Design of gelatin biodegradable nano particles as a carrier system for hydrophilic macromolecules/proteins using nano precipitation technique: A resourceful approach for drug delivery

Heidi Mohamed Abdel-Mageed

Molecular Biology Department, Genetic Engineering and Biotechnology Division, National Research Centre, Cairo, Egypt

* Corresponding Author:

Heidi M. Abdel Mageed, PhD.

Molecular Biology Dept., Genetic Engineering and Biotechnology Division, National Research Centre (NRC), El Behoth St, Dokki, Cairo, Egypt

Tel: (202) – 1002089208, Fax: (202) -2618-6111

E-mail: heidi.abdelmageed@gmail.com

Abstract:

The objective of this study was to design and optimize formulation of biodegradable and biocompatible gelatin nano particle (GNP) using nano-precipitation technique, as potential delivery systems for hydrophilic macromolecules/proteins using bovine serum albumin (BSA) as a model drug. Various parameters were studied, including the concentration of stabilizer, concentration of gelatin, effect of non-solvent nature and the solvent/non-solvent volume ratio. The robustness of the modified methodology was investigated and the prepared GNP was characterized with respect to particle size, PDI using Zetasizer and morphology using Scanning Electron Microscope (SEM). Results showed that among the tested surfactants, poloxamers were able to stabilize the GNP with a minimum concentration of 7% to prepare stable nano particles in the size range 180–240 nm. n-butanol, ethyl ether, acetone and acetonitrile did not allow GNP formation, while on using methanol and ethanol, stable GNP was produced. Increasing the polymer concentration above 25mg/ml greatly increased medium viscosity and caused agglomeration. An optimized formulation (20 mg/ml gelatin, 2% BSA, 7% poloxamer 407 and using ethanol as non-solvent) was prepared with 88% entrapment efficiency (EE %). SEM images showed spherical GNP where, loading BSA did not affect GNP morphology. The cumulative release of BSA after 72 hours was equivalent to 80%. Storage study indicated physical stability GNP after storage at different temperatures for 60 days. The results presented in this study demonstrate and highlight the unexplored potentials of the nano precipitation method for the preparation of nano particles from hydrophilic gelatin polymer for sustained delivery of protein drugs.

Keywords: Gelatin, nano particles, nano precipitation, bovine serum albumin, hydrophilic drug delivery, protein delivery
1. Introduction:

Nano particles are versatile systems in the biomedical field where special interest has been recently raised for nano particles prepared from natural biodegradable biocompatible polymers (Chakraborty et al., 2013; Mirzaeia and Darroudi, 2017). Comparing with other carrier systems, nano particles have superior accumulation, particularly in macrophage rich organs, such as liver, lung and spleen as they are preferentially phagocytosed. Biodegradable nano particles have been extensively employed as drug delivery systems owing to their bioavailability, superior loading yield, prolonged and targeted release, non-toxic and non-immunogenic characteristics (O’Hagan, 1996; Sahoo et al., 2015; Nam et al., 2015).

Gelatin is a denatured protein that is obtained either by partial acid or alkaline hydrolysis or by thermal or enzymatic degradation of animal collagen protein. Two types of gelatin (type A and type B) are commercially available depending on the method of collagen hydrolysis (Pulat and Akalin, 2013). Gelatin has been long used safely in pharmaceuticals, cosmetics, food products, and is considered as GRAS (generally regarded as safe) additive by the United States Food and Drug Administration (US FDA). Gelatin is clinically used as a plasma expander and stabilizer in various protein, gene and vaccine preparations (Zwiorek et al., 2005; Sharma et al., 2015). Gelatin application as a nano material is gaining increasing interest owing to the combined benefit of ‘‘nano-size’’ and its beneficial structural properties including being a naturally occurring macromolecule and of low antigenicity. Gelatin consists of heterogenous polypeptides hence, its structure possesses many available functional groups, which can enable modifications for coupling, conjugation and cross linking. Gelatin based nano particles (GNPs) have great potentials as drug carrier systems being easily available from renewable sources, biocompatible, non-antigenic, of remarkable binding capacity to various active groups in addition to high stability in vivo and on storage. Gelatin nano particles surface can be subjected to various modifications such as attachment to site-specific ligands, or, surface covering with polyethyl glycols for targeted and are used for sustained drug delivery (Sarkar, 2013; Sahoo et al., 2015; Yasmina et al., 2017).

Several methods are used for preparation of GNPs including desolvation, coacervation-phase separation, emulsification-solvent evaporation, reverse phase microemulsion, nano precipitation, self-assembly and layer-by-layer coating (Elzoghby, 2013; Yasmina et al., 2017). Nano precipitation is a recent, rapid, simple and direct method compared to other methods used for preparation of gelatin nano particle. The method uses two miscible solvents whereas, the polymer is soluble in one (the solvent) and should not be soluble in the other (the non-solvent). The gelatin and drug in the aqueous solvent phase are slowly added to the non-solvent phase which should contain a surfactant as a stabilizer, followed by addition of glutaraldehyde to cross-link the nano particles (Khan and Schneider, 2013). GNP are produced at the interface whereas, the diffusion of the solvent causes an interfacial turbulence at the interface between water and non-solvent which is rapidly stabilized by the stabilizing agent, till displacement of the solvent is finished and protein solidification takes place in the nano range (Galindo-Rodriguez et al., 2004; Ganachaud and Katz, 2005). This methodology was reported for cyclosporin A gelatin nano particles with 98% entrapment efficacy (Allemann et al., 1998).
Delivery of protein drugs offers several restrictions and challenges to the formulator, owing to their fragile nature which requires protection during processing and from the biological environment and they usually exhibit low permeability across biological membranes. In addition, they are usually expensive with high does required due to their short half-lives (Simone et al., 2016). Optimization of protein drug delivery has been addressed using various strategies, permeation enhancers (Fasano and Uzzau 1997; Uchida et al., 2001); protease inhibitors (Yamamoto et al., 1994; Marschutz et al., 2006); hydrogels (Musabayane et al., 2000; Kavimandan et al., 2006); and protein–ligand conjugates (Gordon, 2002). In addition, loading into long circulating nano carriers which, have been investigated as a potential carrier system for delivery of proteins. In this context, this study focused on exploring the use of nano precipitation methodology for the encapsulation of a model hydrophilic macromolecule (Bovine Serum Albumin BSA) loaded into gelatin nano particles. The main aim of this research is to prepare gelatin nano particles using nano precipitation methodology that can be loaded with a model hydrophilic macromolecule and studying the effects of formulation parameters on particle size, drug loading and release. This prepared nano particles are proposed to be appropriate for possible application in biological systems since gelatin is a biocompatible and non-immunogenic polymer.

2. Material and Materials:

2.1. Materials:

Gelatin-B (Type B bovine skin extract, 225 blooms, pI~4.9±0.2) having molecular weight 50KDa, glutaraldehyde (25% v/v aqueous solution), and Bovine Serum Albumin (BSA) were purchased from Sigma-Aldrich, USA, were used as received. Poloxamer 407, Poloxamer 188 were obtained from BASF, Germany. Others chemicals including acetone, were of analytical grade and were purchased from local supplier.

2.2. GNP preparation using nano precipitation technique:

GNP was prepared according to methods described in literature with some modifications (Kaul and Amiji, 2002; Khan and Schneider 2013). 200 mg of gelatin were dissolved in 10 mL deionized water at 50 °C. The GNPs were produced by controlled precipitation of the prepared aqueous gelatin solution with ethanol containing 7% w/v srfactant as a stabilizer, under continuous stirring. This step was followed by addition of 0.5mL of a glutaraldehyde solution (2%, w/v) as a cross linking agent, overnight stirring was carried out to allow cross-linking and rigidification of GNP, whereas the ethanol:water ratio was kept at 65:35 v/v in the resulting final mixture (100 ml). The GNP prepared was centrifuged at 25,000 rpm for 90 mins, washed twice with deionized distilled water, and lyophilized to obtain free flowing GNPs. BSA was used as a model of hydrophilic macromolecular drugs. To an aqueous solution of gelatin adjusted to pH 7.0, BSA was added at a final concentration of 2.0% (w/w) and the encapsulated GNP was prepared as described above. The BSA-loaded GNP was separated from the free BSA by centrifugation and consecutive washing.
2.2.1. GNP Formulation Optimization

Gelatin nano particles tend to aggregate during the preparation process, and this process is hastened during the cross-linking reaction. Hence it was important to optimize formulation conditions to obtain stable nano particle dispersions through studying the effect of various parameters.

2.2.1.1. Stabilizer Type and Concentration:

To investigate the suitability of various stabilizers, Poloxamer 407, Poloxamer 188, tween 80 and tween 20 were investigated at various concentrations (1–10%). The gelatin concentration in the solvent phase (20 mgm/ml), solvent/non solvent ratio (1:10) and the glutaraldehyde concentration (0.5 mL of 2% w/v) were kept constant. The effect of the non-solvent volume on the mean particle size was investigated with regard to aqueous phase to ethanol phase.

2.2.1.2. Non solvent Type and volume:

In order to test the effect of non solvents, various non solvents namely ethanol, methanol, acetone, n-butanol, ethyl ether and acetonitrile were studied. Gelatin concentration, mass ratio of gelatin to stabilizer and glutaraldehyde concentrations was kept constant. To investigate the effect of the variation in non-solvent volume on GNP, different solvent/non-solvent volume ratios were tested (0.05-0.13).

2.2.1.3. Initial Gelatin Concentration:

To study the effect of polymer concentration, the gelatin concentration in the solvent phase was varied from 15mg/mL to 35mg/mL. The ratio of solvent/non solvent, mass ratio of gelatin to stabilizer (1/32), glutaraldehyde amount (0.5mL of 2% w/v) and the volume of non-solvent (10 mL of ethanol) were all maintained.

2.3. Determination of entrapment efficiency (EE%)

The total quantity of BSA entrapped (EE%) in GNP was determined using a direct method where, the difference between the total amount of BSA used in the formulation and the final weight of GNP was determined. Five milligrams of GNP were added to 5 ml of 0.5 M phosphate buffer saline (PBS) (pH7.0, 37°C), free GNP (unloaded with BSA) were used as a blank. The free BSA concentration was determined by UV-Vis spectrophotometer at λ = 280 nm in the clear supernatant attained after separation of GNPs with ultracentrifuge at 50,000 g for 3 hours.

\[
EE\%\ (w/w) = 100 \frac{\text{amount of BSA in GNP} - \text{Amount of free BSA}}{\text{Amount of BSA in GNP}}
\]

2.4. Characterization of nano particles

2.4.1 Scanning Electron Microscopy (SEM)

SEM (ESEM™, Quanta 250-EFG, Thermo-Fischer Scientific, USA) was used to image GNP for evaluation of the shape and morphology. Briefly, a small amount of lyophilized GNP was mounted on aluminum stubs, pre-pasted with doubled-side copper tapes. The samples were
sputter coated with a thin layer of gold for 60 sec in a Jeol JSM-6400 (Tokyo, Japan) and placed the specimen chamber at an accelerating voltage of 10 kV.

### 2.4.2. Particle size measurement

The size of nano particles was analyzed by dynamic light scattering (DLS) with a Zetasizer nano-ZS (Malvern Instruments Ltd., UK). The mean diameter and polydispersity index (PI) values were obtained at an angle 90° in 10 mm diameter cells. Samples from GNP suspension were prepared by diluting with 3 ml of double distilled water and sonicating for the 30 s. Particle size measurements were performed at room temperature with a detection angle 90°. All measurements were performed in triplicate.

### 2.5. In vitro release study

10 mg of BSA loaded GNP was placed with 2 ml of buffer solutions (0.5 M phosphate buffer saline (PBS) pH 7.0) in an Eppendorf-type microcentrifuge tubes at 37 °C. At determined time intervals, the tubes were removed from the water-bath and centrifuged at 25,000 rpm for 5 min. afterwards, 500 µl of the supernatant was withdrawn and diluted to 1.0 ml with PBS. The buffer was replaced with fresh one after each withdrawal to maintain constant volume and sink conditions. The amount of BSA in the samples withdrawn was determined by a UV-Vis spectrophotometer. The release profile of BSA from GNP was carried out by measuring the cumulative release over time.

### 2.6. Physical stability

To examine the physical stability of free and BSA loaded GNPs, a short term stability study was carried out. Immediately after GNP preparation (day 0), GNPs aqueous dispersion was divided into three glass vials and stored at three different temperatures (4°C, room temperature (RT 25°C) and 40°C). Stored samples were tested for particle size on days 0, 15, 30, 45 and 60.

### 3. Results:

#### 3.1. Preparation of GNP

Designing and developing a successful protein/peptide drug delivery are important to preserve and protect their biological activity hence; a nano particle-based protein delivery system was investigated for applications in protein drug delivery using nano precipitation method. The simple procedure used in this study successfully produced nano particles from gelatin as a hydrophilic polymer. GNP was formulated instantaneously by rapid diffusion and mixing of the solvent (Table 1). The mechanism of GNP formation has been rationalized by the interfacial unrest occurring as a result of solvent diffusion, this cause a fierce spreading due to solvent/non solvent mutual miscibility. Hence, nano droplets are separated from the interface, which usually require stabilization by a surfactant. Subsequently, the aggregation of the hydrophilic polymer chains induces the formation of the nano particles (Guerrero et al., 1998; Lee et., 2013).
3.2. Stabilizer Type and Concentration

Gelatin is poly-ampholytic, with positive and negative charged groups in the polymer structure. Normally, gelatin molecules intermolecular charge neutralization takes place in the presence of the non-solvent, this usually leads to agglomeration (Gupta and Bohidar, 2009). In addition, gelatin exhibit poor mechanical properties. Therefore, cross linking is considered a crucial step in the preparation of stable GNP. For cross linking, common cross linkers are usually used such as glutaraldehyde, however, this step can lead to inter-particle cross linking occurring simultaneously to intra-particle cross linking, which may as well cause aggregation (Qazvini et al., 2011). Hence, stabilizers in the non-solvent were used in order to overcome possible aggregation during preparation of GNP. Results showed that GNP was not formed in absence of stabilizer, in addition, polysorbate 80 and polysorbate 20 were not able to stabilize GNP dispersion; on the other hand, stabilizing effect of poloxamers was concentration dependent. In addition, Poloxamer concentration influenced the GNP mean size (Figure 1, Table 2). Stabilizer concentrations up to 5% caused a 2-fold increase in GNP size. The increase in size can be attributed to the formation of agglomerates that resulted in an inhomogeneous size distribution, as evident from the elevated value of the poly dispersity index (PDI of 0.4–0.5). However, on increasing the stabilizer concentration up to 6% a reduction in the PDI value to 0.25 was observed. Further increase in stabilizer concentration (7%–10%) produced a constant PDI value. Thereafter, it was deduced that a minimum concentration of 7% poloxamer as a stabilizer is required to prepare stable nanoparticles in the size range 180–720 nm.

![Figure 1: Effect of stabilizer concentration on the particle size and polydispersity. (P407) Poloxamer 407; (P188) Poloxamer 188](image-url)
Table 1: Size characterization of gelatin nano particles.

<table>
<thead>
<tr>
<th>Stabilizer (7%)</th>
<th>Mol. Wt. [g mol(^{-1})]</th>
<th>Size (nm)*</th>
<th>BSA Loaded GNP</th>
<th>EE %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poloxamer 407</td>
<td>12000</td>
<td>180±8 (0.08)</td>
<td>208±6 (0.11)</td>
<td>88%</td>
</tr>
<tr>
<td>Poloxamer 188</td>
<td>8350</td>
<td>240±5 (0.12)</td>
<td>249±7 (0.16)</td>
<td>82%</td>
</tr>
</tbody>
</table>

Formulation: Gelatin concentration: 25mg/mL, nonsolvent: ethanol (10 mL).
*Values in parenthesis represent polydispersity index.

3.3. Non solvent Type and concentration

As presented in Table 2 the final mean particle size was dependent on the type of the dispersing solvent. Results showed that on using simple alcohols (methanol and ethanol) GNP was efficiently prepared. The mean size of GNP measured on using ethanol as the non-solvent was smaller than that obtained with methanol (Table 2) respectively. Special interest has been raised on using alcohols as non-solvents, due to their relatively low dielectric constant (\(\varepsilon\) values). In fact, lower the dielectric constant value will lead to less solubilizing effect of non-solvent on hydrophilic protein, preventing drug leakage. Hence, ethanol is most appropriate non-solvent in this respect as its dielectric constant value is of 24.6, considerably far from that of water (80.1). Moreover, using such solvent of low

Table 2: Effect of different non solvents used for gelatin nano particle preparation mean particle size and polydispersity.

<table>
<thead>
<tr>
<th>Type of non-solvent</th>
<th>Dielectric constant</th>
<th>Stabilizer</th>
<th>S/NS Volume ratio</th>
<th>Size (nm)</th>
<th>PDI</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>24.6</td>
<td>-------------------</td>
<td>0.1</td>
<td>ND</td>
<td>ND</td>
<td>--</td>
</tr>
<tr>
<td>Ethanol</td>
<td>24.6</td>
<td>Poloxamer 407 (1%)</td>
<td>0.1</td>
<td>720±20</td>
<td>0.58</td>
<td>+</td>
</tr>
<tr>
<td>Ethanol</td>
<td>24.6</td>
<td>Poloxamer 407 (5%)</td>
<td>0.1</td>
<td>389±12</td>
<td>0.45</td>
<td>+</td>
</tr>
<tr>
<td>Ethanol</td>
<td>24.6</td>
<td>Poloxamer 188 (1%)</td>
<td>0.1</td>
<td>460±15</td>
<td>0.43</td>
<td>+</td>
</tr>
<tr>
<td>Ethanol</td>
<td>24.6</td>
<td>Poloxamer 188 (5%)</td>
<td>0.1</td>
<td>391±11</td>
<td>0.39</td>
<td>+</td>
</tr>
<tr>
<td>Methanol</td>
<td>32.7</td>
<td>Poloxamer 407 (7%)</td>
<td>0.1</td>
<td>245±6</td>
<td>0.15</td>
<td>+</td>
</tr>
<tr>
<td>Acetone</td>
<td>20.7</td>
<td>Poloxamer 407 (7%)</td>
<td>0.1</td>
<td>ND</td>
<td>ND</td>
<td>--</td>
</tr>
<tr>
<td>n-butanol</td>
<td>17.5</td>
<td>Poloxamer 407 (7%)</td>
<td>0.1</td>
<td>ND</td>
<td>ND</td>
<td>--</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>36.1</td>
<td>Poloxamer 407 (7%)</td>
<td>0.1</td>
<td>ND</td>
<td>ND</td>
<td>--</td>
</tr>
<tr>
<td>Ethyl ether</td>
<td>4.3</td>
<td>Poloxamer 407 (7%)</td>
<td>0.1</td>
<td>ND</td>
<td>ND</td>
<td>--</td>
</tr>
</tbody>
</table>

PI: mean polydispersity index expressed using a 0–1 scale.
Solvent: water, 1 mL. Gelatin concentration: 20 mg/mL.
Key: (+) nano dispersion; (--) visible precipitation, ND: not determined as the formulation produced visible aggregates.
dielectric constant value is of special importance for protection of protein molecular integrity. Brennan and Clarke (1993) reported that the rate of spontaneous deamidation at asparagine residues was considerably decreased in solvents of low dielectric constant values. Finally, ethanol is not of concern considering toxicity. Whereas, it is characterized as Class 3 according to the ICH solvent toxicity scale (Class 3 solvents are of very low risk to human health) (Bilati et al., 2005).

Furthermore, on testing various non-solvents, results revealed that acetone and acetonitrile did not allow GNP formation. Whereas, on the addition of the gelatin solution into these non-solvents heavy aggregation of gelatin occurred with the formation of a visible precipitate. On addition of n-butanol to polymer solution a viscous gel was produced and solvent diffusion to non-solvent was inhibited. Whereas, the addition of ethyl ether to the polymer caused an evident desolvation and polymer precipitation took place as well. The formation of this precipitate can be attributed to either the difference in the dielectric constant values of the solvent and non-solvent or the large difference in the solubility parameter values of water, acetone and acetonitrile (Galindo-Rodriguez et al., 2004; Khan and Schneider, 2013).

The effect of the solvent/non solvent ratio for producing a stable nano-dispersion was investigated (Table 2). GNP prepared in presence of ethanol and poloxamer showed that a low ratio of 1:7.5 produced visible particles, with high PDI value (Figure 2). A solvent/non solvent ratio of 1:10 was able to produce stable GNP whereas, any additional increase in non-solvent volume did not affect the size or the PDI values of GNP. This result is significant as this parameter could be rather modified (as an alternative to polymer concentration) if a higher concentration of nano particles is required in the prepared dispersion. In addition, it would allow reduction in the total volume of solvent used.

![Figure 2: Effect of ethanol volume on particle size of gelatin nano particles. P407 (Poloxamer 407); P188 (Poloxamer 188)](image-url)
3.4. Polymer Concentration

The effect of polymer concentration on particle size was studied at varying gelatin concentrations. Results revealed that increasing the polymer concentration lead to an increase in the mean size of GNP (Figure 3). At concentration higher than 25 mg/ml bigger GNP with high PDI value was produced. Higher polymer concentration would lead to higher viscosity of the solvent phase which would consequently retard diffusion of the solvent phase into the non-solvent phase (Bilati et al., 2005; Hornig et al., 2009).

![Figure 3: Effect of gelatin concentration on the particle size of gelatin nano particles. P407 (Poloxamer 407); P188 (Poloxamer 188)](image)

3.5. Morphology and SEM images

SEM was used to study the shape and morphology of prepared free and BSA loaded optimized GNP (20 mg/ml gelatin, 1:10 water:ethanol concentration, 7% poloxamer 407). SEM micrographs verified that free and BSA loaded GNP were smooth and spherical in shape (Figure 4). SEM images also verifies that loading BSA did not alter the shape or morphology of GNP.
3.6. Particle size analysis

The mean nano particle size and size distribution of prepared GNP are tabulated in Table 1. Both free and BSA loaded gelatin nano particles exhibited a mean particle size between 180–240 nm and with a narrow size distribution. The data presented in Table 1 show that the mean size of GNP of free and BSA loaded ones are very close where; BSA did not affect mean particle size.

3.7. Drug Loading and Release

BSA was used as a model hydrophilic macromolecular as it enables ease of evaluation of loading and release by simple methodology. BSA loaded GNP (water 1ml, Gelatin 25mg/ml, 7% poloxamer 407, ethanol 10 ml) was prepared with an entrapment efficiency of 88%. The in vitro release of BSA was carried out in PBS at 37°C. The release profile of BSA from the GNP matrix exhibited a biphasic model with a burst effect followed by a sustained one (Figure 5). The mechanism of release of BSA from GNP is a multistep process that involves permeation and absorption of solvent through gelatin matrix followed by swelling of GNP and subsequent swelling of BSA molecules. The slow release profile can be explained based on involvement of BSA in the cross-linking process with glutaraldehyde; being of protein nature with primary amino groups. Cross linking occurs as the aldehyde groups found in glutaraldehyde react with amino groups.
found in proteins forming Schiff bases (Barbosa et al., 2014). The cumulative release of BSA after 72 hours was equivalent to 80%. Biphasic release profile of gelatin nano particles has been reported by several authors (Hans and Lowman, 2002; Santoro et al., 2014; Yasmina et al., 2017). In addition, similar slow release profile was reported by other authors where the hydrophilic drug was entrapped in the carrier by cross linking (Azimi et al, 2014; Fathollahipour et al., 2016). The BSA release profile demonstrated the capability of the prepared GNP to support prolonged release of protein/hydrophilic drugs hence, extending the bioavailability of these drugs in vivo.

3.8. Stability Study

Developing a successful drug carrier system demands an acceptable stability of prepared delivery system. Figure 6 represents the physical stability of the free and BSA loaded GNP stored at refrigeration (4°C), room temperature (RT, 25°C) and at 40°C for 60 days. For all three storage conditions tested, the GNP showed practically unchanged mean particle size and all PI values obtained were below 0.2. In addition, no visible aggregation of GNP was observed. These results are promising in terms of practical applications of GNP.
4. Conclusion

In this study gelatin nano particles have been successfully prepared by nano precipitation method in presence of Poloxamer 407 and Poloxamer 188 as stabilizers and ethanol as the non-solvent. A minimum concentration of 7% stabilizer was demanded to avoid agglomeration during the cross-linking process. The mean particle size was affected by polymer concentration, however, on increasing polymer concentration beyond the optimum one, aggregation and precipitation was evident. BSA was chosen as a model hydrophilic macromolecule, results proved that it can be successfully loaded into gelatin nano particles using nano precipitation methodology. GNP release profile from the GNP exhibited a biphasic release profile. Based on the preliminary results presented in this study it can be concluded that the prepared GNP presents a potential biodegradable carrier system for delivery of hydrophilic macromolecular drugs.

References:


Brennan, M., Clarke, S., 1993. Spontaneous degradation of polypeptides at aspartyl and asparaginyl residues: effects of the solvent dielectric. Protein Sci. 2, 331–338


Ijrdo-Journal Of Biological Science

ISSN: 2455-7676

Volume-3 | Issue-11 | November,2017 | Paper-4


Mirzaeia H., Darroudi M. 2017. Zinc oxide nanoparticles: Biological synthesis and biomedical applications. Ceramics Int., 43; 907-914


