0.5 and 2 $ml.min^{-1}$.

Taguchi method

To date, Taguchi's method has been one of the two most effective tools for improving quality, with the other being ISO-9000 (Wang *et al*. 2002).

The fundamental principle of the Taguchi method is to improve the quality of a product by minimizing the effect of the causes of variation without eliminating the causes. Taguchi approach provides systematic, simple and efficient methodology for the optimization of the near optimum design parameters with only a few well-defined experimental sets (Arun *et al*. 2006).

Two major tools used in the Taguchi method are the orthogonal array (OA) and the signal to noise ratio (S/N). OA is a matrix of numbers arranged in rows and columns. Each row represents the level of factors in each run and each column represents a specific level for a factor that can be changed for each run. S/N is indicative of quality and the purpose of the Taguchi experiment is to find the best level for each operating parameter so as to maximize (or minimize) S/N (Jahanshahi *et al*. 2008c). Taguchi methodology for optimization has been represented in the form of a flowchart as shown in **Fig. 1**.

Details of experiments

Usually, to find the influence of controlling parameter on particle size a large number of experiments are needed. In order to avoid this, statistical methods can be used to design the optimum number of experiments. Taguchi method provides a systematic and efficient approach for conducting experimentation to determine near

Table 1 Variable (factors) and their levels employed in Taguchi method

Factors	Levels			
A: pH	7.5	8	8.5	9
B: HSA concentration (mg.ml^{-1})	25	50	75	100
C: Ratio of ethanol/HSA solution	1	2	3	4
D: Ethanol adding rate (ml.min^{-1})	0.5	1	1.5	2

optimum settings of design parameters for performance and cost.

Once the particle size is chosen as the main performance characteristic (the measure of quality), then the design factors, which will have an influence on it, have to be selected. There are no general guidelines for their selection. The choice should be based on the experiences from the previous experiments. Using a special orthogonal array only a small set from all the possible ones is selected. The sense of the orthogonal arrays method lies in choosing the level combinations of the design factors for each experiment. Depending upon the specified performance, the optimum will imply that the product has achieved the target value of the quality measure. Therefore, the aim is to attain quality by reducing the variation around the target. During the experiments, particle size varies from the target value. Determination of the optimal levels of the design variables called factors is based on the assumption that these variations should be as narrow as possible. Thus, knowing the characteristic, i.e., whether a higher or lower value produces the preferred result, the levels of the factors, which are expected to produce the best result, can be predicted. Special criteria depending on the chosen performance characteristic have to be developed in order to identify individual contributions of factors and their interrelationships.

As we was found, four important factor that influence the HSA NP size were pH value, HSA concentration, organic solvent adding rate and the ratio of organic solvent/HSA solution. Therefore in order to minimize the number of experiments, Atomic Design and Analysis of Taguchi Experiments were employed through Qualitek-4 software (version IV). Taguchi's orthogonal array table was used by choosing these four parameters that could affect the particle size. **Table 1** shows the parameters and levels used in this experiment. Here, L_{16} (4^4) ANOVA (analysis of variance) was selected, which represents 16 experiments with four four-level factors.

RESULTS AND DISCUSSION

The effect of different parameters on nanoparticle size

HSA NPs were prepared by a desolvation technique as described previously (Marty *et al*. 1978) and variously modified by our group (Rahimnejad *et al*. 2006; Jahanshahi *et al*. 2007, 2008a). In our experiments, we studied the effects of varying production parameters on the NP properties. Different synthesis parameters were changed, including pH value, HSA concentration, organic solvent adding rate and the ratio of organic solvent/HSA solution. The goal was to prepare small NPs with a narrow size distribution. It has been shown that particle size has a great impact on the uptake of NPs. Desai and co-workers (Amidon 1997) showed that 100 nm size NPs had 2.5-fold greater uptakes compared to 1 μm and 6-fold higher uptakes compared to 10 μm microparticles in a $CaCo_2$ cell line. The results of other researchers also showed that particle size significantly affects cellular and tissue uptake, and, in some cell lines, only the submicron size particles are taken up efficiently in lieu of the larger size microparticles (Desai *et al*. 1996; Zauner *et al*. 2001).

We investigated the effect of these different parameters on the particle size and the polydispersity index, where the polydispersity index measures the second moment of the size distribution of the NP population. A lower polydispersity index indicates a narrower size distribution.

After investigation of the effect of pH on NP size, it was found that with increasing pH value of HSA solution particle size were reduced, apparently due to an increased ionization of the HSA (isoelectric point p*I*=5.3) which leads to repulsion of the HSA molecules and aggregates during particle formation. The results are shown in **Fig. 2**.

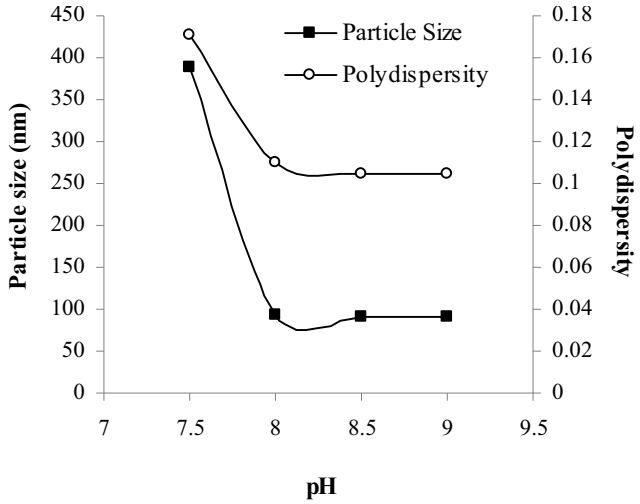

Fig. 2 Influence of the pH value on NP size.

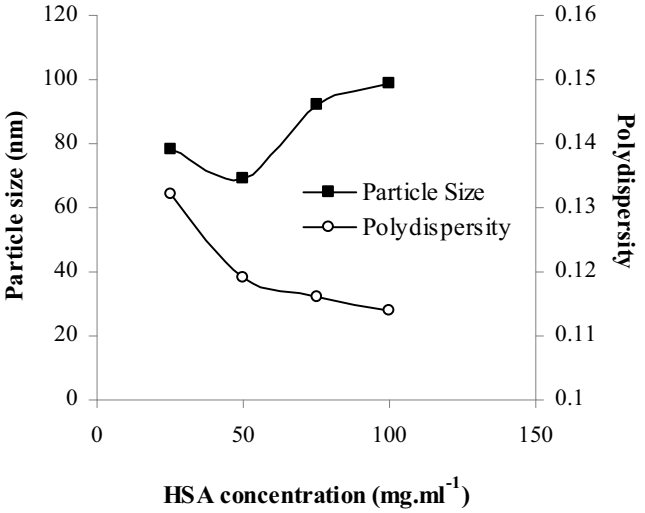

Fig. 3 Diameter and polydispersity index of HSA NPs prepared at different HSA concentration in the presence of 10 mM NaCl solution at pH=9.

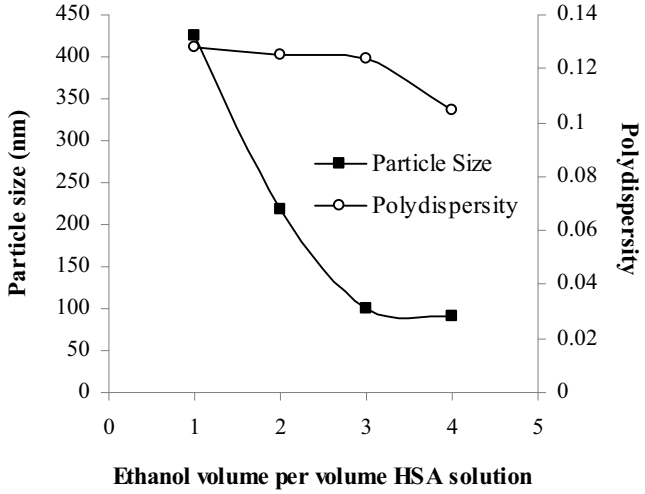

Fig. 4 Diameter and polydispersity index of HSA NPs prepared in 10 mM NaCl solution at pH=9 as a function of the volumetric ratio of ethanol/HSA solution. Initial concentration: 50 mg.ml^{-1}.

The influence of the HSA concentration on particle diameter of resulting samples is shown in **Fig. 3**. In a HSA concentration range between 25 and 100 mg.ml^{-1}, with in-

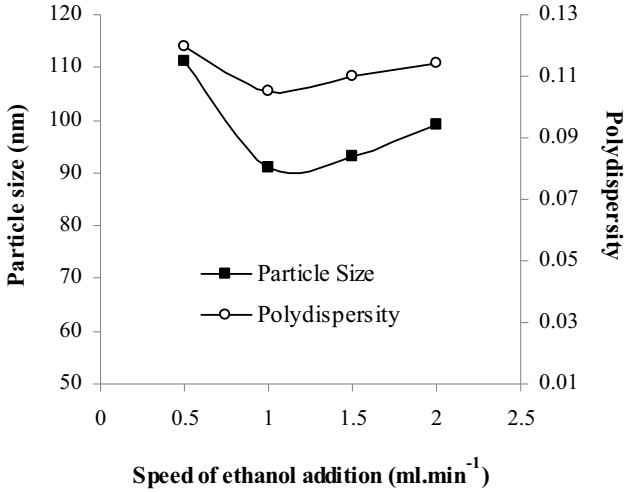

Fig. 5 Diameter and polydispersity index of HSA NPs prepared in 10 mM NaCl solution at pH=9 as a function of the rate of ethanol addition. Initial concentration: 50 mg.ml^{-1}.

creasing HSA concentration the polydispersity of the samples was somewhat decreased.

Fig. 4 shows the effect of the rate of ethanol addition on NP size. The addition of desolvating agent reduced the water available to keep the HSA in solution, resulting in shrinkage of the hydrated HSA chains. At a certain point the hydration was too low and the protein chains participated as NPs so according to **Fig. 4** as the ratio increased the particle size was decreased.

In order to influence the resulting particle size, the rate of ethanol addition during the desolvation procedure was varied. **Fig. 5** shows the effect of rate of ethanol addition on NP size.

Taguchi's orthogonal array design

The number of experiments to be conducted for four factors and four signal levels under full-factorial testing is 256. Any process will give the best possible output when all of the factors operate at the optimum level. If 'm' factors are selected with 'n' signal levels, the total number of experiments to be conducted is 'n^m'. If the total number of factors and signal levels involved is greater, the number of experiments to be conducted becomes very large. Taguchi suggested the use of an orthogonal array (OA), which is the basis for conducting 'fractional factorial' experiments. The OA is selected based on the number of factors, interactions between them and the number of signal levels of each factor (Ross 1996; Jahanshahi *et al*. 2008b). **Table 2** shows 'L_{16}' standard OA used for the four factors, each set at four signal levels.

Table 2 Experimental measured values for particle size of HSA NPs and S/N ratio (Taguchi orthogonal array table of L_{16}).

Exp. No.	Experimental conditions				Particle size	S/N Ratio
	A	B	C	D	(nm)	(dB)
1	1	1	1	1	185	-45.3
2	1	2	2	2	175	-44.9
3	1	3	3	3	99	-39.9
4	1	4	4	4	111	-40.9
5	2	1	2	3	93	-39.4
6	2	2	1	4	91	-39.2
7	2	3	4	1	87	-38.8
8	2	4	3	2	92	-39.3
9	3	1	3	4	78	-37.8
10	3	2	4	3	69	-36.8
11	3	3	1	2	99	-39.9
12	3	4	2	1	92	-39.3
13	4	1	4	2	75	-37.5
14	4	2	3	1	77	-37.7
15	4	3	2	4	79	-37.9
16	4	4	1	3	92	-39.3

Signal-to-noise ratio evaluation

As an evaluation tool for determining the robustness of the design, 'signal-to-noise' ratio (S/N) is the most important component of the factor design. In the Taguchi method, the term 'signal' represents the desirable target (higher percentage of approved castings) and 'noise' represents the undesirable value. In our study smaller the better type of S/N ratio is used in analysis for better accuracy. For smaller the better case, S/N ratio is obtained by (Atil and Unv 2000; Jahanshahi *et al*. 2008c):

$$\eta = -10\log_{10}((1/n)\sum_{i=1}^{n} y_i^{\,2})$$

where η is the average S/N, 'n' is the number of experiments conducted at level 'i' and 'y_i' is the approved percentage of parameter 'y'. A robust system will have a high S/N. S/N should be as large as possible for higher values of approved percentages. **Table 2** shows the average S/N for each at the signal level and factors, respectively.

ANOVA analysis

Statistical methods are powerful tools for extracting useful information contained in data and ANOVA is one of the most frequently used tools. ANOVA is particularly useful in analyzing data from the statistically designed experiments and can decompose variations for any type of data and helps in quickly calculating the magnitude of influence of the cause being considered (Arun *et al*. 2006).

ANOVA is an analytical method to square the dispersion of specific numbers. The variance of the particle size in **Table 2** (analysis of variance) was calculated, and the results are shown in **Table 3**. The purpose of the ANOVA is to investigate which factors significantly affect the quality characteristic.

Determination of optimal conditions using Taguchi method

The optimum conditions can be determined through the response table of ANOVA-TM software. The level average graph of the raw data is illustrated in **Fig. 6**, which shows that data analyzed by the Taguchi method is in good agreement with experimental finding and it can be understand from that the pH value reveals that it has the most important influence on the particle diameter. The ratio of organic solvent/HSA solution has the second most important effect on the NP size. From **Table 3**, it can be seen that the pH value and ratio of organic solvent/HSA solution are the significant parameters for affecting the size. Therefore, based on the S/N and ANOVA analysis, the optimal parameters for NP size are the pH value at level 4, the organic solvent adding rate at level 3, the ratio of organic solvent/HSA solution at level 4, initial HSA concentration at level 3.

Under these conditions the program estimated the HSA NP diameter as 46.625 nm, while in the experiment 53 nm was achieved for the NP diameter.

Fig. 7 illustrates the HSA NP size and size distribution in the optimum condition. There is good agreement between

Table 3 The ANOVA table of particle size.

Factors	Degree of freedom	Sums of squares	Variance	F-Ratio
A	3	10008.25	3336.083	10.293*
B	3	640.25	213.416	0.658
C	3	3070.25	1023.416	3.157*
D	3	1810.75	603.583	1.862
Error	3	972.25	324.083	
Total	15	16501.75		

*Main significant parameter.

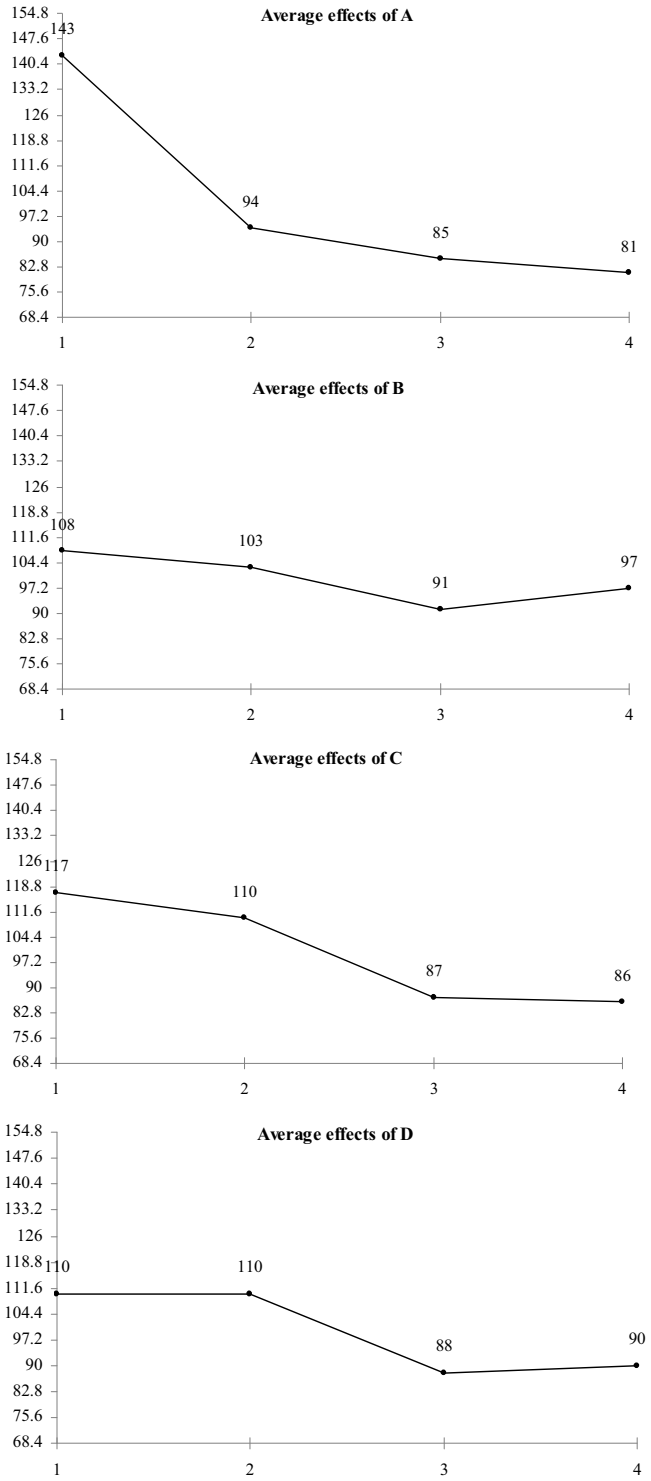

Fig. 6 Response graph for significant factors. The horizontal axis shows the different levels of the each significant factor. The lines represent the trend of each factor with respect to different levels.

the predicted and experimental particle size being observed. Consequently, particle size in the fabrication of HSA NP can be decreased through the Taguchi method approach.

Physical characteristics of HSA nanoparticles

Simple coacervation process of HSA NP was evaluated based on the particle size. The particle sizes as well as the light intensity counts of the samples were determined by photon correlation spectroscopy (PCS) for all the experiments. The SEM image of HSA NPs is shown in **Fig. 8**. It was clear that the most of the resulting protein NPs were smooth and semispherical. AFM technique has been widely applied to provide surface and morphological information

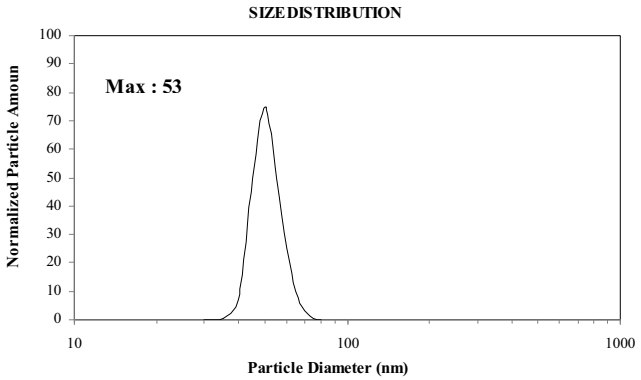

Fig. 7 Size distribution and diameter size of the fabricated HSA NP in the optimal condition.

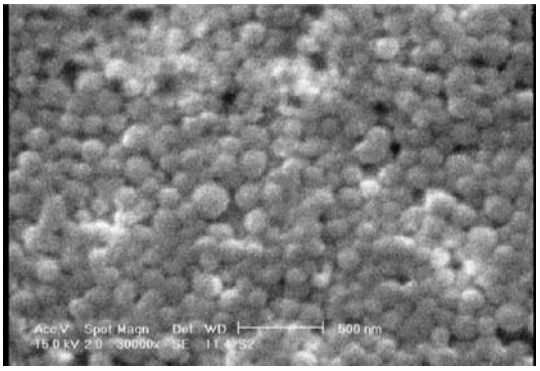

Fig. 8 SEM image of HSA NP.

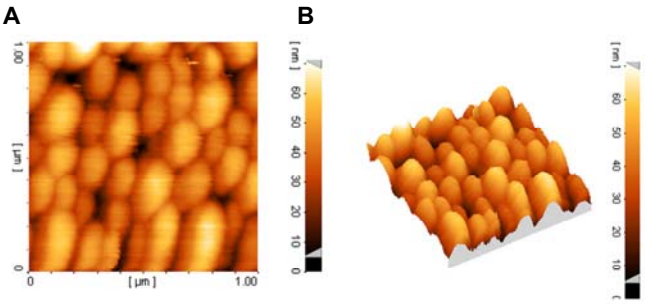

Fig. 9 Topography of HSA NPs with AFM. (**A**) Two dimension. (**B**) Three dimension.

on nanometer scale. The images of the shape and surface characteristic of the NPs were obtained successfully by AFM (**Fig. 9**). Based on these characteristics; HSA NPs were good enough to be candidate for loading drugs on/in them.

CONCLUSION

Our systematic investigation of the synthesis parameters shows that it is possible to prepare HSA NPs with different particle sizes and a small size distribution. The NPs size fabricated from HSA was influenced by several process variables including pH value, HSA concentration, ratio of desolvating agent/ HSA and rate of addition of the desolvating agent. The interrelationships between the mentioned parameters are complex and therefore the analysis of this system for optimizing the factors is a time and labor consuming work. Taguchi method was adopted for the design of experiments and analysis of experimental data was carried out successfully by maximizing S/N ratio and ANOVA.

The best result (minimum size of HSA NPs) were attained at pH 9, 75 $mg.ml^{-1}$ HSA, ratio of organic solvent/ HSA solution 4 and rate of ethanol addition 1.5 $ml.min^{-1}$. The NP size at the determined condition was less than 53 nm. This result in comparison with existing literature is more favorable in terms of minimum size which is suitable

for drug delivery systems. Loading the drug on these NPs will be the next step of the work and subject of our further publication. It can be anticipated that where preparative scale fabrication of such NPs is successful, the application of such delivery systems in nanobiotechnology will contribute to de-bottlenecking of current biopharmaceutical industries.

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REFERENCES

Amidon GL (1997) The mechanism of uptake of biodegradable microparticles in Caco-2 cells is size dependent. *Pharmaceutical Research* **14**, 1568-1573

Arnedo A, Espuelas S, Irache JM (2004) Albumin nanoparticle as carriers for a phosphodiester oligonucleotide. *International Journal of Pharmaceutics* **244**, 59-72

Arun K, Shakti K, Aggarwal KK (2006) Identification of strategy parameters for particle swarm optimizer through Taguchi method. *Journal of Zhejiang University-Science A* **7(12)**, 1989-1994

Atil H, Unver Y (2000) A different approach of experimental design: Taguchi method. *Pakistan Journal of Biological Science* **3**, 1538-1540

Bravo-Osuna I, Ponchel G, Vauthier C (2007) Tuning of shell and core characteristics of chitosan-decorated acrylic nanoparticles. *European Journal of Pharmaceutical Sciences* **30**, 143-154

Desai MP, Labhasetwar V, Amidon GL, Levy RJ (1996) Gastrointestinal uptake of biodegradable microparticles: effect of particle size. *Pharmaceutical Research* **13**, 1838-1845

He X-M, Carte DC (1992) Atomic structure and chemistry of human serum albumin. *Nature* **358**, 209-215

Heller J, Baker RW (1980) Theory and practice of controlled release of bioactive materials. In: Baker RW (Ed) *Controlled Release of Bioactive Materials*, Academic Press, New York, pp 1-17

Irving B (2007) Nanoparticle drug delivery system. *Innovations in Pharmaceutical Technology*, 58-62

Jahanshahi M, Babaei Z (2008) Protein nanoparticle: A unique system as drug delivery Vehicles. *African Journal of Biotechnology* **7**, 4926-4934

Jahanshahi M, Njafpour GD, Rahimnejad M (2008a) Applying the Taguchi method for optimized fabrication of bovine serum albumin (BSA) nanoparticles as drug delivery vehicles. *African Journal of Biotechnology* **7**, 362-367

Jahanshahi M, Raoof JB, Hajizadeh S, Jabari Seresht R (2008b) Synthesis and subsequent purification of carbon nanotubes by arc discharge in NaCl solution. *Physica Status Solidi* **A**, 1-5

Jahanshahi M, Sanati MH, Babaei Z (2008c) Optimization of parameters for the fabrication of gelatin nanoparticles by the Taguchi robust design method. *Journal of Applied Statistics* **35**, 1345-1353

Jahanshahi M, Sanati MH, Hajizadeh S, Babaei Z (2008d) Gelatin nanoparticle fabrication and optimization of the particle size. *Physica Status Solidi* **A**, 1-5

Jahanshahi M, Sanati MH, Minuchehr Z, Hajizadeh S, Babaei Z (2007) Controlled fabrication of gelatin nanoparticles as a drug carrier. *Nanotechnology and its Application. American Institute of Physics* **929**, 228

Jahanshahi M, Williams S, Lyddiatt A, Shojaosadati SA (2004) preparation and purification of synthetic protein nanoparticles. *IET Nanobiotechnology* **151**, 176-182

Kayser O, Lemke A, Hernández-Trejo N (2005) The impact of nanobiotechnology on the development of new drug delivery systems. *Current Pharmaceutical Biotechnology* **6**, 3-5

Kratz F, Fichtner I, Beyer U, Schumacher P, Roth T, Fiebig HH, Unger C (1997) Antitumor activity of acid labile transferring and albumin doxorubicin conjugates *in vitro* and *in vivo* human tumor xenograft models. *European Journal of Cancer* **33**, 175

Langer K, Anhorn M. G, Steinhauser I, Dreis S, Celebi D, Schrickel N, Faust S, Vogel V (2008) Human serum albumin (HSA) nanoparticles: reproducibility of preparation process and kinetics of enzymatic degradation. *International Journal of Pharmaceutics* **347**, 109-117

Li P, Dai YN, Zhang JP, Wang AQ, Wei Q (2008) Chitosan-alginate nanoparticles as a novel drug delivery system for nifedipine. *International Journal of Biomedical Science* **4**, 221-228

Lin W, Coombes GA, Garnett C, Davies C, Schacht E, Davis S, Illun L (1994) Preparation of sterically stabilized human serum albumin nanospheres using a novel dextranox-MPEG crosslinking agent. *Pharmaceutical Research* **11**, 1588-1592

Malik DK, Baboota S, Ahuja A, Hasan S, Ali J (2007) Recent advances in protein and peptide drug delivery systems. *Current Drug Delivery* **4**, 141-151

Marty JJ, Oppenheimer RC, Speiser P (1978) Nanoparticles-a new colloidal drug delivery system. *Pharmaceutica Acta Helvetiae* **53**, 17-23

Masumura Y, Maeda H (1986) A new concept for macromolecular therapeutics in cancer chemotherapy: Mechanism of tumoritropic accumulation of proteins of the antitumor agent smances. *Cancer Research* **46**, 6387- 6392

Michaelis K, Hoffmann MM, Dreis S, Herbert E, Alyatdin RN, Michaelis M, Kreuter J, Langer K (2006) Covalent linkage of apolipoprotion E to albumin nanoparticles strongly enhances drug transport in to the brain. *Journal of Pharmacology and Experimental Therapeutics* **317**, 1246-1253

Muller B, Leuenberger H, Kissel T (1996) Albumin nanospheres as carriers for passive drug targeting: an optimized manufacturing technique. *Pharmaceutical Research* **13 (1)**, 32-27

Peppas LB (1995) Recent advances on the use of biodegradable microparticles and nanoparticles in controlled drug delivery. *International Journal of Pharmaceutics* **116**, 1-9

Rahimnejad M, Jahanshahi M, Najafpour GD (2006) Production of biological nanoparticles from bovine serum albumin for drug delivery. *African Journal of Biotechnology* **5**, 1918-1923

Ross PJ (1996) *Taguchi Techniques for Quality Engineering* (2nd Edn), McGraw Hill, New York

Takakura Y, Fujita T, Hashida M, Sezaki H (1990) Diposition characteristics of macromolecules in tumor-bearing mice. *Pharmaceutical Research* **7**, 339-346

Wang SM, Giang YS, Ling YC (2002) Taguchi's method in optimizing the experimental conditions of simultaneous supercritical fluid extraction and chemical derivatization for the gas chromatographic-mass spectrometric determination of amphetamine and methamphetamine in aqueous matrix. *Forensic Science Journal* **1**, 47-53

Weber C, Kreuter J, Langer K (2000) Desolvation process surface characteristics of HSA nanoparticles. *International Journal of Pharmaceutics* **196**, 197-200

Zauner W, Farrow NA, Haines AM (2001) *In vitro* uptake of polystyrene microspheres: effect of particles size, cell line and cell density. *Journal of Controlled Release* **71**, 39-51