IJPSR (2014), Vol. 5, Issue 6 **(Review Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 17 January, 2014; received in revised form, 28 March, 2014; accepted, 25 April, 2014; published 01 June, 2014

GELATIN NANOPARTICLE: PREPARATION, CHARACTERIZATION AND APPLICATION IN DRUG DELIVERY

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Keywords:

Nanotechnology, site specific delivery, gelatin, immunogenicity, intravenous

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ABSTRACT: Nanotechnology plays an important role in advanced biology and medicine research particularly in the development of potential site-specific delivery systems with lower drug toxicity and greater efficiency. In terms of nanopharmaceutics, gelatin was already considered as interesting biodegradable base material in the early days of particle development. The interest was based on the facts that gelatin has been known for its low immunogenicity for many years and is administered intravenously since it is an ingredient of various registered blood substitutes. One advantage offered by the amino acid side-chains of the gelatin matrix molecule is the option of multiple further modifications. Therefore, gelatin nanoparticles can be used as a great carrier for the drugs delivery intravenously with few surface modifications.

INTRODUCTION: Gelatin (or gelatine, from Latin: gelatus = stiff, frozen) is a translucent, colorless, brittle (when dry), flavorless solid substance, derived from collagen obtained from various animal by-products. It is commonly used as a gelling agent in food, pharmaceuticals, photography, and cosmetic manufacturing. Substances containing gelatin or functioning in a similar way are called gelatinous.

Gelatin is an irreversibly hydrolyzed form of collagen, and is classified as a foodstuff. It is found in most gummy candies as well as other products such as marshmallows, gelatin dessert, and some ice cream, dip and yogurt.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(6).2149-57

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(6).2149-57

Household gelatin comes in the form of sheets, granules, or powder. Instant types can be added to the food as they are; others need to be soaked in water beforehand.

Composition and properties: Gelatin is a mixture of peptides and proteins produced by partial hydrolysis of collagen extracted from the skin, bones, connective tissues, of animals such as domesticated cattle, chicken, pigs, and fish. Food-grade gelatin is produced mainly from two raw materials, beef skin and pig hides. Photographic and pharma grades of gelatin are generally made from beef bones, although some beef bone gelatin is used by the food industry. Gelatin is an animal protein unlike many other gelling agents used by the food industry. The natural molecular bonds between individual collagen strands are broken down into a form that rearranges more easily. Gelatin melts to a liquid when heated and solidifies when cooled again. Together with water, it forms a semi-solid colloid gel.

Gelatin forms a solution of high viscosity in water, which sets to a gel on cooling, and its chemical composition is, in many respects, closely similar to that of its parent collagen1. Gelatin is also soluble in most polar solvents.

Gelatin solutions show viscoelastic flow and streaming birefringence. If gelatin is put into contact with cold water, some of the material dissolves, but not all. The solubility of the gelatin is determined by the method of manufacture. Typically, gelatin can be dispersed in a relatively concentrated acid. Such dispersions are stable for 10–15 days with little or no chemical changes and are suitable for coating purposes or for extrusion into a precipitating bath.

Gelatin gels exist over only a small temperature range, the upper limit being the melting point of the gel, which depends on gelatin grade and concentration (but is typically less than 35°C) and the lower limit the freezing point at which ice crystallizes. The upper melting point is below human body temperature, a factor which is important for mouth feel of foods produced with gelatin [2].

Mechanical properties of gelatin gels (for example the gel strength, which is quantified using the Bloom test) are very sensitive to temperature variations, previous thermal history of the gel, and time. The viscosity of the gelatin/water mixture increases with concentration and when kept cool (≈ 4°C).

PRODUCTION OF GELATIN: The worldwide production amount of gelatin is about 375,000 tons per year (roughly 827 million lb). On a commercial scale, gelatin is made from by-products of the meat and leather industry. Recently, fish by-products have also been considered because they eliminate some of the religious obstacles surrounding gelatin consumption [2]. Gelatin is derived from pork skins, pork, horses, and cattle bones, or split cattle hides [3]. The raw materials are prepared by different curing, acid, and alkali processes which are employed to extract the dried collagen hydrolysate. These processes[4] may take up to several weeks, and differences in such processes have great effects on the properties of the final gelatin products [5].

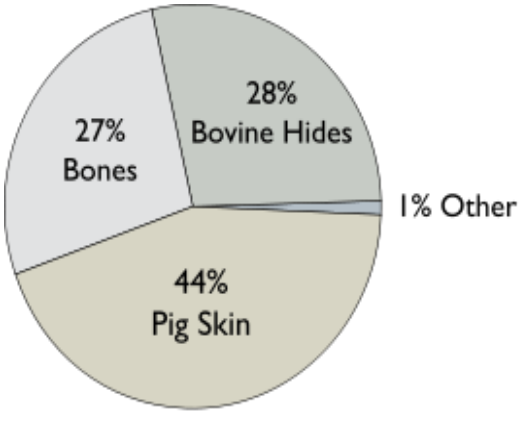
Gelatin can also be prepared in the home. Boiling certain cartilaginous cuts of meat or bones will result in gelatin being dissolved into the water. Depending on the concentration, the resulting stock (when cooled) will naturally form a jelly or gel. This process is used for aspic.

While there are many processes whereby collagen can be converted to gelatin, they all have several factors in common. The intermolecular and intramolecular bonds which stabilize insoluble collagen rendering it insoluble must be broken, and the hydrogen bonds which stabilize the collagen helix must also be broken[1]The manufacturing processes of gelatin consist of three main stages:

1. Pretreatments to make the raw materials ready for the main extraction step and to remove impurities which may have negative effects on physiochemical properties of the final gelatin product,

2. The main extraction step, which is usually done with hot water or dilute acid solutions as a multi-stage extraction to hydrolyze collagen into gelatin, and finally,

3. The refining and recovering treatments including filtration, clarification, evaporation, sterilization, drying, rutting, grinding and sifting to remove the water from the gelatin solution, to blend the gelatin extracted, and to obtain dried, blended and ground final product.



Materials Used in Gelatin Production

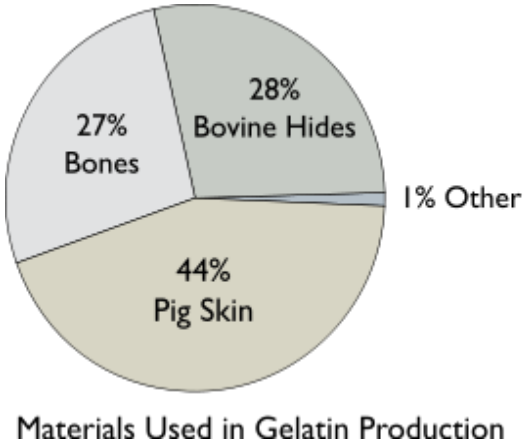


FIG. 1: MATERIALS USED IN GELATIN PRODUCTION

Medical and Nutritional properties: Although gelatin is 98-99% protein by dry weight, it has less nutritional value than many other complete protein sources. Gelatin is unusually high in the non-essential amino acids glycine and proline (i.e., those produced by the human body), while lacking certain essential amino acids (i.e., those not produced by the human body). It contains no tryptophan and is deficient in isoleucine, threonine, and methionine. The approximate amino acid composition of gelatinis: glycine 21%, proline 12%, hydroxyproline 12%, glutamicacid10%, alanine 9%, arginine 8%, aspartic acid 6%, lysine 4%, serine 4%, leucine 3%, valine 2%, phenylalanine 2%, threonine 2%,isoleucine 1%, hydroxylysine 1%, methionine and histidine <1%and tyrosine <0.5%.

These values vary, especially the minor constituents, depending on the source of the raw material and processing technique [6]. Several Russian researchers offer the following opinion regarding certain peptides found in gelatin: "gelatin peptides reinforce resistance of the stomach mucous tunic to ethanol and stress action, decreasing the ulcer area by twice" [7].

Gelatin is also a topical haemostatic. A piece of gelatin sponge of appropriate size is applied on bleeding wound, pressed for some time and tied in bandage. Haemostatic action is based on platelets damage at the contact of blood with gelatin, which activates the coagulation cascade. Gelatin also causes a tamponading effect - blood flow stoppage into a blood vessel by a constriction of the vessel by an outside force [8].

Gelatin has also been claimed to promote general joint health. A study at Ball State University sponsored by Nabisco, the former parent company of Knox gelatin, found that gelatin supplementation relieved knee joint pain and stiffness in athletes [9].

It has been claimed that oral gelatin consumption has a beneficial therapeutic effect on hair loss in both men and women [10, 11, 12, 13, 14, 15, 16, 17]. In addition there are scientific publications that present evidence that consumption of oral gelatin has beneficial effect for some fingernail changes and diseases [18, 19, 20, 21, 22].

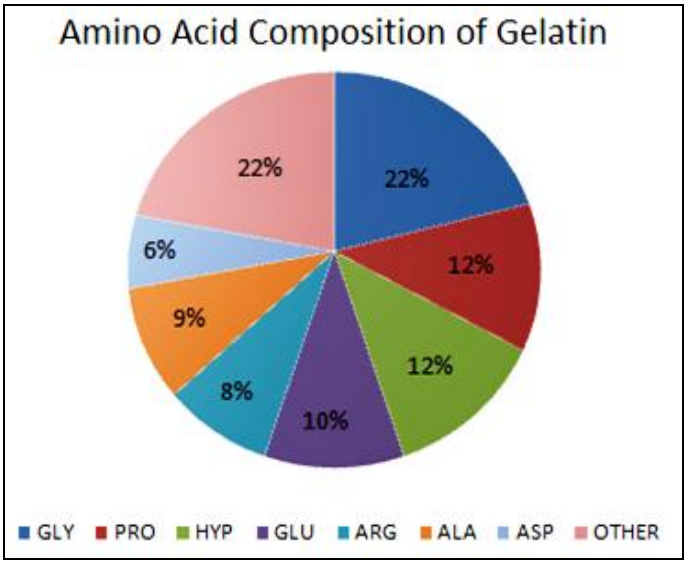


FIG. 2: AMINO ACID COMPOSITION OF GELATIN

NANOPARTICLES: Over the past three decades, there has been a considerable research interest in the area of developing drug delivery using nanoparticles (NPs) as carriers for small and large molecules. Targeting delivery of drugs to the diseased lesions is one of the most important aspects of drug delivery system. Various polymers have been used in the formulation of nanoparticles for drug delivery research to increase therapeutic benefit, while minimizing side effects.

The concept of drug targeting and controlled drug delivery is used in attempts to improve the therapeutic index of drugs by increasing their localization to specific organs, tissues or cells and by decreasing their potential toxic side effects at normal sensitive sites [23]. Controlled drug delivery involves the association of a drug with a carrier system, thereby allowing modulation of the pharmacokinetic properties and biodistribution of

the drug. Different nanosized carriers, such as nanoparticles [24], polymeric micelles [25], liposomes [26], surface-modified nanoparticles [27] and solid lipid nanoparticles [28], have been developed and suggested for achieving these goals. Though liposomes have been used as potential carriers with unique advantages including protecting drugs from degradation, properties like targeting to site of action and reduction of toxicity also have been explored. On the other hand, nanoparticles possess certain advantages over the liposomal delivery systems, such as greater stability during storage, stability *in vivo* after administration and ease of scale-up during manufacture [29].

The advantages of using nanoparticles as a drug delivery system include the following [30]:

1. Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration.

2. They control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects.

3. Controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents. Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction; this is an important factor for preserving the drug activity.

4. Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance.

5. The system can be used for various routes of administration including oral, nasal, parenteral, intraocular etc.

Protein Nanoparticles: The most important advantage of colloidal drug carrier systems is the possibility of drug targeting by a modified body distribution as well as the improvement of the cellular uptake [31] of a number of substances. As a result undesired toxic side effects of the free drug can be avoided, for example with methotrexate [32].

Protein nanoparticles can be utilized for the pulmonary delivery of protein therapeutics or can be incorporated into biodegradable polymer microspheres/nanospheres for controlled release depot or oral delivery. No Protein nanoparticles can be utilized for the pulmonary delivery of protein therapeutics or can be incorporated into biodegradable polymer microspheres/nanospheres for controlled release depot or oral delivery. Nowadays active research is focused on the preparation of nanoparticles using proteins like albumin, gelatin, gliadin and legumin.

Gelatin Nanoparticle: Gelatin is a natural polymer derived from collagen and is commonly used for pharmaceutical and medical applications, because of its biodegradability and biocompatibility in physiological environments [33].

Gelatin is one of the protein materials that can be used for the production of nanoparticles. In terms of nanopharmaceutics, gelatin was already considered as interesting biodegradable base material in the early days of particle development[34]. The interest was based on the facts that gelatin is biodegradable, non-toxic, easy to crosslink and to modify chemically and has therefore an immense potential to be used for the preparation of colloidal drug delivery systems such as microspheres and nanoparticles [35 36].

Fortunately, formulations containing gelatin in the outer layer (hard and soft gelatin capsules) are prone to inter or intramolecular cross-linking of gelatin with time, temperature and humidity. Because of this tendency, the very use of gelatin in pharmaceutical formulations has been put to question [37]. On the other hand, the material is used widely despite efforts to replace it with other substances [38].

Addition of a chemical cross-linker like glutaraldehyde, gives gelatin stability, shape and a raised circulation time *in vivo* as compared to unmodified particles [39 40], and release is a function of cross-linking density of these nanoparticles.

This structural change improves the performance, properties and characteristics of gelatin like insolubility at high temperatures, reduced swelling in water and less permeability to cell membranes [41].

Different types of Gelatin: Two different gelatins, A and B with different isoelectric points (IEP), are formed following either acid or base hydrolysis, respectively [42]. Gelatin type A is derived from acid processed collagen, while type B is obtained by alkaline collagen treatment, resulting in a difference in isoelectric points, being 7 – 9 for gelatin type A and 4 – 5 for gelatin type B.

Characteristic features of gelatin are the high content of the amino acids glycine, proline (mainly as hydroxyproline) and alanine. Gelatin molecules contain repeating sequences of glycine, proline and alanine amino acid triplets, which are responsible for the triple helical structure of gelatin[43]. The primary structure of gelatin offers many possibilities for chemical modification and covalent drug attachment. This can be done either within the matrix of the particles or on the particle surface only[44]. In the first case, chemical modifications have to be done to the gelatin macromolecules before nanoparticles are formed, while in the latter case the particle surface is used [45]. These properties, combined with the high potential of nano-sized delivery systems make gelatin-based nanoparticles a promising carrier system for drug delivery.

Preparation and characterization of nanoparticles: The preparation of biodegradable nanoparticles was achieved by the two step method called desolvation.

Gelatin type A 175 bloom: Gelatin type A (0.25 g) from porcine skin (175 Bloom Sigma-Aldrich, Steinheim, Germany) was dissolved in 25 ml water and heated below 40°C along with magnetic stirring. About 25 ml of acetone was added to the gelatin for complete desolvation and rapid sedimentation. The precipitate formed in the sample was redissolved in 25 ml water under heating and the pH was adjusted to 2.5. Gelatin was again desolvated by addition of 25 ml acetone. After 10 min of stirring, 500 µl of glutaraldehyde (8% v/v) and glutaraldehyde (10% v/v) was added to 25 ml of the gelatin sample for cross-linking the molecules. After 30 min of stirring, 500 mg L-cysteine was added. The sample was centrifuged for 2 min to remove the cysteine and purified three-fold by centrifugation for 12 min at 10,000 rpm and redispersion in acetone/water mixture (30/70). The sample obtained after last dispersion was magnetically stirred with slight warming to remove the acetone. The particle size was measured using dynamic light scattering to be 300 nm at both 8% (v/v) and 10% (v/v) and later by AFM.

Gelatin type B 75 bloom: About 0.25 g gelatin type B 75 bloom was dissolved in 25 ml water and about 25 ml acetone was added for completely desolvating and sedimenting the gelatin molecules. The pH was adjusted to 8.76 in the gelatin samples. The gelatin was again desolvated by adding acetone about 25 ml and then 500 µl $CaCl_2$ (1 M) was added to cross-link the particles by magnetic stirring. The gelatin nanoparticles were made free of acetone by evaporation method. The particle size was determined by dynamic light scattering to be 110 nm at pH 8.76 as well as AFM.

Preparation Methods: A lot of available macromolecules are used in preparation of nanoparticle. These macromolecules consist of proteins such as albumin, gelatin, legumin, vicillin and polysaccharides such as alginate or agarose. These substances have extensive usage in preparation of biomaterial because of their natural properties such as biodegradability and biocompatibility.

Among of above mentioned macromolecules, albumin and gelatin have been used widely. There are two basic methods for preparation of nanoparticles:

1. **Emulsification method:** Initially, its method was set forth by Scheffel and his coworkers (1972) in order to prepare albumin sphere to a high volume of pre-heated oil (over 120°C) drop by drop. This process will result a rapid evaporation of existed water and albumin irreversible destruction. This process will also cause formation of nanoparticles. The resulted suspension was put into cold- ice bath.

2. **Desolvation method:** The disadvantage of the emulsion methods for particles preparation is the need for applying organic solvents, for the removal both of the oily residues of the preparation process and of surfactants required for emulsion stabilization. Therefore, as an alternative method for the preparation of protein nanoparticles a desolvation process derived from the coacervation method of microencapsulation was developed. In this method, particles in aqueous will formed by coacervation process and later on will be stabilized by cross linking agent such as glutaraldehyde.

   A new method was offered by Marty and his coworkers (1978) the foundation of this method was using a desolvation factor such as natural salts or alcohol which should be added to protein solution slowly. By adding this factor, protein third structure will changed. When we have reached to a certain level of a desolvation, protein clump will be formed. In the next stage, nanoparticles will result by this polymerization clump crosslinkage with a chemical factor that is glutaraldehyde[46]. In order to obtain dispersed nanoparticles not in a mass form, we must stop the system before particles start to accumulate. System turbidity will be increased owing to this desolvation factor. Particles accumulation will form alone with increasing system's turbidity. In order to stop such kind of accumulation and creating ideal nanodispersion, we must use a resolvating agent.

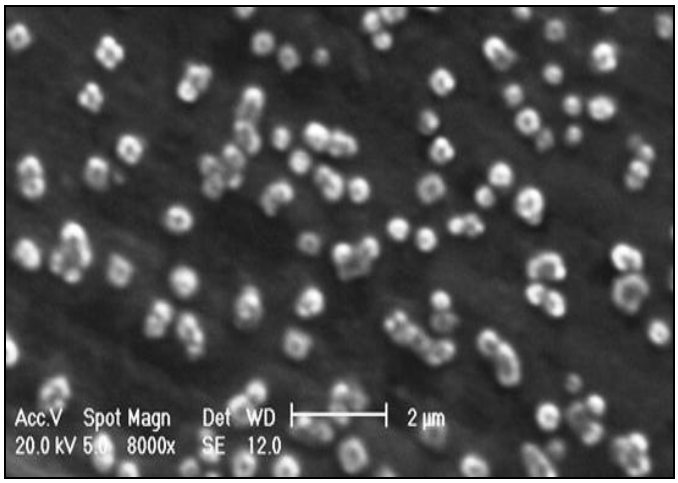


FIG. 3: SCANNING ELECTRON MICROGRAPHS OF NANOPARTICLES PREPARED USING 175-BLOOM GELATIN (Zu Lu, Teng-Kuang Yeh, Max Tsai, *et al*, 2004).

Loading and Release:

Drug loading: Drug may be bound to nanoparticles either

(i) By polymerization in the presence of the drug- in most cases in the form of a solution (incorporation method) or

(ii) By adsorbing the drug after the formation of nanoparticles by incubating them in the drug solution.

Depending on the affinity of the drug to the polymer, the drug will be surface adsorbed, dispersed in the particle polymer matrix in the form of a solid solution [47], or solid dispersion, or in some case, the drug may be covalently bound to the polymer. Therefore it is apparent that a large amount of drug can be entrapped by the incorporation method when compared to the adsorption [48]. The macromolecule or protein shows greatest loading efficiency when it is loaded at or near its isoelectric point when it has minimum solubility and maximum adsorption.

The drug loading of the nanoparticles is generally defined as the amount of drug bounded per mass of polymer (usually moles of drug per mg polymer or mg drug per mg polymer) it could also be given on a percentage basis based on the polymer.

Determination of drug entrapment: Binding of drug to the protein nanoparticles was measured by centrifuging part of the particle suspension. For determination of drug entrapment, the amount of drug present in the clear supernatant after centrifugation was determined (*w*) by UV-spectrophotometry, fluorescence spectrophotometer or by a validated HPLC method. A standard calibration curve of concentration versus absorbance was plotted for this purpose. The amount of drug in supernatant was then subtracted from the total amount of drug added during the formulation (*W*). Effectively, (*W- w*) will give the amount of drug entrapped in the pellet. Then percentage entrapment is given:

$$\text{Drug entrapment (\%)} = \frac{(W - w)}{W} \times 100$$

Finally, the encapsulation efficiency refer to the ratio of the amount of drug encapsulated/absorbed to the total (theoretical) amount of drug used, with regard to the final drug delivery system of the dispersion of nanoparticles.

Drug release: Release profiles of the drugs from nanoparticles depend upon the nature of the delivery system. In the case of nanospheres, drug is uniformly distributed/dissolved in the matrix and the release occurs by diffusion or erosion of the matrix. If the diffusion of the drug is faster than matrix degradation, then the mechanism of drug release occurs mainly by diffusion, otherwise it depends upon degradation.

Many theoretically possible mechanisms may be considered for the release drug from protein nanoparticles:

(a) Liberation due to polymer erosion or degradation,

(b) Self- diffusion through pores,

(c) Release from the surface of the polymer,

(d) Pulsed delivery initiated by the application of an oscillating magnetic or sonic field [49].

In many case, some of these processes may coexist, so that the distinction between the mechanisms is not always trivial. When drug release occurs by a self-diffusional process, a minimum drug loading is necessary before drug release is observed. This is easy to under- stand since the process involves diffusion through aqueous channels created by the phase separation and dissolution of the drug itself. This mechanism rarely occurs with drug loaded nanoparticles since, as explained before, the encapsulation efficiency of most drugs is generally too low. Infact, release from the surface and erosion or bulk polymer degradation is usually the most important processes affecting the liberation of drug from nanoparticles.

Methods for quantifying drug release *in vitro* are:

a) Side-by-side diffusion cells with artificial or biological membranes;

b) Equilibrium dialysis technique;

c) Reverse dialysis sac technique;

d) Ultracentrifugation;

e) Ultrafiltration; or

f) Centrifugal ultrafiltration technique [50].

Gelatin Nanoparticle for delivery of Amphotericin B and other Lipophilic drugs: Our aim in this review to develop a nanoparticulate carrier of amphotericin B (AmB) for controlled delivery as well as reduced toxicity. Amphotericin B is a polyenen antifungal drug, often used intravenously for systemic fungal infections. Nanoparticles of different gelatins (GNPs) (type A or B) can be prepared by two-step desolvation method and optimized for temperature, pH, amount of cross-linker, and theoretical drug loading. AmB-loaded GNPs can be characterized for size, polydispersity index (PI), shape, morphology, surface charge, drug release, and hemolysis.

Some other lipophilic drugs like propofol causes severe pain on intravenous injection and if it is formulated with Gelatin nanoparticles can lead to an another support media for such drugs. This can also helpful in minimizing the pain at site.

CONCLUSION: Drug targeting technology represents one of the frontier areas of science, which involves a multidisciplinary scientific approach with great potential to positively impact our health care. Drug targeting of a therapeutic moiety to the site of drug action within the body offers numerous advantages compared to the use of conventional dosage forms, including improved efficacy, reduced toxicity and overcoming drug resistance.

Protein nanoparticles hold promise as drug delivery systems for parenteral, peroral and ocular administration as well as adjuvant for vaccines. Due to their greater stability and due to their easier manufacturing they offer advantages over other colloidal carriers such as liposomes and cell ghosts.

These properties, combined with the high potential of nanosized delivery systems make gelatin-based nanoparticles a promising carrier system for drug delivery.

In terms of nanopharmaceutics, gelatin was already considered as interesting biodegradable base material in the early days of particle development [51]. The interest was based on the facts that gelatin has been known for its low immunogenicity for many years and is administered intravenously since it is an ingredient of various registered blood substitutes. One advantage offered by the amino acid side-chains of the gelatin matrix molecule is the option of multiple further modifications. This could be used for coupling of ligands to improve targeting diseased tissues, to enhance specific cellular uptake or affect intracellular distribution [52].

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How to cite this article:
Singh K and Mishra A: Gelatin Nanoparticle: Preparation, characterization and application in Drug Delivery. Int J Pharm Sci Res 2014; 5(6): 2149-47.doi: 10.13040/IJPSR.0975-8232.5(6).2149-57