doi.org/10.36721/PJPS.2020.33.1.REG.221-228.1

MINI-REVIEW

Opportunities and challenges in the techniques used for preparation of gelatin nanoparticles

Saeed Ahmad Khan*
Department of Pharmacy, Kohat University of Science and Technology, Kohat, Pakistan

Abstract: Gelatin nanoparticles have attracted substantial interest as drug delivery vehicle. The presence of many functional groups in gelatin structure offer several opportunities for cross linking and targeting. However, there has always been constraints in preparation of stable and mono disperse gelatin nanoparticles avoiding aggregation during cross-linking; hence, different approaches have been adopted for preparation of stable nanoparticles from gelatin. This review examines the techniques employed for preparation of gelatin nanoparticles and in particular the advantages and disadvantages are discussed. Further, this review also presents a direction for future research in circumventing the issue of crosslinking in gelatin nanoparticles.

Keywords: Protein nanoparticles, desolvation, coacervation, nanoprecipitation

INTRODUCTION

"Gelatin" is derived from latin word "gelatus", which means "stiff, frozen". Frenchman named Denis Papin (1647-1712) was the first to obtain gelatinous mass by boiling animal bones (This, 2010). Recently, gelatin, is the term used for purified proteins obtained from collagen (Chauhan *et al*., 2012, Foox and Zilberman, 2015). The source of collagen is mainly bones and skin of animals. However, fishes are also used for isolation of gelatin, now a days (Gómez-Guillén *et al*., 2011, Karim and Bhat, 2009, Choi and Regenstein, 2000). There are two different types of gelatin available commercially (type A & type B). The hydrolysis method determines the type of gelatin obtained from collagen. For instance, acid hydrolysis does not affect the amide groups of glutamine and asparagine, which results in an higher isoelectric point (IEP), i.e., 7-9 (Type A gelatin) (Patel *et al*., 2008), while alkaline hydrolysis forms aspartate and glutamate from asparagine and glutamine, respectively. Hence imparting more carboxyl groups, due to which the IEP is lowered, i.e., 4.5-6.0 (Type B gelatin) (Ninan *et al*., 2010).

Gelatin does not dissolve in cold water. However, at temperature higher than 35-40°C, gelatin dissolves in water, which is termed as "sol". (Tanaka, 2003). At colder conditions, gelatin-water system changes into a three dimensional "gel", induced by intra-molecular hydrogen bonding, at concentrations >1% (Gao *et al*., 2014, Parker and Povey, 2012).

Commercially, gelatin is available in different bloom strength (Choi and Regenstein, 2000). Bloom strength is the weight required in gram to push the surface of a gel (prepared with 6.67% w/w, developed at 10°C for 16-18h), 4 mm inward without breaking the surface, using a cylindrical plunger (0.5 inch in diameter). Bloom strength is affected by the α- and β-chain components in gelatin, which depends upon molecular weight of gelatin (Mariod and Adam, 2013). It is a critical parameter to estimate the mechanical properties of gelatin based devices.

Gelatin has a versatile range of applications. For instance, in food industry, gelatin is used as gelling agent and coating agent (Cheng *et al*., 2014). Biomedical field has immense utilization of gelatin in tissue engineering (Olad and Farshi Azhar, 2014, Chhabra *et al*., 2014). Pharmaceutically, gelatin is used in capsules, emulsions, gels and vaccines (Hoffmann and Reger, 2014, Sovilj *et al*., 2013, Pal *et al*., 2013, Buchweitz *et al*., 2013, Jamil *et al*., 2014, Boks *et al*., 2007). Because of intuitive properties, gelatin has gained novel interests in nanoparticulate drug delivery systems (Azarmi *et al*., 2006 , Rajan and Raj, 2013, Azimi *et al*., 2014, Lu *et al*., 2015, Joachim *et al*., 2014). Nanoparticles have shown huge promises in delivery systems. Intracellular uptake is favored because of submicron size compared to larger carriers (Daear *et al*., 2015).

Gelatin nanoparticles (GNPs)

Gelatin nanoparticles were explored in 1980s for the first time (Oppenheim *et al*., 1978, Victoria and Speiser, 1975). Gelatin nanoparticles offer biodegradability and biocompatibility, which are important attributes for any material to be applicable in drug delivery and it's *in vivo* application (Panyam and Labhasetwar, 2003, Prow *et al*.). Drugs can be loaded inside nanoparticles matrix or

**Corresponding author:* e-mail: saeedkhan663@hotmail.com

attached to the surface of nanoparticles. As evident from the chemical structure of gelatin, it offers many reactive functional groups, which provides many prospects for cross linking and surface modification (Balthasar *et al*., 2005, Azarmi *et al*., 2008, Gupta *et al*., 2014). These virtuous properties make gelatin nanoparticles attractive drug delivery systems (Coester *et al*., 2006, Elzoghby, 2013).

Preparation techniques of GNPs

Various researchers have adopted various techniques, for preparation of gelatin nanoparticles. All these techniques have many distinct advantages and disadvantages, summarized in table1. Some of the common techniques are, emulsion/solvent evaporation (Bajpai and Choubey, 2006, Cascone *et al*., 2002), reverse phase preparation (Gupta *et al*., 2004), inverse miniemulsion (Ethirajan *et al*., 2008), coacervation (Leo *et al*., 1997a, Leo *et al*., 1997b, Leo *et al*., 1999, Oppenheim *et al*., 1978, Victoria and Speiser, 1975), desolvation (Mohanty and Bohidar, 2003, Vandervoort and Ludwig, 2004, Kaul and Amiji, 2002, Kommareddy and Amiji, 2005, Azarmi *et al*., 2006, Coester *et al*., 2000), and more recently nanoprecipitation (Lee *et al*., 2010, Khan and Schneider, 2014, Khan *et al*., 2015), which are discussed in detail as follows.

Simple emulsion method

Emulsion method was introduced by Cascone *et al*., (Cascone *et al*., 2002). The technique rely on emulsification and homogenization of gelatin aqueous solution in poly(methylmethacrylate) (PMMA) dissolved in chloroform/toluene (schematically shown in fig. 1). The nanoparticles are subsequently cross linked with glutaraldehyde. The size range reported was 100-200 nm. However, the size of nanoparticles depend on the homogenization speed, since the emulsion droplet are affected by the energy applied for size reduction (Bajpai and Rajpoot, 2001).

The chemical architecture and behavior of nanoparticles was found to be affected by the concentration of gelatin and amount of cross linker used. Moreover the type of gelatin also influences the loading and release behavior of nanoparticles, for instance, higher release of drug is observed when type B gelatin was used(Bajpai and Choubey, 2005, Bajpai and Choubey, 2006).

The use of PMMA and solvents like chloroform and toluene are some of the constraint in emulsion technique (Horowitz *et al*., 1988). Furthermore, the polydispersity index (PDI) of the produced nanoparticles was not reported. Therefore, the stability and reproducibility of nanoparticles produced by emulsion method could not be assessed.

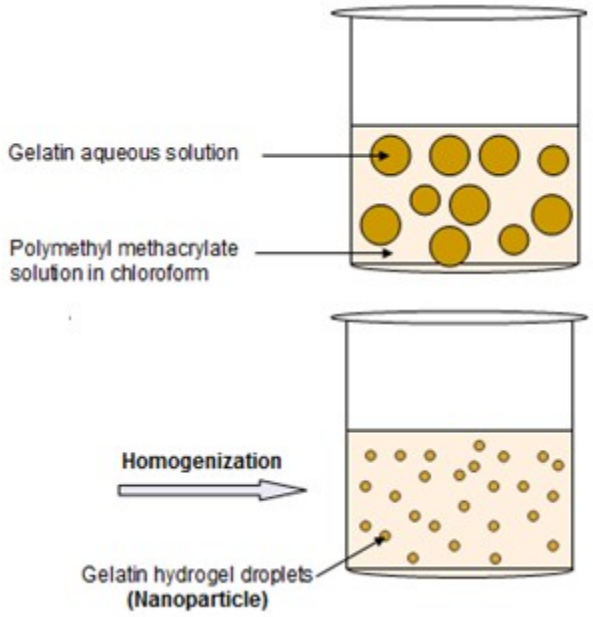

Fig. 1: Schematic representation of gelatin nanoparticles preparation by simple emulsion technique (Khan, 2014)

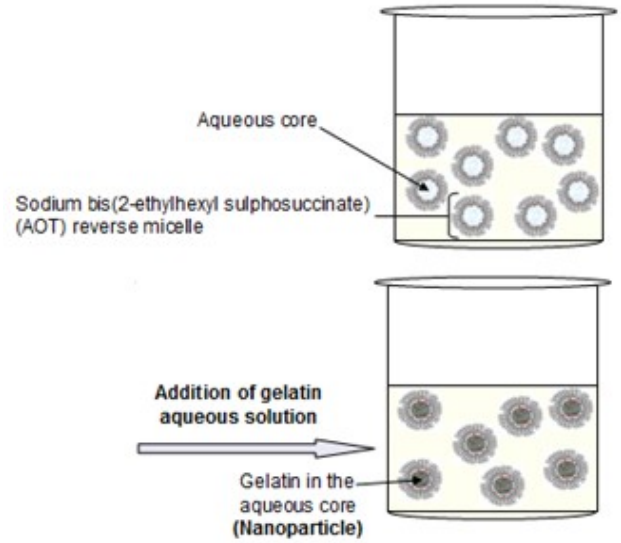

Fig. 2: Schematic representation of gelatin nanoparticles preparation by reverse phase preparation technique (Khan, 2014).

Reverse phase preparation technique

This technique rely on formation of reverse micelles of surfactant bis(2-ethylhexyl sulphosuccinate) in-hexane (Gupta *et al*., 2004). Gelatin solution is confined in the aqueous core of reverse micelles. Subsequent cross linking of gelatin converts the aqueous phase to gelatin nanoparticles, schematically shown in figure 2. This technique may offer good loading efficiency of hydrophilic drugs, since there will be minimal distribution of drug to the nonpolar external medium. Besides, the small size of particles produced, i.e., around 40 nm, is distinct advantage of this technique. Nevertheless,

removal of n-hexane and surfactant AOT is a serious challenge, since harsh washing steps affect the size and morphology of particle (Janes *et al*., 2001). Though, in one report calcium chloride solution was used to precipitate AOT from nanoparticles suspension as calcium diethylhexyl sulphosuccinate ($Ca(DEHSS)_2$) (Banerjee *et al*., 2002). However, the precipitating cake may contain nanoparticles, hence decreasing the nanoparticle yield.

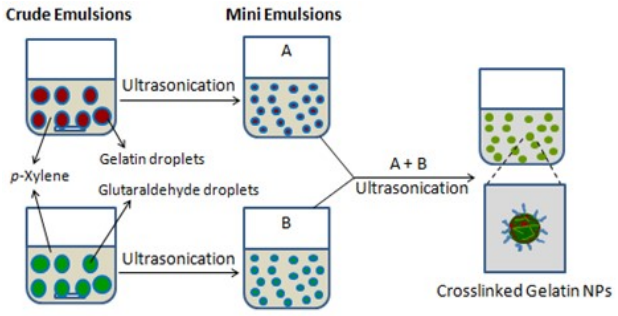

Fig. 3: Schematic representation of gelatin nanoparticles preparation by inverse miniemulsion technique (Khan, 2014).

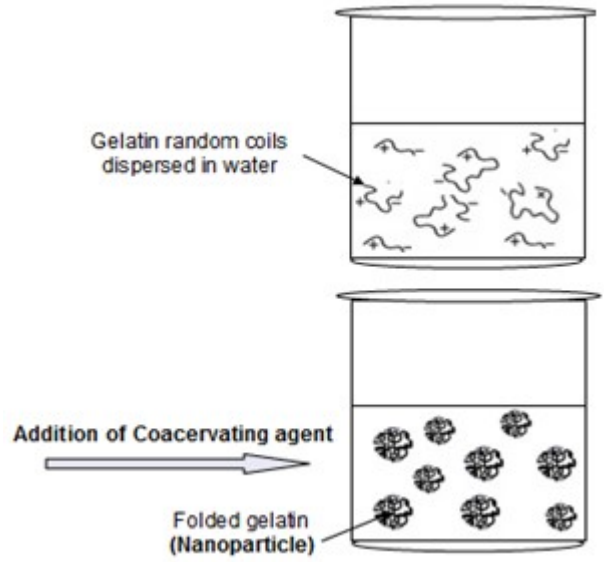

Fig. 4: Schematic representation of gelatin nanoparticles preparation by coacervation technique (Khan, 2014).

Inverse miniemulsion technique

According to this technique, gelatin aqueous solution and glutaraldehyde aqueous solution is emulsified in *p*-Xylene (i.e. emulsions A and B). Miniemulsions are formed by sonication, which hare subsequently mixed to fuse the droplets using sonication in an ice bath to produce cross linked gelatin nanoparticles, as shown schematically in fig. 3. The principle of nanoparticles formation by inverse miniemulsion and fission phenomena (Landfester, 2001, Ethirajan *et al*., 2008, Sarika *et al*., 2015). This technique may also offer good loading for hydrophilic drugs, since the continuous phase is a nonpolar solvent. However, this technique yield nanoparticles with higher polydispersity, which may not be desired in many cases where reproducibility is critical. Moreover, repeated sonication may also negatively affect some of the loaded drugs such as proteins or peptides. Besides, removal of *p*-Xylene requires extensive washing of nanoparticles (hence, affecting the size, morphology and loading efficiency) are some of the main disadvantages of inverse miniemulsion procedure.

Coacervation technique

This technique is based on addition of coacervating agent to the gelatin solution (Oppenheim *et al*., 1978). Coacervating agent separates the system to two liquid-liquid phases, i.e., coacervate phase and solvent phase. The coacervate phase is gelatin rich dense phase. Typically, coacervating agent dehydrates gelatin molecules. The gelatin molecules in tern tend to roll up as nanoparticles (Leo *et al*., 1997a, Victoria and Speiser, 1975), as shown in fig. 4. The nanoparticles are cross linked with glutaraldehyde. Nevertheless, GNPs by sodium sulfate induced coacervation have higher polydispersity and larger sizes because of aggregation (Leo *et al*., 1997b, Leo *et al*., 1999). The aggregation process could not be prevented by using tween 20 as stabilizer (Oppenheim, 1981).

Desolvation technique

Desolvating agent (e.g., alcohol) is added to gelatin aqueous solution at controlled pH under continuous stirring. Typically nanoparticles are produced when composition of solutions changes to 65% hydro-alcoholic solution (Mohanty and Bohidar, 2003, Gupta *et al*., 2006, Mohanty *et al*., 2005, Mohanty *et al*., 2006). Nanoparticles are crosslinked using glyoxal (Kaul and Amiji, 2005b) or glutaraldehyde (Vandervoort and Ludwig, 2004). Nanoparticles produced by desolvation are in the range of 200-500nm (Kaul and Amiji, 2002, Kaul and Amiji, 2004, Kaul and Amiji, 2005b, Kaul and Amiji, 2005a, Kommareddy and Amiji, 2005, Kommareddy and Amiji, 2007b, Kommareddy and Amiji, 2007a, Vandervoort and Ludwig, 2004, Ofokansi *et al*., 2010). However, polydispersity and colloidal stability are serious issues in the nanoparticles produced by desolvation technique (Coester *et al*., 2000). Besides, a slight excess of ethanol may induce complete phase separation, which challenges the reproducibility (Zwiorek, 2007). Desolvation of gelatin aqueous solution can be influenced by many factors, e.g., temperature, pH, composition of ethanol and molecular weight of gelatin (Farrugia and Groves, 1999). The ethanol induced aggregation process is exclusively investigated by Mohanty *et al* (Mohanty *et al*., 2005, Mohanty and Bohidar, 2003, Mohanty *et al*., 2006).

The stability issues in conventional desolvation was addressed by Coester *et al*. in 2000 (Coester *et al*., 2000).

Table 1: Advantages and disadvantages of different preparation techniques for gelatin nanoparticles (Khan, 2014)

Preparation Method	Size (nm)	Positive aspects	Negative aspects	Reference
Desolvation	200-500	Simple procedure	Agglomeration, Polydispersity and stability issues.	(Kaul and Amiji, 2002)
Two step desolvation	100-300	Homogeneous size	Narrow pH range, Specific molecular weight requirement.	(Coester et al., 2000, Lee et al., 2013)
Emul./solvent evaporation	100-200	Homogeneous size	Difficult procedure of washing for nanoparticles isolation	(Cascone et al., 2002)
Reverse phase preparation	40	Small size	Nanoparticles isolation	(Gupta et al., 2004)
Inverse miniemulsion	150-200	No special gelatin needed	High polydispersity and difficult procedure	(Ethirajan et al., 2008)
Nanoprecipitation	200-350	Simple and straight forward procedure	High amount of surfactant needed.	(Khan and Schneider, 2013a)

In this context, two-step desolvation technique was introduced. In two-step desolvation technique the low molecular weight portion of gelatin is discarded in first desolvation step. The high molecular weight portion (precipitate) is dissolved in water, pH is adjusted farther from isoelectric point and subsequently re-desolvated using acetone. Two step desolvation is the most common techniques for preparation of GNPs (Zwiorek, 2007, Azarmi *et al*., 2006 , Zillies, 2007, Narayanan *et al*., 2013, Karthikeyan *et al*., 2013, Khan and Schneider, 2013b, Karthikeyan *et al*., 2015, Hamarat Sanlier *et al*., 2015, Fuchs, 2010). It yields particles in the size range of 100-300 nm. However, this technique requires a narrow pH range before the second desolvation step i.e., 2.3 to 4.0. Since type A gelatin was employed in this technique, therefore, the aforementioned pH values are quite far from isoelectric point (IEP), i.e., pH 7-9 for gelatin type A. pH values far from IEP is strict requirement of two step desolvation, because the net charge on gelatin is dependent on solution pH. Conditions where pH was adjusted above 4.0 lead to aggregation and precipitation, because at this pH the positive charge on gelatin is weaker to induce inter-particle repulsion. Therefore, severe acidic conditions are critical for production of homogeneous small size particles (Zwiorek, 2007). This makes it unrealistic in conditions where low pH can negatively affect the activity of drugs, such as proteins (Khan and Schneider, 2013b).

Besides, the temperature, cross linker amount, and speed of acetone addition are important factors affecting nanoparticles size and homogeneity (Zwiorek, 2007). For instance, Temperature before first desolvation step induced significant change in size and polydispersity of nanoparticles. More specifically, gelatin solutions with 35°C and 60°C before first desolvation step yielded larger but monodisperse particles. However, temperature before second desolvation did not affect the resulting particles significantly (Zwiorek, 2007). With regards to crosslinker amount, the size of nanoparticles is inversely related to crosslinker amount, i.e., smaller sizes are produced with higher crosslinker amount (Zwiorek, Cascone *et al*., 2002). Moreover, rate of acetone additions before second desolvation is not decisive upto 5mL/min. However, above this limit, particles tend to aggregate. Thus the size and polydispersity increases substantially (Zwiorek *et al*., 2004).

Farrugia & Groves reported that higher fraction of the high molecular weight fraction is necessary for formation of GNPs in single desolvation step (Farrugia and Groves, 1999). However, Zillies *et al*., (Zillies, 2007) negated this assumption. They thoroughly investigated different fractions of gelatin after each desolvation step, using Asymmetrical Flow Field Flow Fractionation (AF4) and Multi Angle Light Scattering (MALS). It was found that gelatin containing large quantity of high molecular weight component (higher than 104 kDa) could not produce nanoparticles by single desolvation. Rather decrease in very low molecular weight components (lower than 65 kDa) is important for homogeneity and stability of GNPs (Zillies, 2007, Zwiorek, 2007). More specifically, gelatin nanoparticles can be formed by single desolvation, if the gelatin used contain less than 20% (w/w) of low molecular weight peptides (lower than 65 kDa). Thus, the unavoidable first desolvation step, if ordinary gelatin is used, may compromise the nanoparticles yield. Nevertheless, two-step desolvation yields homogeneous gelatin nanoparticles. For this reason it is the most widely employed technique for gelatin nanoparticles preparation.

Nanoprecipitation technique

With the intention to produce gelatin nanoparticles without pretreatment of gelatin bulk material, such as changing the native pH of gelatin solution or discarding low molecular weight fraction, Nanoprecipitation

technique was introduced (Lee *et al*., 2010, Lee *et al*., 2012). Nanoprecipitation can be done with two miscible solvents, i.e., solvent and nonsolvent. Polymer is dissolved in solvent and is precipitated in the nonsolvent. Typically, gelatin dissolved in water is added drop wise into ethanol containing stabilizer. The turbulence generated due to diffusion of solvent cause a violent dispersion of solvent phase in nonsolvent, since both are miscible. Consequently, droplets of nano-size are formed from the interface (Rodriguez *et al*., 2004, Fessi *et al*., 1989, Guerrero *et al*., 1998, Khan and Schneider, 2013b). Gelatin molecule present in these droplets aggregate to form nanoparticles stabilized by poloxamer present as stabilizer, schematically shown in fig. 5. Glutaraldehyde is added to obtain stable cross linked nanoparticles (Khan, 2014).

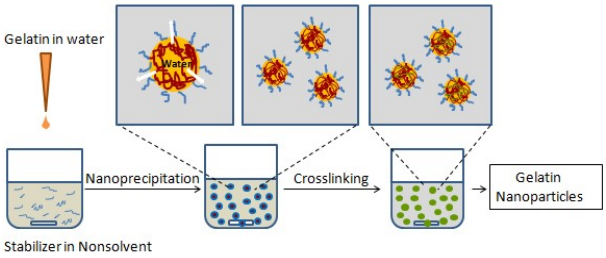

Fig. 5: Schematic representation of gelatin nanoparticles preparation by nanoprecipitation technique (Khan, 2014).

Tunable size of gelatin nanoparticles without changing the intuitive attributes of gelatin is one of the advantage which nanoprecipitation technique offers. However, poloxamer concentration as high as 7% w/v is required for stability of nanoparticles during cross linking. Besides, several other parameters in the nanoparticles preparation process, such as type of stabilizer, nonsolvent type and gelatin concentration are critical for size, homogeneity and colloidal stability of GNPs (Khan and Schneider, 2013a).

Viewpoint and outlook for future work

Gelatin nanoparticles have extensively been investigated as a promising nanocarrier for many drugs. In order to improve mechanical stability and drug release from gelatin nanoparticles, cross linking has always been a mandatory step. However, the generalized reaction of cross linkers with proteins is a critical challenge in application of gelatin particles for the delivery of arbitrary biologicals. Since cross linker not only cross links gelatin but also therapeutic peptides and proteins. Consequently, leading to biological inactivity of proteinaceous drugs. For this reason, alternative approaches need to be sought, in order to circumvent the issue of cross linking. One step in this direction was our study where the structural integrity of GNPs was kept intact by entrapping them in Eudragit® E 100 nanospheres (Khan and Schneider, 2014). For this purpose, nanoprecipitation-emulsion solvent evaporation hybrid technique was employed. The system provides a good opportunity for stabilization of gelatin nanoparticles. However, the efforts are required to make the system more biocompatible by using biodegradable polymers.

REFERENCES

Azarmi S, Huang Y, Chen H, Mcquarriea S, Abramse D, Road W, Finlay W H, Miller G G and Löbenberga, R. (2006). Optimization of a two-step desolvation method for preparing gelatin nanoparticles and cell uptake studies in 143B osteosarcoma cancer cells. *J. Pharm. Pharm.,* **9**(1): 124-132.

Azarmi S, Roa W H and Löbenberg R (2008). Targeted delivery of nanoparticles for the treatment of lung diseases. *Adv. Drug Deliv. Rev.,* **60**(8): 863-875.

Azimi B, Nourpanah P, Rabiee M and Arbab S (2014). Producing Gelatin Nanoparticles as Delivery System for Bovine Serum Albumin. *Iran. Biomed. J.,* **18**(1): 34-40.

Bajpai A and Choubey J (2005). Release study of sulphamethoxazole controlled by swelling of gelatin nanoparticles and drug-biopolymer interaction. *J. Macromol. Sci. Pure and Appl. Chem.,* **42**(3): 253-275.

Bajpai A and Rajpoot M (2001). Release and diffusion of sulfamethoxazole through acrylamide-based hydrogel. *J. Appl. Polym. Sci.,* **81**(5): 1238-1247.

Bajpai A K and Choubey J (2006). In vitro release dynamics of an anticancer drug from swellable gelatin nanoparticles. *J. Appl. Polym. Sci.,* **101**(4): 2320-2332.

Balthasar S, Michaelis K, Dinauer N, Von Briesen H, Kreuter J and Langer K (2005). Preparation and characterisation of antibody modified gelatin nanoparticles as drug carrier system for uptake in lymphocytes. *Biomaterials,* **26**(15): 2723-2732.

Banerjee T, Mitra S, Singh AK, Sharma, RK and Maitra A (2002). Preparation, characterization and biodistribution of ultra fine chitosan nanoparticles. *Int. J. Pharm.,* **243**: 93-105.

Boks RH, Wijers MJ, Hofland J, Takkenberg JJ and Bogers AJ (2007). Low molecular starch versus gelatin plasma expander during CPB: Does it make a difference? *Perf.,* **22**(5): 333-337.

Buchweitz M, Brauch J, Carle R and Kammerer DR (2013). Application of ferric anthocyanin chelates as natural blue food colorants in polysaccharide and gelatin based gels. *Food Res. Int.,* 51: 274-282.

Cascone M, Lazzeri L, Clarmignani C and Zhu Z (2002). Gelatin nanoparticles produced by a simple w/o emulsion as delivery system for methotrexate. *J. Mat. Sci. Mat. Med.,* **13**(5): 523-526.

Nagar P, Chauhan I and Yasir M (2012). Insights into Polymers: Film Formers in Mouth Dissolving Films. *Drug Inv. Today,* **3**: 280-289.

Cheng YH, Hung KH, Tsai TH, Lee, CJ, Ku RY, Chiu AWH, Chiou, SH and Liu C (2014). Sustained delivery

of latanoprost by thermosensitive chitosan–gelatin-based hydrogel for controlling ocular hypertension. *Acta biomat.,* **10**:(10) 4360-4366.

Chhabra H, Gupta P, Verma P J, Jadhav S and Bellare JR (2014). Gelatin–PMVE/MA composite scaffold promotes expansion of embryonic stem cells. *Mat. Sci. Eng.,* **37**(1):184-194.

Choi S S and Regenstein J (2000). Physicochemical and sensory characteristics of fish gelatin. *J. Food Sci.,* **65**: 194-199.

Coester C, Kreuter J, Von Briesen H and Langer K (2000). Preparation of avidin-labelled gelatin nanoparticles as carriers for biotinylated peptide nucleic acid (PNA). *Int. J. Pharm.,* **196**(2): 147-149.

Coester C, Nayyar P and Samuel J (2006). In vitro uptake of gelatin nanoparticles by murine dendritic cells and their intracellular localisation. *Eur. J. Pharm. Biopharm.,* **62**(3): 306-314.

Daear W, Lai P, Anikovskiy M and Prenner E J (2015). Differential Interactions of Gelatin Nanoparticles with the Major Lipids of Model Lung Surfactant: Changes in the Lateral Membrane Organization. *J. Phys. Chem. B,* **119**(17): 5356-5366.

Elzoghby A O (2013). Gelatin-based nanoparticles as drug and gene delivery systems: Reviewing three decades of research. *J. Control. Release,* **172**(3): 1075-1091.

Ethirajan A, Schoeller K, Musyanovych, A, Ziener U and Landfester K (2008). Synthesis and optimization of gelatin nanoparticles using the miniemulsion process. *Biomacromol.,* **9**(9): 2383-2389.

Farrugia C A and Groves M J (1999). Gelatin behaviour in dilute aqueous solution: Designing a Nanoparticulate Formulation. *J. Pharm. Pharmacol.,* **51**: 643-649.

Fessi H, Puisieux F, Devissaguet JP, Ammoury N and Benita S (1989). Nanocapsule formation by interfacial polymer deposition following solvent displacement. *Int. J. Pharm.,* **55**: R1-R4.

Foox M and Zilberman M (2015). Drug delivery from gelatin-based systems. *Expert Opin. Drug Deliv.,* **12**(9):1547-63.

Fuchs S (2010). Gelatin Nanoparticles as a modern platform for drug delivery. Ph.D. dissertation, 2007, Ludwig-Maximilians-Universität München.

Gao M, Liu N, Li Z, Wang W, Li C, Zhang H, Chen Y, Yu Z and Huang Y. (2014). A gelatin-based sol–gel procedure to synthesize the LiFePO4/C nanocomposite for lithium ion batteries. *Solid State Ion.,* **258**: 8-12.

Gómez-Guillén MC, Giménez B, López-Caballero ME and Montero MP (2011). Functional and bioactive properties of collagen and gelatin from alternative sources: A review. *Food Hydrocol.,* **25**: 1813-1827.

Guerrero DQ, Allémann E, Fessi H, Doelker E (1998). Preparation techniques and mechanisms of formation of biodegradable nanoparticles from preformed polymers. *Drug Dev. Ind. Pharm.,* **24**(12): 1113-1128.

Gupta A, Reena and Bohidar HB (2006). Free-energy landscape of alcohol driven coacervation transition in aqueous gelatin solutions. *J. Chem. Phys.,* **125**(5):054904.

Gupta AK, Gupta M, Yarwood S J and Curtis A S (2004). Effect of cellular uptake of gelatin nanoparticles on adhesion, morphology and cytoskeleton organisation of human fibroblasts. *J. Control. Release,* **95**:197-207.

Gupta B, Tummalapalli M, Deopura B L and Alam M S (2014). Preparation and characterization of *in-situ* crosslinked pectin–gelatin hydrogels. *Carbohydr. Polym.,* **106**: 312-318.

Hamarat Sanlier S, Yasa M, Cihnioglu AO Abdulhayoglu M, Yilmaz H and AKG (2015). Development of gemcitabine-adsorbed magnetic gelatin nanoparticles for targeted drug delivery in lung cancer. *Artif. Cells Nanomed. and Biotech.*, **44**(3):943-9

Hoffmann H and Reger M (2014). Emulsions with unique properties from proteins as emulsifiers. *Adv. Colloid and Interface Sci.,* **205**: 94-104.

Horowitz SM, Frondoza CG and Lennox DW (1988). Effects of polymethylmethacrylate exposure upon macrophages. *J. Orthop. Res.,* **6** (6): 827-832.

Jamil R K, Taqavian M, Sadigh Z-A, Shahkarami M-K, Esna-Ashari F, Hamkar R, Hosseini S-M and Hatami A (2014). Evaluation of the thermal stability of a novel strain of live-attenuated mumps vaccine (RS-12 strain) lyophilized in different stabilizers. *J. Virol. Methods* **199**: 35-38.

Janes KA, Calvo P and Alonso MJ (2001). Polysaccharide colloidal particles as delivery systems for macromolecules. *Adv. Drug Deliv. Rev.,* **47**: 83-97.

Joachim E, Kim ID, Jin Y, Kim KK, Lee JK and Choi H (2014). Gelatin nanoparticles enhance the neuroprotective effects of intranasally administered osteopontin in rat ischemic stroke model. *Drug Deliv. Transl. Res.,* **4**(5-6): 395-399.

Karim AA and Bhat R (2009). Fish gelatin: Properties, challenges, and prospects as an alternative to mammalian gelatins. *Food Hydrocol.,* **23**: 563-576.

Karthikeyan S, Hoti SL and Prasad NR (2015). Resveratrol loaded gelatin nanoparticles synergistically inhibits cell cycle progression and constitutive NF-kappaB activation, and induces apoptosis in non-small cell lung cancer cells. *Biomed. Pharmacother.,* **70**: 274-282.

Karthikeyan S, Rajendra Prasad N, Ganamani A and Balamurugan E (2013). Anticancer activity of resveratrol-loaded gelatin nanoparticles on NCI-H460 non-small cell lung cancer cells. *Biomed. Pre. Nutr.,* **3**: 64-73.

Kaul G and Amiji M (2002). Long-circulating poly (ethylene glycol)-modified gelatin nanoparticles for intracellular delivery. *Pharm. Res.,* **19**:1061-1067.

Kaul G and Amiji M (2004). Biodistribution and targeting potential of poly (ethylene glycol)-modified gelatin nanoparticles in subcutaneous murine tumor model. *J. Drug Target.,* **12**: 585-591.

Kaul G and Amiji M (2005). Cellular interactions and in

vitro DNA transfection studies with poly (ethylene glycol) modified gelatin nanoparticles. *J. Pharm. Sci.,* **94** (1): 184-198.

Kaul G and Amiji M (2005). Tumor-targeted gene delivery using poly (ethylene glycol)-modified gelatin nanoparticles: in vitro and in vivo studies. *Pharm. Res.,* **22** (6): 951-961.

Khan SA (2014). Gelatin Nanoparticles as Potential Nanocarriers for Macromolecular Drugs. PhD, Philipps University Marburg, Germany.

Khan SA, Ali H, Ihsan A and Sabir N (2015). Tuning the size of gelatin nanoparticles produced by nanoprecipitation. *Col. J.,* **77**: 672-676.

Khan SA and Schneider M (2013). Improvement of nanoprecipitation technique for preparation of gelatin nanoparticles and potential macromolecular drug loading. *Macromol. Biosci.,* **13**(4): 455-463.

Khan SA and Schneider M (2013). Nanoprecipitation versus two step desolvation technique for the preparation of gelatin nanoparticles. Proc. SPIE 8595, Col. Nano. Biomed. Appl. VIII, 85950H (February 22, 2013); doi: 10.1117/12.2002419.

Khan SA and Schneider M (2014). Stabilization of gelatin nanoparticles without crosslinking. *Macromol. Biosci.,* **14** (11): 1627-1638.

Kommareddy S and Amiji M (2005). Preparation and evaluation of thiol-modified gelatin nanoparticles for intracellular DNA delivery in response to glutathione. *Bioconj. Chem.,* **16** (6): 1423-1432.

Kommareddy S and Amiji M (2007). Biodistribution and pharmacokinetic analysis of long circulating thiolated gelatin nanoparticles following systemic administration in breast cancer bearing mice. *J. Pharm. Sci.,* **96**(2): 397-407.

Kommareddy S and Amiji M (2007). Poly(ethylene glycol)-modified thiolated gelatin nanoparticles for glutathione-responsive intracellular DNA delivery. *Nanomedicine: Nanotech. Bio. Med.,* **3**(1): 32-42.

Landfester K (2001). The generation of nanoparticles in miniemulsions. *Adv. Mater.,* **13**: 765-768.

Lee EJ, Khan SA and Lim KH (2010). Gelatin Nanoparticle Preparation by Nanoprecipitation. *J. Biomat. Sci. Polym. E.,* **22**(4-6) 753-771.

Lee EJ, Khan SA, Park JK and Lim KH (2012). Studies on the characteristics of drug-loaded gelatin nanoparticles prepared by nanoprecipitation. *Bioprocess and Biosyst. Eng.,* **35**(1-2): 297-307.

Lee SJ, Yhee JY, Kim SH, Kwon IC and Kim K (2013). Biocompatible gelatin nanoparticles for tumor-targeted delivery of polymerized siRNA in tumor-bearing mice. *J. Control. Release,* **172**(1): 358-366.

Leo E, Angela Vandelli M, Cameroni R and Forni F (1997). Doxorubicin-loaded gelatin nanoparticles stabilized by glutaraldehyde: Involvement of the drug in the cross-linking process. *Int. J. Pharm.,* **155**: 75-82.

Leo E, Arletti R, Forni F and Cameroni R (1997). General and cardiac toxicity of doxorubicin-loaded gelatin nanoparticles. *Farmaco,* **52**(6-7): 385-388.

Leo E, Cameroni R and Forni F (1999). Dynamic dialysis for the drug release evaluation from doxorubicin-gelatin nanoparticle conjugates. *Int. J. Pharm.,* **180**: 23-30.

Lu CT, Jin RR, Jiang YN, Lin Q, Yu WZ, Mao KL, Tian FR, Zhao YP and Zhao YZ (2015). Gelatin nanoparticle-mediated intranasal delivery of substance P protects against 6-hydroxydopamine-induced apoptosis: An *in vitro* and *in vivo* study. *Drug Des., Dev. Ther.,* **7**(9): 1955-62

Mariod AA and Adam HF (2013). Gelatin, source, extraction and industrial applications. *Acta Scientiarum Pol. Tech. Aliment.,* **12**:135-147.

Mohanty B, Aswal V, Goyal P and Bohidar H (2005). Small-angle neutron and dynamic light scattering study of gelatin coacervates. *Ind. Acad. Sci.,* **63**: 271-276.

Mohanty B, Aswal VK, Kohlbrecher J and Bohidar HB (2006). Length Scale Hierarchy in Sol, Gel, and coacervate phases of gelatin. *J. Polym. Sci. B: Polym. Phys.,* **44**: 1653-1667.

Mohanty B and Bohidar HB (2003). Systematic of alcohol-induced simple coacervation in aqueous gelatin solutions. *Biomacromol.,* **4**(4):1080 -1086.

Narayanan D, Geena MG, Lakshmi H, Koyakutty M, Nair S and Menon D (2013), Poly-(ethylene glycol) modified gelatin nanoparticles for sustained delivery of the anti-inflammatory drug Ibuprofen-Sodium: An *in vitro* and *in vivo* analysis. *Nanomedicine: Nanotech. Bio. Med.,* **9**(6): 818-828.

Ninan G, Jose J and Abubacker Z (2010). Preparation and characterization of gelatin extracted from the skins of rohu (*Labeo rohita*) and common carp (*Cyprinus carpio*). *J. Food Process. Pres.,* **35**: 143-162.

Ofokansi K, Winter G, Fricker G and Coester C (2010). Matrix-loaded biodegradable gelatin nanoparticles as new approach to improve drug loading and delivery. *Eur. J. Pharm. Biopharm.* **76**(1):1-9.

Olad A and Farshi A F (2014). The synergetic effect of bioactive ceramic and nanoclay on the properties of chitosan–gelatin/nanohydroxyapatite–montmorillonite scaffold for bone tissue engineering. *Ceram. Int.,* **40**: 10061-10072.

Oppenheim CR, Marty JJ and Speiser P (1978). Injectable compositions, nanoparticles useful therein, and process of manufacturing same. U.S. Patent 4107288.

Oppenheim RC (1981). Solid colloidal drug delivery systems: Nanoparticles. *Int. J. Pharm.,* **8**: 217-234.

Pal K, Paulson AT and Rousseau D (2013). Biopolymers in Controlled-Release Delivery Systems. *In:* Ebnesajjad S and Andrew W. Handbook of Biopolymers and Biodegradable Plastics. William Andrew Publishing. Boston, UK.

Panyam J and Labhasetwar V (2003). Biodegradable nanoparticles for drug and gene delivery to cells and tissue. *Adv. Drug Deliv. Rev.,* **55** (3):329-347.

Parker NG and Povey MJW (2012). Ultrasonic study of the gelation of gelatin: Phase diagram, hysteresis and

kinetics. *Food Hydrocol.,* **26**: 99-107.

Patel ZS, Yamamoto M, Ueda H, Tabata Y and Mikos AG (2008). Biodegradable gelatin microparticles as delivery systems for the controlled release of bone morphogenetic protein-2. *Acta Biomater.,* **4**(5): 1126-1138.

Prow TW, Grice JE, Lin LL, Faye R, Butler M, Becker W, Wurm EMT, Yoong C, Robertson TA, Soyer HP and Roberts MS (2011). Nanoparticles and microparticles for skin drug delivery. *Adv. Drug Deliv. Rev.,* **63**(6): 470-491.

Rajan M and Raj V (2013). Formation and characterization of chitosan-polylacticacid-polyethylene glycol-gelatin nanoparticles: A novel biosystem for controlled drug delivery. *Carbohyd. Polym.,* **98**(1): 951-958.

Rodriguez SG, Allemann E, Fess H and Doelker E (2004). Physicochemical parameters associated with nanoparticle formation in the salting-out, emulsification-diffusion, and nanoprecipitation methods. *Pharm. Res.,* **21**(8): 1428-1439.

Sarika P, Kumar PA, Raj DK and James NR (2015). Nanogels based on alginic aldehyde and gelatin by inverse miniemulsion technique: Synthesis and characterization. *Carbohyd. Polym.,* **119**(30): 118-125.

Sovilj V, Milanović J and Petrović L (2013). Viscosimetric and tensiometric investigations of interactions between gelatin and surface active molecules of various structures. *Food Hydrocoll.,* **32**: 20-27.

Tanaka F (2003). Thermoreversible gelation driven by coil-to-helix transition of polymers. *Macromol.* **36**(3-4): 5392-5405.

This H (2010). Kitchen mysteries: Revealing the science of cooking, Columbia University Press, New York, USA.

Vandervoort J and Ludwig A (2004). Preparation and evaluation of drug-loaded gelatin nanoparticles for topical ophthalmic use. *Eur. J. Pharm. Biopharm.,* **57**(2): 251-261.

Victoria PSO and Speiser (1975). Injectable compositions. U.K Patent GB1516348.

Zillies JC (2007). Gelatin nanoparticles for targeted oligonucleotide delivery to kupffer cells–analytics, formulation development, practical application. Ph.D dissertation, Ludwig-Maximilians-Universität München.

Zwiorek K (2007). Gelatin Nanoparticles as Delivery System for Nucleotide-Based Drugs. Ph.D dissertation, Ludwig-Maximilians-Universität, München.

Zwiorek K, Kloeckner J, Wagner E and Coester C (2005). Gelatin nanoparticles as a new and simple gene delivery system. *J. Pharm. Pharm. Sci.,* **7**(4): 22-28.