Fabrication and evaluation of gelatin nanoparticles for delivering of anti - cancer drug

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Abstract

The aim of present study was to prepare gelatin nanoparticle for drug and gene delivery applications. These nanoparticles were prepared by two-step desolvation method. The body distribution of colloidal drug delivery systems was mainly influenced by two physicochemical properties namely particle size and surface characteristics. The influence of several factors on the fabrication process including the amount of desolvating agent, concentration of gelatin, temperature, the amount of glutaraldehyde, organic solvent addition rate and agitation speed was investigated herein. The smallest size of achieved nanoparticles was 168 nm and the largest size was 460 nm which is suitable for drug delivery. The amount of cross linker and organic solvent addition rate have less effect on produced nanoparticle size. The study indicated that a minimum size could be obtained with temperature 60° C, 75 ml acetone and 250 µL glutaraldehyde. To characterize the nanoparticle sample, AFM and SEM were employed whilst size distribution was measured by photon correlation spectroscopy. The mechanistic of the optimum conditions for preparing protein nanoparticles as well as their characterization are discussed.

Keyword: *gelatin; drug carrier; nanoparticle evaluation; photon correlation spectroscopy*

1. INTRODUCTION

In recent years the improvement of drug therapy in terms of a more controlled body distribution to reduce side effects was investigated. Different new drug carrier systems in the micro- and nanometer size range were generated to overcome these problems [1]. Colloidal drug delivery systems, which have been developed, include liposomes and nanoparticles. The nanometer size-ranges of these delivery systems offer certain distinct advantages for drug delivery [2]. Nanoparticles are solid colloidal particles ranging in size from about 10 to 1000 nm.

They consist of macromolecular materials and can be used as adjuvant in vaccines, or as drug carriers, in which the active principle (drug or biologically active material) is dissolved, entrapped, encapsulated and/or to which the active principle is adsorbed or attached [3].

Conventional drug therapy requires periodic doses of therapeutic agents. For most drugs conventional methods of drug administration are effective, but some drugs are unstable or toxic and have narrow therapeutic ranges [4]. Some drugs also possess solubility problem. In such cases a method of continuous administration of therapeutic agents is desirable. To overcome these problems, controlled drug delivery systems were introduced. The principal advantage of this technology is that the carrier polymer matrix systems allow much less active agents to be used for the desire of activity [5]. Polymeric nanoparticles have attractive physicochemical properties such as size, surface potential, hydrophilic-hydrophobic balance etc. and for this reason they have been recognized as

potential drug carriers for bioactive ingredients such as anticancer drugs, vaccines, oligonucleotides, peptides, etc [6]. Although various biodegradable nanoparticles of natural polymers such as starch, chitosan, liposomes etc. are largely in use as drug carriers in controlled Drug-delivery technology, however, gelatin nanoparticles represent a promising carrier system for controlled drug delivery [7].

Gelatin has a number of advantages as a nanoparticle material.It is a natural macromolecule, nontoxic and is of non-carcinogenic nature. It possesses a relatively low antigen city and laboratories have a great deal of experience in its use parental formulations. The formation of gelatin based nanoparticles has not been extensively investigated even though its first use as a base for nanoparticles was described more than 25 years ago [8].

Thus being motivated by the application potential of gelatin in biomedical and pharmaceutical fields in the present paper, we prepared gelatin nanoparticles in narrow size range through two-step desolvation method and the effectiveness parameters on its manufacture such as temperature, gelatin concentration, agitation speed and etc. would be discussed in here.

In this work the effect of different imperative factors on the fabrication of gelatin nanoparticle with desolvation method are studied. The main goal is to consider these factors and optimize the particle size and size distribution of gelatin nanoparticles as drug delivery vehicle. The effects of manufacturing conditions such as temperature, gelatin concentration, agitation speed, acetone addition rate etc. upon the fabrication of such nanoparticles are strongly investigated.

2. EXPERIMENTAL

2.1. Materials

Gelatin type A (from porcine skin), glutaraldehyde grade 1.25% aqueous solution, HCl and acetone were obtained from Sigma, Poole, UK. Trypsin-EDTA was purchased from Gibco (New York, NY, USA). Double distilled water was used for all the experiments. All chemicals were of analytical grade and used as received.

2.2. Preparation and purification of gelatin nanoparticles

Gelatin nanoparticles were prepared using a desolvation technique. Gelatin type A (1.25 g) was dissolved in distilled water (25 mL) under constant heating temperature range. Acetone (25 mL) was added to the gelatin solution as a desolvating agent to precipitate the high molecular weight (HMW) gelatin. The supernatant was discarded and the HMW gelatin re-dissolved by addition 25 mL distilled water and stirring at 600 rpm under constant heating. The pH of the gelatin solution was adjusted at 2.5. Acetone (75 mL) was added drop-wise to form nanoparticles. At the end of the process, glutaraldehyde solution (250 μL) was used for preparing nanoparticles as a cross-linking agent, and stirred for 12 h at 600 rpm. The particles were purified by threefold centrifugation and redispersion in acetone (30%) in milliQ water. After the last redispersion, the acetone was evaporated using concentrator (speed vacuum). The resultant nanoparticles were stored at 2-8 °C. The following parameters were changed to study their effect on the characteristics of the nanoparticles: temperature, rate of agitation, concentration of gelatin, concentration of acetone and cross-linker.

2.3. Determination of Nanoparticle Size and Distribution

The size distribution of the prepared Gelatin nanoparticle was analyzed by photon correlation spectroscopy (PCS). Analyzed sample in the PCS device should consist of well dispersed particles in liquid medium. In such conditions the particles are in constant random motion, refer to as Brownian motion and PCS measures the speed of this motion by passing a laser. PCS determines the average particle size and Polydispersity Index (PI) which is a range of measurement of the particle sizes within measured samples. The accurate measurement of particle size must be below 0.7 (70%).

2.4. Scanning Electronic Microscopic (SEM)

For electronic microscopic Scanning micrographs, samples were taken from nanoparticles that were experimentally obtained. Samples were dipped into liquid nitrogen for 10 min, then freeze dried

for 24 h in the freeze drier, EMITECH, model IK750, Cambridg, UK. The sample was fixed on the aluminum stub and coated with gold palladium by Polaron machine model SD515, EMITECH, Cambridg, UK, at 20 nm coating thickness. Finally the sample was examined under SEM using Stereo scan model S360 brand SEM – Leica Cambridg, Cambridg, UK.

2.5. Atomic Force Microscopic (AFM)

Atomic force microscope (AFM) pictures were taken using an Auto probe CP Research AFM system, model AP- 2001 (Thermo microscopes, USA). Samples were prepared by spreading a drop of nanoparticle solution on a degreased glass plate uniformly. The ability of the atomic force microscope to create three-dimensional micrographs with resolution down to the nanometer and Angstrom scales has made it an essential tool for imaging surfaces in applications ranging from semiconductor processing to cell biology. In addition to this topographical imaging, however, the AFM can also probe mechanical and other fundamental properties of sample surfaces, including their local adhesive or elastic (compliance) properties.

3. RESULT AND DISCUSSION

3.1. The effect of different parameters on gelatin nanoparticle size

A method of preparing gelatin nanoparticles by two step desolvation method has been described by Coester et al [9] and subsequently modified by Jahanshahi et al [7, 14] . In our experiments, we studied the effects of varying production parameters on the nanoparticle size. Different synthesis parameters were changed, including temperature, concentration of gelatin, acetone addition rate, amount of glutaraldehyde, agitation speed and amount of desolvating agent. The goal was to prepare small nanoparticles with a narrow size distribution. It has been shown that particle size has a great impact on the uptake of nanoparticles. Desai and co-workers [10] showed that 100 nm size nanoparticles 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 [11-12].

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 nanoparticle population. A lower polydispersity index indicates a narrower size distribution. To study the effect of temperature on the particle size of the nanoparticles, only the temperature was changed in the experiments and all other parameters were kept constant. Acetone was used as desolvating agent (75 ml) and glutaraldehyde (250 μl) as crosslinker. The results are shown in Fig.1.

During these investigations, it was found that the preparation of nanoparticles at ambient temperature (25 °C) was not possible due to the fact that gelatin formed a highly viscous gel at this temperature. The results at 40, 50, 55 and 60 °C showed that temperature has an impact on the particles size. The smallest nanoparticles were prepared at 60 °C with gelatin A. Decreasing the temperature from 60 to 40 °C increased the particle size. This might be explained by the gelling properties of gelatin.

However, one of the investigated factors on particle size is agitation speed. Fig.2 shows a minimum size of particle (209 nm) was obtained at agitation speed of 700 rpm. Generally, the size of particle is expected to reduce with increasing trend of agitation speed [13]. The big nanoparticles were produced in agitation speed lower than 400 rpm. The particle size was unaffected with agitation speed of higher than 700 rpm (data not shown).

To study the effect of the concentration of glutaraldehyde as a cross-linking agent, 200, 300, 400 and 500 μL aliquots of a 25% v/v aqueous glutaraldehyde solution were added to the nanoparticles. The nanoparticles were prepared at 50 °C using gelatin A under the conditions described above. In these experiments, acetone was used as the desolvating agent. These amounts of the cross-linker were sufficient to stabilize the particles. Lower amounts were not sufficient to cross link the nanoparticles since a steep increase in the particle size was observed upon storage (data

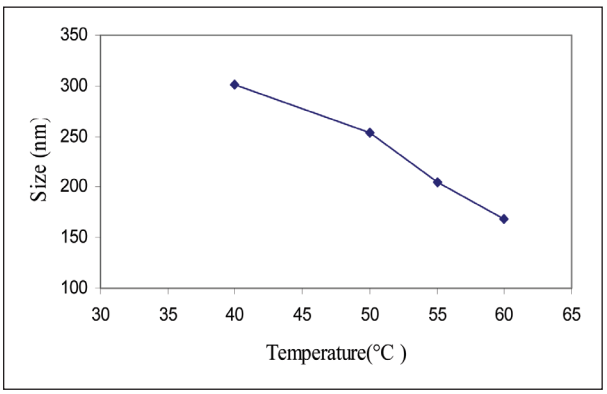

Figure 1: Temperature effect on nanoparticle size

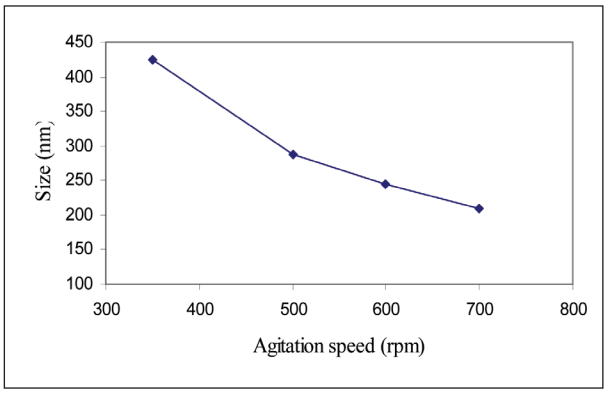

Figure 2: Agitation speed effect on nanoparticle size

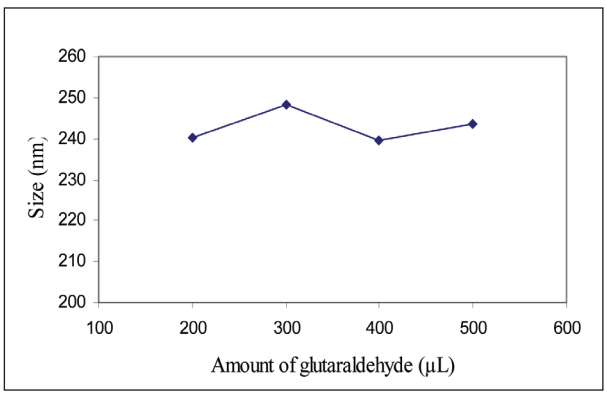

Figure 3: Influence of the amount of glutaraldeyde on nanoparticle size

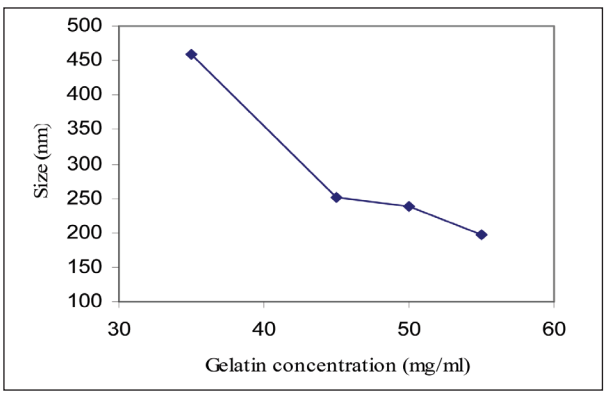

Figure 4: Influence of gelatin concentration on nanoparticle size

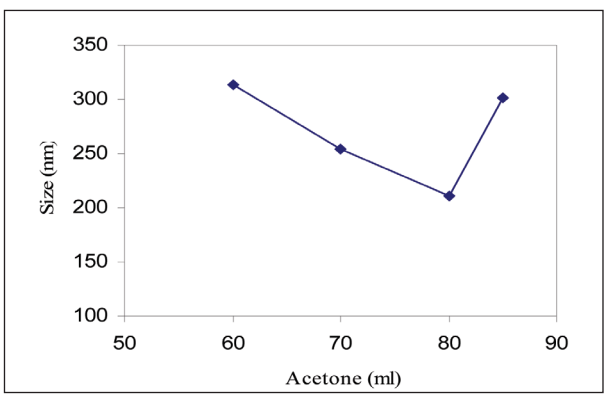

Figure 5: Influence of the amount of acetone on nanoparticle size

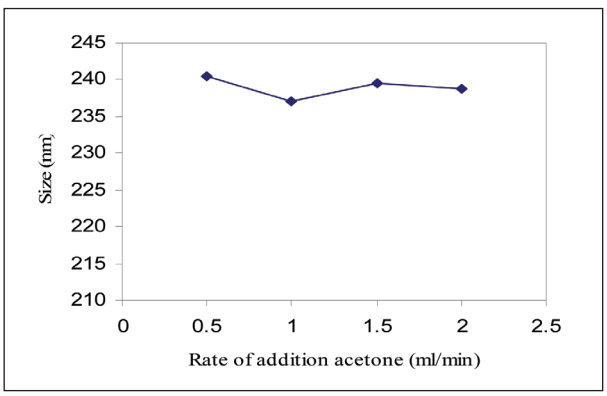

Figure 6: Effect of organic solvent addition rate on nanoparticle size

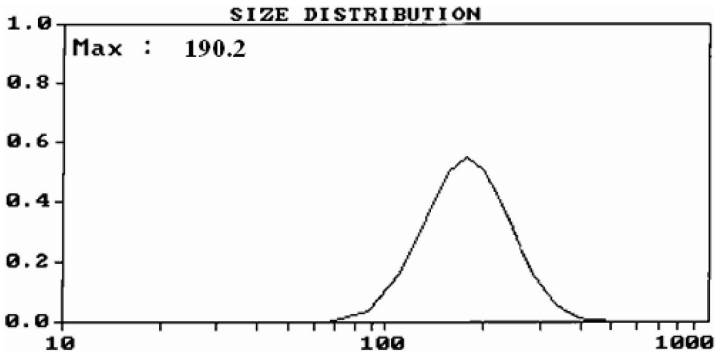

Figure 7. The size distribution of the fabricated gelatin nanoparticle in the optimal condition

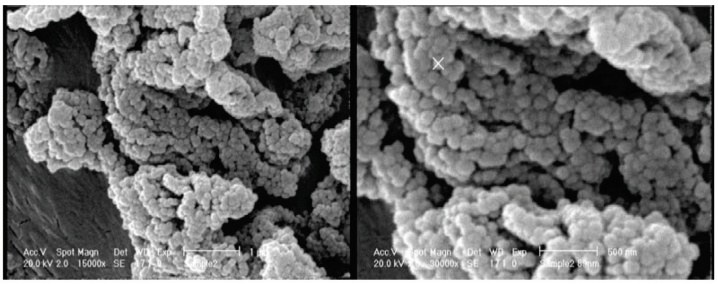

Figure 8. The SEM images of gelatin nanoparticles: 1 μm scale (left), 500 nm scale(right), fabricated by two-step desolvation method.

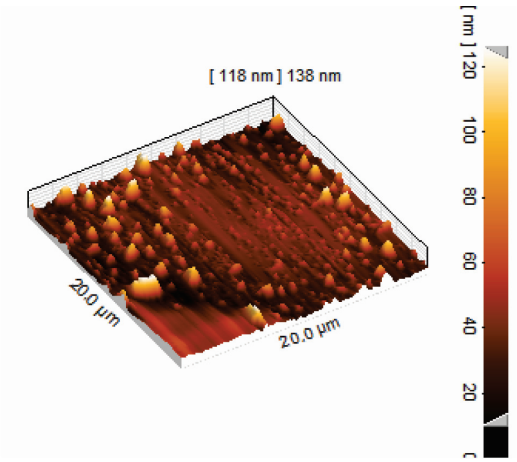

Figure 9. AFM image of gelatin nanoparticles prepared with two-step desolvation method.

not shown). This can be attributed to the swelling of the gelatin in aqueous media after the organic solvent was removed [14]. Increasing the amount of glutaraldehyde from 200 to 500 μL did not show any significant effect on the particle size of the nanoparticles, as shown in Fig. 3.

The concentration of gelatin also had an effect on the particle characteristics. In order to study the effect of the concentration of gelatin, different concentrations such as 35, 45, 50, 55 mg/ml were prepared in 40 °C. Fig. 4 shows that increasing gelatin concentrations decrease nanoparticle size.

The influence of different acetone concentrations (60, 70, 80 and 85 ml) on nanoparticle size also investigated herein and these experiments shown that increasing acetone amounts decrease nanoparticle size (Fig.5).

The last investigated parameter is organic solvent (i.e. acetone) addition rate. Increasing or decreasing the rate of addition acetone did not show any significant effect on the particle size of the nanoparticles (Fig.6).

3.2. Physical Characterization of Nanoparticles

The particle sizes as well as the light intensity counts of the samples were determined by photon correlation spectroscopy (PCS). Fig. 7 illustrates the gelatin nanoparticle size distribution and size in one of the condition considered above. This figure shows that produced nanoparticles have a reasonable size distribution which is favorable for drug delivery.

In addition, the morphology and size distribution of the prepared gelatin nanoparticles were examined with Scanning Electronic Microscopy as well as Atomic Force Microscopy. A SEM image is shown in Figure 8, which clearly shows smooth and spherical nanoparticles with a diameter of 100-300 nm. In addition, AFM pictures are indicated that no hair cracks or heterogeneity appear on the nanoparticles surface. This obviously presents a morphological evidence for solid and smooth nanoparticles which is vital for delivering system.

4. CONCLUSION

The present study showed that gelatin nanoparticle can be prepared with predictable size, in size range between 168 and 460 nm by two - desolvation method. The experimental data presented herein indicated that a number of parameters such as temperature, initial gelatin concentration, agitation speed and amount of acetone have more effect on particle size. In contrast, acetone (desolving agent) addition rate and Glutaraldehyde concentration were shown which have less effect on particle diameter. The smallest size of nanoparticles was obtained at temperature 60 °C and the largest size of those was obtained at agitation speed of 350 rpm. Based on the SEM and AFM analyze, the protein nanoparticle as it has been assembled herein, not only mimics the size and surface chemistry of nanoparticles such as viruses and plasmid, but also can be used as drug delivery vehicles in its own right. To the best of our knowledge the current study is one of the first one have demonstrated optimized fabrication of protein nanoparticles. Loading the drug on these nanoparticles will be the next step of the work and subject of our further publication.

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References

1. Conrad Coester, Paras Nayyar, John Samuel. In vitro uptake of gelatin nanoparticles by murine dendritic cells and their intracellular localization. 114 European Journal of Pharmaceutics and Biopharmaceutics 62, 306–314 (2006).

2. A.Saxena, K.Sachin, H.B. Bohidar, A. K. Verma. Effect of molecular weight heterogeneity on drug encapsulation efficiency of gelatin nanoparticles. Colloids and Surfaces B: Biointerfaces 45, 42-48 (2005).

3. Kumaresh S. Soppimath, Tejraj M. Aminabhavi, Anandrao R. Kulkarni, Walter E.Rudzinski. Biodegradable polymeric nanoparticles as drug delivery devices. Journal of Controlled Release 70, 1-20 (2002).

4. Vinod Labhasetwar, Cunxian Song, Robert J. Levy. Nanoparticle drug delivery system for restenosis. Adv Drug Del Rev 24, 63-85 (1997).

5. Muller R.H. and Keck C.M. Challenges and Solutions for the Delivery of Biotech Drugs-A Review of Drug Nanocrystal Technology and Lipid Nanoparticles. J.Biotechnol., 113, 151-170 (2004).

6. Majeti N. V.Ravi Kumar. Nano and microparticles as controlled drug delivery devices. J Pharm Pharmaceut sci 3(2), 234-258 (2000).

7. M. Jahanshahi, H.Aghajani and Tau.C.Ling. Assembly and purification of nanostructure bioprod-

ucts: protein nanoparticle characterization and nano-stick exterior coating adsorbents. International Journal of nanoscience and nanotechnology1(1), 9-20 (2005).

8. Shirzad Azarmi, Yuan Huang, Hua Chend, Steve McQuarriea, Douglas Abramse, Wilson Road, Warren H. Finlayf, Gerald G. Millera, Raim Löbenberga. Optimization of a two-step desolvation method for preparing gelatin nanoparticles and cell uptake studies in 143B osteosarcoma cancer cells. J Pharm Pharmaceut Sci 9(1), 124- 132 (2006).

9. Coester CJ, Langer K, Van Briesen H, Kreuter J. Gelatin nanoparticles by two-step desolvation-a new preparation method, surface modifications and cell uptake. J Microencapsul. 17, 187-193 (2000).

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

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

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

13. M. Jahanshahi, AW Pacek, AW Nienow, A Lyddiatt. Fabrication by three-phase emulsification of pellicular adsorbent customized for liquid fludized bed adsorption products. J. Chem. Technol. Biotechnol. 78, 1111-1120 (2003).

14. M. Jahanshahi, Gh. Najafpour and M. Rahimnejad,. Applying the Taguchi method for optimized fabrication of BSA nanoparticles as drug delivery vehicles. . African Journal of Biotechnology, Vol (7), 3. (2008).