Research Article

Gelatin-Based Nanoparticles as Drug Delivery System of Lornoxicam Gel

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Accepted on: 28-07-2016; **Finalized on:** 30-09-2016.

ABSTRACT

The purpose of contemporary study was to project GNP by using of two step desolvation method. Biodegradable hydrophilic gelatin nanoparticles used as a delivery system of anti-inflammatory lornoxicam after gel formulation using each of hydroxyl propyl methyl cellulose (HPMC) and carbopol as gelling agent. The size and shape of the nanoparticles were examined by optical microscope and transmission electron microscopy, particles with a mean diameter of 240.6 nm and 0.1 poly dispersibility index PDI were produced and the percentage of entrapment efficiency was found to be 87.1%. The optimum amount of LOR loading was obtained. Four formulas were prepared F1, F2 are slandered drug gel and F3 and F4 are GNP-LOR gel. Permeation of drug through membrane was determined by Franz diffusion cell. Further stability studies were carried out at 4°C for a period of 8 weeks. Vivo study was carried on white albino male rats to compare between different lornoxicam gel formulations. Results show that the two step desolvation is an appropriate method for preparing GNP as a delivery vehicle for LOR gel which gives sustained drug release. LORF3 which has carbopol as gelling agent was of lower release rate as regard carbopol was found to be a good choice for formulating LOR as topical formulation.

Keywords: Gelatin, nanoparticle, desolvation, transmission electron microscopy.

INTRODUCTION

Lornoxicam is a non-steroidal anti-inflammatory drug (NSAID), it is a compound in the same chemical class as piroxicam, meloxicam and tenoxicam, with potant anti-inflammatory antipyretic and analgesic activity[1]. Lornoxicam is a new nonsteroidal anti-inflammatory drug of oxicam class. It is distinguished from established oxicams by a relatively short elimination half-life[2]. Lornoxicam inhibits the COX-1/COX-2 system, the production of interleukin 6, and the inducible NO synthase[3]. It may be applied by the intramuscular or intravenous route; its bioavailability after oral application is approximately 90%. Although its elimination half life is only about four hours, the duration of effect is approximately eight hours, analogous to other acidic antipyretic analgesics. The analgesic potency of lornoxicam is remarkable. Lornoxicam niosomal gel which is drug delivery transporters for transdermal system was defining[4].

In recent years, nanoparticles have received a growing attention as a delivery system of different bioactive molecules. Nanoparticles are solid colloidal particles ranging in size from 1 to 1,000 nm. They can be used to entrap, encapsulate or absorb active agents (drug or other biological active agents)[5]. These particles can be prepared from a variety of natural and synthetic materials, such as proteins, polysaccharides, and synthetic polymers. Although various biodegradable nanoparticles of natural polymers, such as starch, chitosan, liposomes, etc. are largely in use as drug carrier system for controlled drug delivery technology[6]. The interest was based on the fact that gelatin is low cost, biodegradable, biocompatible, non-toxic, and easy to crosslink and to modify chemically and has therefore an immense potential to be used for the preparation of drug delivery systems such as protein containing nanoparticles[5].

Various methods, including Nano encapsulation[7], water-in-oil emulsion[8,9], desolvation[10], and coacervation-phase separation[11] have been used to prepare GNP. There are some studies that use these methods to produce GNP containing different bioactive molecules[12-17].

Although all of these methods have several advantages, there are some limitations. In case of water-in-oil emulsion technique, a large amount of surfactant is required to produce the small-sized GNP, which needs a complicated post-process.

The coacervation method is a process of phase separation followed by cross-linking step, while the non-homogeneous cross-linking occurs in this method and has unsatisfied loading efficiency[18]. Indeed, GNP prepared by many of these methods was found to be large in size and has a high polydispersity index (PDI) due to heterogeneity in molecular weight of the gelatin polymer.

An easier GNP preparation method, two-step desolvation, was developed that enabled the production of GNP with a reduced tendency for aggregation[19,20]. In this method, after the first desolvation step, the low molecular gelatin fractions presented in the supernatant were removed by decanting, and the high molecular fractions presented in the sediment were redesolved. However, there are some studies that use two-step desolvation to produce GNP containing different bioactive molecules[21-24]. Also, few studies have been conducted in production of protein-loaded GNP by this method.

OBJECTIVE

In the present study, a two-step desolvation method was used to produce GNP containing lornoxicam drug. At the first step, the optimum formula of gelatin nanoparticle according to size and PDI was investigated. Then, lornoxicam loaded GNP gel was prepared using gelling agent HPMC and carbopol. The size, shape, entrapment efficiency EE % and drug release of nanoparticles were investigated. Finally vivo study was estimated.

MATERIALS AND METHODS

Materials

Gelatin type A (Bloom 175), glutaraldehyde (25% v/v aqueous solution), acetone, lornoxicam, hydroxypropyl methylcellulose (HPMC 4000), carbopol and potassium dihydrogen orthophosphate were purchased from Sigma Chemical Company (USA). All other chemicals were of analytical grade and were used as received without any further modification.

GNP Preparations

GNP was prepared by two-step desolvation method. The mechanism of GNP formation was based on applying a desolvating agent to reduce water available and keep the hydrated gelatin chain in solution, resulting in agglomeration and subsequent formation of particles.

Two-steps Desolvation

Glutaraldehyde cross-linked GNP was prepared by a two-step desolvation method previously reported by Coester, with slight modifications. Gelatin (5% w/v) was dissolved in water by constant heating at 50±1C. A desolvating agent, that is, acetone was added to the aqueous gelatin solution (25 ml) to precipitate the high molecular weight (HMW) gelatin and supernatant was separated and removed. The HMW gelatin was then dissolved again by adding 25 ml distilled water with continuous stirring at 200 rpm using hot plate cum magnetic stirrer (Lab Tech, LMS – 1003, Korea) under constant heating at 50±1C. pH of solution was adjusted to 3.0 with 0.1N HCl. Lornoxicam was added followed by addition of acetone (70 ml) drop wise under constant stirring (200 rpm) for 70 min. the formed GNP were cross linked with 200 µl of glutaraldehyde solution (25% v/v aqueous solution) at room temperature for 10 min. Aceton evaporated from the dispersion by rotary evaporator, water containing nanoparticles freeze-dried, and then faint yellow freely flowing powder of LOR-loaded GNP was obtained.

The study started in August 2015 till June 2016 and carried in the research lab of Pharmaceutics and Pharmaceutical Technology Department.

Optimization of the Two Steps Desolvation Technique

Temperatures at the First and Second Desolvation Step

Temperatures range of 35-60°C chosen to be tested. The upper limit of the tested temperature spectrum was constituted with respect to the boiling temperature of the later applied desolvation reagent acetone. The lower limit was constituted with respect to the expected sol-gel transition temperature.

Amount of Gelatin

Three different concentrations were chosen to be tested, 5%, 10% and 15% w/w represent 1.25, 2.5 and 3.75 gm. gelatin and according to GNP size beside poly-dispersity PDI the appropriate weight used to continue the study.

pH Value

The net charge of low charge density polyelectrolytes such as gelatin in solution is strongly affected by the prevalent pH conditions. Hence, changing the pH led to strong alterations in final particle size. The pH region observed was between pH 2.0 and 4.0. Since gelatin type A has an IEP of pH 9.0, it had a positive net charge at all tested conditions. Under standardized conditions as previously described, it was possible to produce gelatin nanoparticles within a range of pH 2.0 – 4.0. Above pH 4.0 the produced gelatin nanoparticles tended strongly towards aggregation and precipitation. Obviously, this pH value is too close to the IEP of gelatin type A. Thus the remaining net charge of gelatin is too weak to prevent the freshly in-situ formed nanoparticles from instabilities.

Amount of Acetone Addition during the Second Desolvation

In this study different amount of acetone addition were studied as 50, 60, 70, 80 and 90 ml. The resulting nanoparticles were influenced by acetone variation. Mean particle sizes along with their corresponding PDI were differing with either decrease or increase with different amount of acetone.

Amount of Crosslinking Reagent Applied

In the present study gelatin nanoparticles prepared according to the standard conditions and varied the amount of applied GTA from 100-500 µl. Each condition was prepared in triplicates.

Stirring Speed

Variation of speed of hot plate with stirring during preparation of gelatin nanoparticles applied in order to study the effect of different rpm on GNP sizes and PDI. The speeds vary as 200. 300, 400, 500 and 600 rpm.

Drug Concentration

Three different drug concentrations were encapsulated in GNP as 2, 3 and 5 mg lornoxicam. The optimal concentration chosen according to loaded GNP size and their corresponding PDI.

Differential Scanning Calorimetric (DSC)

Thermal analysis was used to elucidate any interactions between LOR and investigated polymers. DSC was carried out using Shimadzu, DSC 60 thermal analyzer with a liquid nitrogen cooling accessory. The analysis was performed under purge of dry nitrogen gas (40 mL min^{-1}). A sample

of 2–5 mg was placed in an aluminum crucible cell and was firmly crimped with the lid to provide an adequate seal. The samples were heated from ambient temperature to 300°C at a preprogrammed heating rate of 10°C min^{-1}. All samples were analyzed in the same manner.

Characterization of GNP

Optical Microscopy

Slight volumes of the GNP made were spread on a glass slide and examined for the vesicle structure and the presence of insoluble drug crystals using light microscopy (Olympus, Philippines). Photomicrographs were taken at a magnification of CK X41 using digital camera fitted with microscope (Nikon cool-pix S220, Japan).

Size Analysis and Zeta Potential

Mean size and poly-dispersity index of nanoparticle formulations were measured at 25°C by photon correlation spectroscopy, using a Malvern particle size analyzer (Malvern Instruments, UK) equipped with the Malvern PCS software. Light scattering was monitored at 25°C at a scattering angle of 90°. The poly-dispersity index of nanoparticles was performed as a measurement of the size distribution of system. The surface charge (Zeta potential) was determined by measuring the electrophoretic mobility of the nanoparticles.

Transmission Electron Microscopy

Transmission electron microscopy (TEM) on a Philips EM 2681 instrument (Philips, The Netherland). The liquid dispersion of nanoparticles (one drop) was placed over a 400-mesh carbon-coated copper grid followed by negative staining with phosphotungestic acid solution (3% w/v, adjusted to pH 4-7 with KOH) and placed at the accelerating voltage of 200 KV for TEM.

Determination of Entrapment Efficiency

For determination of drug entrapment, the amount of drug present in the clear supernatant after centrifugation at 24000 rpm for 20 min at 4°C was determined (C_f) by UV spectrophotometry at376 nm. A standard calibration curve of drug was plotted for this purpose. The amount of drug in supernatant was then subtracted from the total amount of drug added during the preparation (C_t). Effectively, (C_t - C_f) will give the amount of drug entrapped. The percentage entrapment is obtained by (C_t - C_f) × 100/C_t.

GNP Loaded with Lornoxicam as Gel

Gel Preparation

The exact amount of HPMC was dispersed in warm water with continuous stirring to form gel. The drug/methylparaben mixture was prepared and the final volume was adjusted by addition of water. To prepare the carbopol gel, it was dispersed in warm distilled water with stirring. Triethanolamine quantity sufficient was added to allow for the complete swelling. Then the amount of LOR and methylparaben were prepared and added. GNP loaded with LOR formula was prepared, acetone evaporated by controlled rotary evaporator, water lifted containing nanoparticles frozen at -80°C and transferred to lyophilize. Finally freely flowable powder obtained and the amount of which containing the exact drug concentration was used to prepare the same previous two types of gel. The composition of gel formulations is given in (Table 1).

In-vitro LOR Release

In vitro release study of lornoxicam from gels (LORF1-LORF4) was performed by using 2 gram of each gel formulation. The amount of gel was accurately weighed and placed on a cellophane membrane (MWCO 12–14,000) previously immersed in phosphate buffer of pH 7.4. The loaded membrane was mounted on the Franz diffusion cell with a diffusional area of 1.76 cm^2. The receptor phase contained 7.1 mL of phosphate buffer. The buffer solution temperature was maintained at 37°C ± 0.5 with constant stirring. Accurate samples (2 mL) were withdrawn at time intervals 0.25, 0.5, 0.45, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 20, and 24 hr. The volume of each withdrawn sample was replaced by the same volume of dissolution medium maintained at the same temperature to keep constant volume. The released amount of LOR was measured spectrophotometrically at 376 nm.

Stability of Lornoxicam GNP Gel

The stability of vesicles to retain the drug was assessed by keeping the Lornoxicam gel at two different temperature conditions like refrigeration temperature (4°C), room temperature (25±2°C) in aluminum foil sealed glass vials. The samples were withdrawn at different time intervals over a period of 2 month and they were observed visually and under optical microscope for the change in consistency and appearance of drug crystals upon storage[24]. Drug content was determined at weeks 4 and 8 by placing 1 gm. of each formula F1, F2, F3 and F4 in 10 ml phosphate buffered saline pH 7.4, followed by agitation in an incubator shaker (100 rpm/min)for 24 hrs. at room temperature. After suitable dilutions drug solubilized was analyzed spectrophotometrically (Table 2). The mean particle size of GNP drug loaded dispersion, surface charge (Zeta potential) and PDI was determined by using a Malvern zeta sizer (Malvern Instruments, UK) on the spot and after 3 months (Table 3).

***In–Vivo* Characterization**

Selection of Animals

White albino rate weighing between 190-200 g were selected for study. The animals were divided in to three groups, each consisting of six rats. Group I was treated with plain gel base without drug, Group II was treated with lornoxicam with carbopol standard gel, finally Group III was treated with lornoxicam encapsulated in GNP gelled with carbopol.The study was conducted by complete cross over design.

Procedure

The animals were fasted over-night and all groups were treated by applying 1 g of gel on the left paw of the rats, with respective gel. The part of application was sealed with dressings and it was left in place for 2 hrs. The dressing was then removed and the gel remaining on the surface was wiped off with cotton. The animals were then injected with 0.1 ml of 1% w/v carrageenan solution in plantar region of left hind paw and the paw volume was measured after 1hr, 2hr, 3hr, 4hr, 5hr, 6hr, 7hr, 8hr, and 10 hrs. Micrometer was used. The right paw served as a reference which was not treated with formulations, but inflamed using the same solution paw for comparison. The percentage difference between right and left paw volumes was taken as percent edema produced. The percent edema produced with test samples were subtracted from percent edema produced in control group to obtain percent edema inhibition by respective groups. Percent inhibition of edema is directly proportional to the anti-inflammatory activity. Results are shown in (Table 4).

RESULTS AND DISCUSSION

Optimization of GNP according to Size Analysis and Zeta Potential

Preformulation studies showed the absorption maxima for Lornoxicam at 376 nm and the developed Spectrophotometric method obeyed beer's law with linearity range of 5-30µg/ml (Figure1).

Effect of Temperature

Contrary to expectance, variations of the gelatin solutions' temperatures did not induce significant differences in the resulting particles. At least, concerning the temperature before the first desolvation step a slight trend could be seen. The highest (60°C) and the lowest (35°C) temperature conditions led to the biggest and most inhomogeneous particles (Figure 2 A). In terms of the temperature at the second desolvation step, only a minimal trend could be observed favoring 50-55 °C as optimal temperature for the production of small nanoparticles (Figure 2 B). So it can be concluded that the chosen standard conditions (50°C for both) also seem to represent the ideal setup as well.

Amount of Gelatin

Size of GNP are affected by variation of gelatin weight 5%, 10% and 15% w/w represented 1.25, 2.5 and 3.75 gm. Nanoparticles increase in size with increasing gelatin weight and the most appropriate weight was 5% w/w that gives a suitable GNP size.(Figure 3)

pH Value

Within the favorable pH region, increasing acidic conditions correlated with the reduction of the particle's mean sizes. The produced nanoparticles had sizes range 193.9 and 770.1 nm (Figure 4). Highly homogenous nanoparticles with PDI below 0.1 could be produced between pH 2.5 and 3.0. Performing the drop wise addition of 70 mL acetone to generate the nanoparticles it was noticed that a first turbidity, indicating phase separation, and the generation of nanoparticles appeared at a later point of acetone addition by shifting the pH towards lower values.

The net charge of the gelatin molecules was apparently too intense, so that the intermolecular repulsion forces stabilizing the characteristic three-dimensional protein shape prevented it from collapse and aggregation induced by the desolvation agent.

In the pH regions where nanoparticles can be produced, the same reasons seem to be responsible for the smaller particle sizes. Intermolecular electrostatic repulsion forces obviously hinder the inter-molecular co-aggregation.

Amount of Acetone

Mean particle sizes along with their corresponding PDI increased exponentially with increasing amount of acetone added drop wise during second desolvation step as 50, 60, 70, 80 and 90 ml. Nanoparticle sizes range as 93.74, 96.88, 123.8, 142.8 and 103.8. The resulting nanoparticles were not negatively influenced up to a rate of 1 mL/min. 70 ml acetone was chosen for optimal GNP formula with corresponding Nano size 123.8 and PDI 0.08. (Figure 5).

Amount of Crosslinking Reagent

Crosslinking has to be done to prevent the in situ formed nanoparticles from disintegration when the desolvation agent acetone is evaporated. It can be performed with non-zero length and zero length agents.

Zero-length crosslinking agents (e.g. EDC) activate carboxylic acid residues to react directly with amine groups on adjacent protein chains. For gelatin nanoparticles glutaraldehyde (GTA) is the agent of choice, since it is a highly reactive and efficient crosslinking agent. GTA is a non-zero length cross linker as it operates by intra-particulate bridging of residual amino groups.

Variation in amounts of GTA applied lead to mean particle size range as 129.4 to 209.7. Particle size reduction is decelerated from an amount of 200 µl GTA on. With regards to measured PDI values, 200 µl represent an optimum with the most homogeneous nanoparticles resulting with 129.4 sizes and 0.1 PDI. The slight increase of the PDI values beyond this point might be addressed to stronger interparticulate aggregation tendencies which are facilitated by higher amounts of GTA (Figure 6).

Stirring Speed

During formula trial stirrer speed increased starting with 200 rpm up to 600 rpm in order to study the influence on mean GNP sizes which as regard ranging from 163.9 – 1890. The particle size and PDI increase with higher speed applied. The suitable speed chosen is 200 rpm resulting Nano size as 163.9 and corresponding PDI 0.1. (Figure7).

Drug Concentration

After optimal formula have prepared with 1.25 gm. gelatin, temperature 50 °C, 3.0 pH, 200 rpm stirring speed, 200 µl GTA and 70 ml drop wise acetone added during second desolvation step, three different lornoxicam concentrations are used for drug loaded 2, 3 and 5 mg. The resulting mean sizes of GNP are 199.5, 240.6 and 268.5 nm with PDI 0.1, 0.1 and 0.2 respectively. Drug of 3 mg. concentration with appropriate GNP size and small PDI was chosen for gel preparation (Figure 8).

Optical Microscopy

The morphology of all GNP formulations was determined by optical microscope equipped with digital camera. The photomicrograph of optimal formula was shown in (Figure 9). These photomicrographs confirmed the formation of vesicular structures. The microscopic appearance of all formulations showed spherical vesicles. Unfortunately, information concerning microstructure of GNP not be visualized by the low-magnification power of optical microscope, therefore transmission electron microscope was employed to elucidate morphology of Nano particles.

Transmission Electron Microscopy

TEM of plane GNP were of uniform and definite shape (Figure 10). The nanoparticles were found in between the size range of 52.0 nm to 84.9 nm, whereas the mean particle size by Zeta seizer was 240.6 nm. The larger size measured by zeta seizer was probably due to the formation of aggregates.

Differential Scanning Calorimetric (DSC) Studies

DSC study has been used to detect formulation incompatibility due to interactions between drug and excipients. DSC thermogram of LOR and used excipients are shown in (Figure 11). DSC thermogram of LOR showed a sharp exothermic peak at 235.2°C, which matches to its melting point indicating decomposition of LOR[25]. DSC thermogram of LOR with HPMC showed the LOR exothermic peak at 230.7°C which might be due to decomposition of LOR. Therefore, LOR is compatible with HPMC due to absence of interaction.

Moreover, DSC curve of LOR with carbopol showed an exothermic peak at 229.3°C followed by an upward line higher than the baseline of the curve which may be attributed to the fusion of the drug with decomposition. These DSC data of LOR and carbopol may be indicated to molecular dispersion of drug molecules with carbopol particles.

GNP Size Determination

The size and size distribution are important characteristics of a Nano encapsulation product used for drug delivery because of the dependence of drug release rate on the size and size distribution. (Figure 10) shows TEM of plane GNP size distribution with average of 70.2 nm. The average particle size and PDI of GNP by Zeta seizer were 184.5 nm and 0.2, respectively. The average particle size and PDI of drug loaded GNP by Zeta seizer was 240.6 nm and 0.1, respectively. These results demonstrate that two-steps desolvation is more suitable to get GNP with desirable size and PDI. As shown in mentioned figure smooth and spherical GNP was produced. The photograph clearly indicates that no hairline cracks or heterogeneity appear on the nanoparticles surface. These data present morphological evidence for the smooth nanoparticles. (Figure 9) C shows Zeta potential of LOR loaded GNP.

Encapsulation Efficiency (EE %)

The entrapment efficiency is the most important parameter from pharmaceutical point of view in nanoparticle formulations. A high percentage of entrapment would mean less time and effort involved in removal of unentrapped material. Percentage encapsulation efficiency was measured by "centrifuge method", in which unentrapped drug was calculated spectrophotometrically and entrapped drug was determined using formula $EE\% = (C_t - C_f) \times 100/C_t$. For the optimal formula loaded with 3 mg lornoxicam. Entrapment efficiency was found to be approximately 87 %.

In-vitro Release Studies

In vitro LOR diffused through cellophane membrane was recorded in (Figure 12). It was observed that *in vitro* release data of standard LOR carbopol gel F1 started slowly, that is because of increases the viscosity of the formulation and hence lowers the release rate of the drug, but eventually the LOR release was the higher for standard LOR HPMC gel F2. Taking in consideration that drug concentration was 0.5 mg in each formula. Drug concentration loaded in GNP was 0.5mg.

According to this study, lower LOR release and drug control was observed in GNP-LOR F3 gel formulation and was found to be the most appropriate formulation for LOR gel. GNP–LOR F3 containing carbopol as gelling agent has lower release rate than GNP–LOR F4 which has HPMC as gelling agent and both were of higher release rate than F1 and F2 and all formula are containing the same drug concentration. So as a result from this experiment carbopol was found to be a good choice for formulating GNP-LOR gel as topical formulation.

Stability Studies

Stability studies were performed on HPMC and carpobol GNP gel for of 8 weeks by subjecting them to aging at 4°C. The samples were stored at 4°C and at room temperature 25°C for 8 weeks and stability and drug content per gram of all these samples was determined after 4 and 8 weeks.

A direct relationship between the percentage leaching of the drug out of the vesicles and aging was observed i.e. as the storage period increases, the degree of leaching increased. It was observed that GNP gelled with carpobol was more stable, (Table 2). Literatures showed that zeta

potential and particle size is not the primary consideration in enabling the drug loading process even under iso-osmotic conditions[26]. The relative consistency of the zeta potential value upon storage indicated that there was no significant drug diffusion out from the nanoparticles in to the aqueous phase. After 3 months storage, the gelatin nanoparticles size difference was observed as shown in (Table 3).

In-vivo Study

The results showed that GNP-LOR gel formulation F3 (Group III) gives sustained anti-inflammatory effect with maximum percentage inhibition of edema with reference to control and as compared to Group II at 6 hrs.

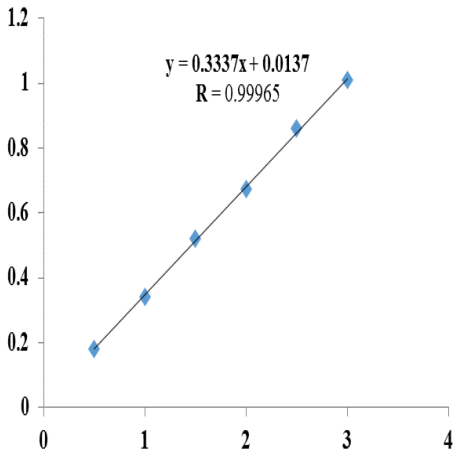

Figure 1: Calibration Curve of LOR

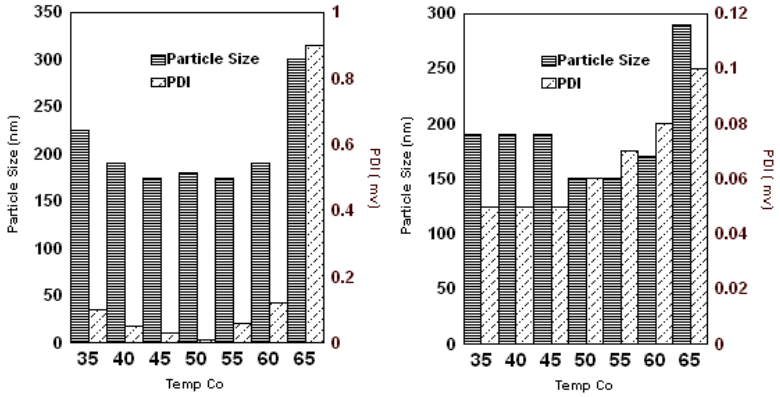

Figure 2: Correlation between the temperature of the gelatin solutions before each desolvation step and the resulting nanoparticles; A: temperature before the first desolvation step; B: temperature after the second desolvation step (n=3)

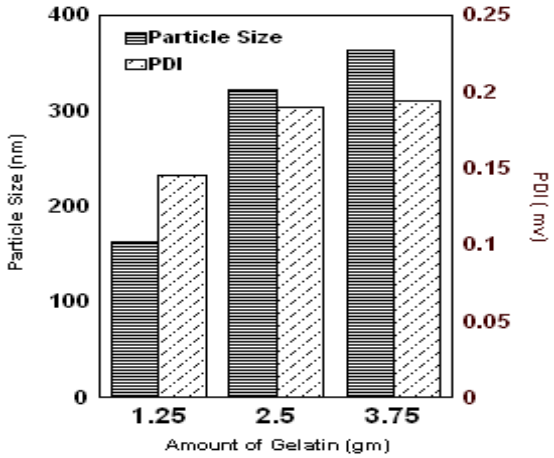

Figure 3: Correlation between Amount of Gelation and the resulting Nanoparticles quality (n=3)

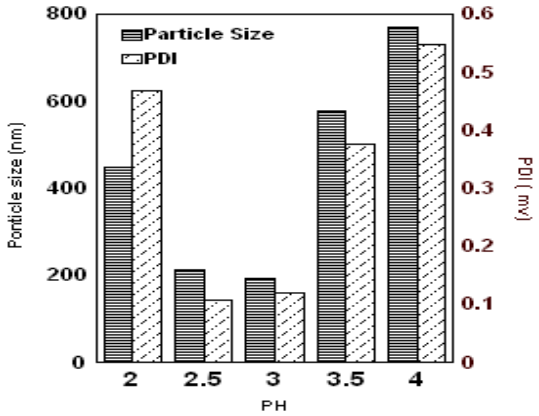

Figure 4: Correlation between the adjusted PH value before the second desolvation step and the resulting nanoparticles (n=3)

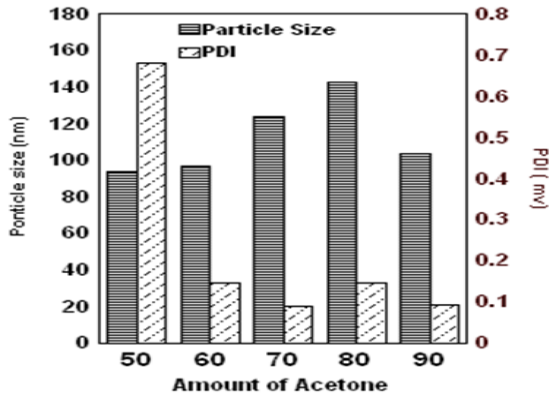

Figure 5: Correlation between the amount of acetone used in second desolvation step and the resulting nanoparticles quality (n=3)

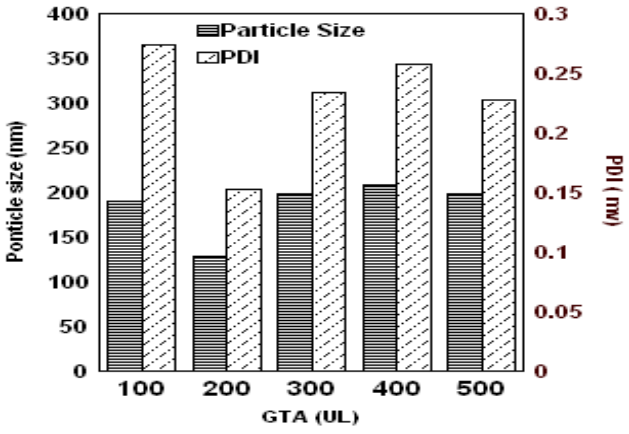

Figure 6: Correlation between the amounts of GTA applied for crosslinking and the resulting nanoparticles quality (n=3)

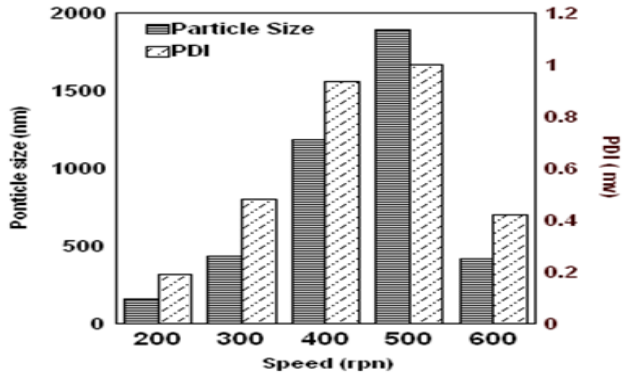

Figure 7: Correlation between the speed of stirrer during the second desolvation step and the resulting particle quality (n=3)

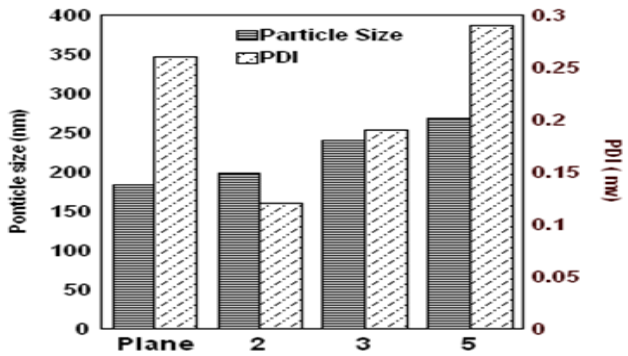

Figure 8: Correlation between weight of drug encapsulated in GNP and the resulting particle quality (n=3)

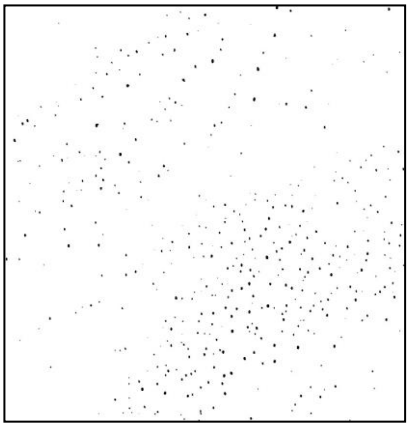

Figure 9: A: optical microscope photograph for plane GNP

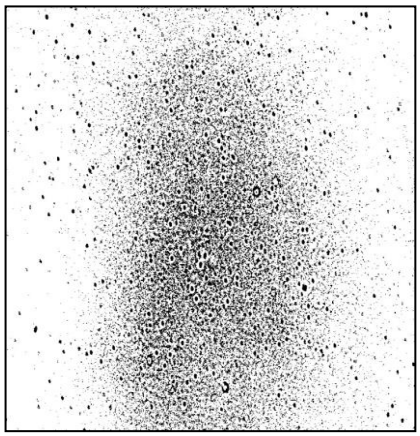

Figure 9B: Optical Microscope photograph for LOR loaded GNP

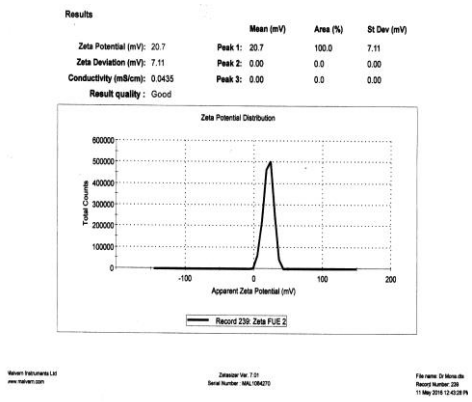

Figure 9C: Zeta Potential of LOR loaded GNP

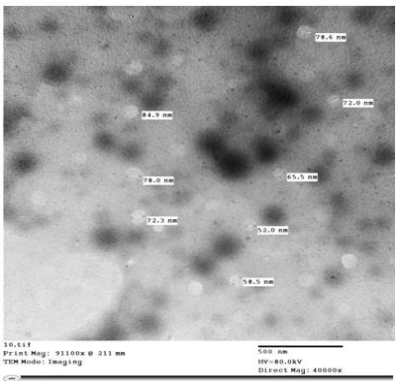

Figure 10: TEM Photograph for plane GNP

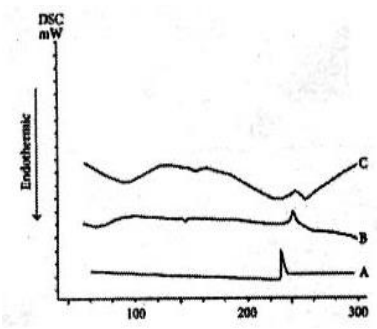

Figure 11: DSC thermograms of (a) LOR, (b) LOR –HPMC and (c) LOR-Carbopol

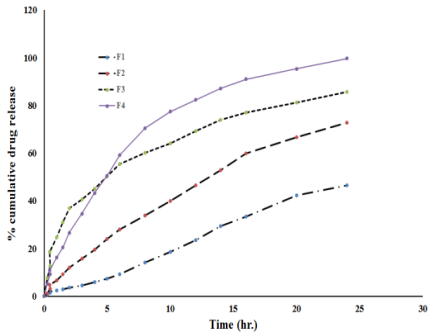

Figure 12: % Cumulative Amount of LOR Released from Different Gel Formulations

Table 1: Composition of LOR Gel Formulation

Materials	Formulation Composition (% w/w)			
	LOR F1	LOR F2	LOR F3	LOR F4
LOR	0.5	0.5	0.5 GNP	0.5 GNP
MPMC	-	10	-	10
Carbopol 974	0.5	-	0.5	-
Triethanolamine	Q.S	-	Q.S	-
Methyl paraben	0.2	0.2	0.2	0.2
Water to	100	100	100	100

Table 2: Drug Content Determination

S. No.	Formulations	Drug Content (mg/gm)				
		1st Day	4 Weeks		8 Weeks	
1	F1	0.5	0.32	0.26	0.20	0.16
2	F2	0.5	0.29	0.25	0.15	0.14
3	F3	0.5	0.31	0.28	0.19	0.18
4	F4	0.5	0.28	0.24	0.16	0.15

Table 3: Malvern Zeta Sizer and Transmission Election Microscopy Results of LOR Loaded GNP

Size of GNP	After Preparation	After 3 Months
TEM	**52.0 nm**	**93.0 nm**
Malvern Zeta Sizer	**206.1**	**557.8**
Polydispersity	**0.06**	**0.09**

Table 4: *In vivo* Evaluation of Anti-inflammatory Activity (Mean Reduction in Edema)

S. No.	Time (hrs.)	Gel base (GP I)	Standard LOR gel with Carbopol (GP II)	GNP Loaded with LOR, gel with Carbopol (GP III)
		Mean ± S.D (ml)	Mean ± S.D (ml)	Mean ± S.D (ml)
1	1	0.65 ± 0.04	0.455 ± 0.02[a] (63.33%)*	0.45 ± 0.05[a] (66.66%)*
2	2	0.75 ± 0.02	0.407 ± 0.03[a] (97.20%)*	0.47 ± 0.05[a] (72.05%)*
3	3	0.90 ± 0.02	0.43 ± 0.02[a] (90.38%)*	0.48 ± 0.03[a] (77.14%)*
4	4	0.85 ± 0.05	0.52 ± 0.02[a] (65.61)*	0.45 ± 0.03[ab] (85.71%)*
5	5	0.85 ± 0.01	0.53 ± 0.01[a] (55.44%)*	0.42 ± 0.03[ab] (93.33%)*
6	6	0.80 ± 0.05	0.54 ± 0.04[a] (53.33%)*	0.40 ± 0.02[ab] (100%)*
7	7	0.70 ± 0.06	0.61 ± 0.05[a] (13.24%)*	0.40 ± 0.01[ab] (100%)*
8	8	0.68 ± 0.03	0.50 ± 0.06[a] (10.11%)*	0.40 ± 0.01[ab] (100%)*
9	10	0.65 ± 0.02	0.45 ± 0.01[a] (8.00%)*	0.40 ± 0.01[ab] (100%)*

[a]: Statistically significant ($p < 0.05$) as compared to control; [b]: Statistically significant ($p < 0.05$) as compared to Group II;

*: % inhibition of edema with reference to control

CONCLUSION

It can be concluded from this study that desirable lornoxicam loaded GNP, can be prepared using two-step desolvation method. By using 3% w/v of drug loading as optimum particles with a mean diameter of 240.6 nm and EE% of 87.1 % were produced. According to results obtained from this study, carbopol was found to be a suitable and stable vehicle for formulation of GNP - LOR gel science the formula gave sustained effect with maximum % inhibition of edema.

Acknowledgement: Author is grateful to research lab of Department of Pharmaceutics and Pharmaceutical Technology, FUE University, Cairo for providing facilities for research.

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Source of Support: Nil, **Conflict of Interest:** None.