Oleic Acid Coated Gelatin Nanoparticles Impregnated Gel for Sustained Delivery of Zaltoprofen: Formulation and Textural Characterization

Savita Pawar¹, Vishal Pande²*

¹ R.C. Patel College of Pharmaceutical Education and Research, Shirpur, India 425405.
² Sanjivani College of Pharmaceutical Education and Research, Kopargaon, India 423603.

Abstract

**Purpose:** In the present study, we have formulated zaltoprofen loaded, surface decorated, biodegradable gelatin nanogel and evaluated its texture characterization.

**Methods:** The method used to prepare gelatin nanoparticles (GNP) was ‘two step desolution’ and its surface decoration was performed with oleic acid (OA). The GNP was optimized by DOE software. Nanogels were evaluated for particle size entrapment efficiency, texture properties, SEM, in-vitro, ex-vivo drug release studies, in-vitro characterization, stability and in vivo evaluation of nanogel for anti-inflammatory activity was carried out by carrageenan induced rat paw edema method as an anti-inflammatory experimental model.

**Results:** The formulated GNP with particle size and entrapment efficiency of optimized batch was found to be 247.1 nm and 76.21% respectively. The SEM of GNP shows smooth and spherical shape. In-vitro and Ex-vivo drug release shows that there was 69.47% and 78.59% drug released within 48 hrs. It follows Ritger peppas model, which indicates sustained drug release. The good texture properties of nanogel were observed from texture analysis graphs.In vivo studies of our formulation give significant results compared to the marketed nanogel. Stability data revealed stability of nanogel formulation up to 3 months.

**Conclusion:** The present approach can provide us promising results of the sustained analgesic activity and the stability of drug within the GNP.

Introduction

Nanogel may be defined as the nanosized hydrogel systems which are highly crosslinked system in the nature involving polymer system which are either copolymerized or monomers.¹ Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 1-1000 nm. Nanoparticles are receiving considerable interest to delivery of therapeutic drug.² Several natural and synthetic polymers have been used for the preparation of micro or nanoparticles.³ The topical drug delivery systems are gaining increasing attention and several drugs have been successfully delivered by this route for both local and systemic action.⁴ Nanogel is attracting researchers because of its properties like, encapsulation stability, responses to stimuli, passive and active targeting, easy to synthesis and much more.⁵ In recent years most of NSAIDs have been designed to deliver the drug in the form of topical gels to avoid the gastro intestinal irritation to overcome first pass effect and to enhance the drug concentration at the site of action. As a vehicle they have a better potential to administer drug topically as compared to ointment because they are non-sticky require low energy during the formulation, they are stable and have an aesthetic value.⁶

Polymeric nanoparticles release the drugs mostly in subcutaneous layer of skin, by surface modification of GNP with oleic acid as a penetration enhancer helps to improved drug permeation through skin.⁷ To improve the drug absorption from the skin surface nanoparticulate carriers have proven to be advantageous & effective.⁷,⁸ Polymeric nanoparticles have been widely studied as a carrier for the drug and used in various drug delivery systems due to their submicron size, controlled, sustained release properties biocompatibility with tissue and cells.⁹ Nanoparticles based on negatively charged hydrophilic polymers show a strong increase in biodehesive properties .Various synthetic like , poly (glycolic acid) , poly (l-lactic acid) , poly (glycerol adipate) etc. & natural like , collagen , alginate , fibrin etc. substance used in controlled release drug delivery systems.¹⁰,¹¹ Gelatin is a natural polymer that is derived from collagen. It is commonly used for pharmaceutical & medical application due to its properties like low cost, inexpensive & readily available, low antigenic, due to its structure it can be modified by many ways like; coupling
of targeting legend, crosslinkers, hydrophilicity of gelatin gives its diffusion mediated drug release, biodegradability.\textsuperscript{12} Hence gelatin used as a delivery vehicle for the controlled release of bioactive molecule. The use of GPNPs as vehicles for topical drug delivery, providing a reservoir system for release into the skin has also been studied. Also, the GNP loaded with drug can be used in topical ophthalmic use.\textsuperscript{5,13} The current area of interest in the drug encapsulated nanoparticle given by topical route & NP mobility through gel was studied by traditional diffusion cell experiments.\textsuperscript{13}

Rheumatoid arthritis is a chronic autoimmune inflammatory disease, drugs for RA treatment applied topically gives better results as well as reduction in the GI toxicity / irritation produced by oral route. The Hydrophobic drug chosen was zaltoprofen, in order to improve the aqueous solubility of drug, complexes with HP-β-CD were prepared.\textsuperscript{14} Nanoparticulate suspension of gelatin loaded with zaltoprofen can be coated with oleic acid & converted in to gel by adding poloxamer to enhance viscosity. Optimizations using factorial designs, where all the factors are studied in all possible combinations, are considered to be the most efficient in estimating the influence of individual variables and their interactions using minimum experiments. The application of factorial design in pharmaceutical formulation development has played a key role in understanding the relationship between the independent variables and the responses to them.\textsuperscript{15}

The objective of present study was to develop a nanogel & its (TPA) i.e. texture profile analysis.\textsuperscript{16} NSAID drugs need to sustained the drug release mainly in case of chronic inflammatory disorders like Rheumatoid arthritis (RA), which causes serious morbidity and disability for the patients.\textsuperscript{17} For that purpose we have prepared polymeric nanoparticles loaded with zaltoprofen by two step desolvation method, coating of these nanoparticles was performed with oleic acid and these nanoparticles were incorporated in the gel. The prepared gel retards the drug release from polymeric matrix. Therefore, the goal of present study was first to develop reproducible & homogeneous preparation of zaltoprofen loaded GPNPs & application of these particulate carriers for topical drug delivery in the form of gel. Secondly to investigate texture properties of the prepared nanogel & its in-vivo toxicity studies.

**Materials and Methods**

**Material**
Zaltoprofen was obtained as a gift sample from Tonira Pharmaceuticals, Ankaleshwar, Pluronic F-127 purchased from Sigma Aldrich, Gelatin type A (Bloom 175) derived from porcine skin purchased from Sigma Aldrich, HP-β-CD purchased from Himedia laboratories Ltd, Mumbai, Glutaradehyde (25% V/V aqueous solution) was purchased from RFCL lab. Pvt. Ltd. Mumbai, All other solvents used were of analytical grade and used as received.

**Factorial design parameters and experimental conditions**
A 3-level factorial design was implemented to study the effect of two factors on (independent variables), factor level and responses (dependent variables). The levels, factors and responses are given in Table 1. Experimental design of the different batches of GNP is given in Table 2.

---

**Table 1.** Factors (independent variables), factor levels and responses (dependent variables) used in 3-level factorial experimental design.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Type of factors</th>
<th>Factor level used</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-1</td>
<td>0</td>
</tr>
<tr>
<td>X₁ Glutaraldehyde (µl)</td>
<td>30</td>
<td>50</td>
<td>70</td>
</tr>
<tr>
<td>X₂ Agitation speed (RPM)</td>
<td>500</td>
<td>600</td>
<td>700</td>
</tr>
</tbody>
</table>

**Table 2.** Experimental design for preparation of different batches and Results for particle size and entrapment efficiency.

<table>
<thead>
<tr>
<th>Std</th>
<th>Run</th>
<th>Batch code</th>
<th>Factors</th>
<th>Agitation speed (RPM)</th>
<th>Particle size (nm)</th>
<th>Entrapment efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1</td>
<td>GNP 1</td>
<td>50</td>
<td>600</td>
<td>261</td>
<td>74.87</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>GNP 2</td>
<td>70</td>
<td>700</td>
<td>303</td>
<td>67.34</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>GNP 3</td>
<td>70</td>
<td>500</td>
<td>423</td>
<td>63.74</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>GNP 4</td>
<td>50</td>
<td>700</td>
<td>247</td>
<td>76.21</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>GNP 5</td>
<td>30</td>
<td>600</td>
<td>553</td>
<td>43.9</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>GNP 6</td>
<td>30</td>
<td>500</td>
<td>754.1</td>
<td>37.89</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>GNP 7</td>
<td>30</td>
<td>700</td>
<td>514</td>
<td>57.34</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>GNP 8</td>
<td>50</td>
<td>500</td>
<td>289</td>
<td>60.17</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>GNP 9</td>
<td>70</td>
<td>600</td>
<td>412.3</td>
<td>64.23</td>
</tr>
</tbody>
</table>
**Formulation of surface decorated nanogel**

To improve aqueous solubility of zaltoprofen, we have prepared a zaltoprofen – Hydroxy propyl beta cyclodextrin (HP-β-CD) complex in 1:2 molar ratio using the kneading method. Gelatin nanoparticles were first prepared by two step desolvation method as described by Coester.\textsuperscript{17,18} With a slight modification in the previous method and by using DOE – Software we have optimized GNP by using different conc. of crosslinking agent and agitation speed. The 250 mg gelatin (type A) was dissolved in double distilled water under cont. heating (40°C). The resulting solution was kept at the room temperature for 10 minutes then immediately added 5ml of ethanol in gelatin solution as a desolvating agent to precipitate high molecular weight (HMW) gelatin. The supernatant was discarded & the HMW gelatin was re-dissolved by adding 5ml double distilled water containing zalt-o-cyclodextrin complex containing nearly 50 mg of zaltoprofen & stirred at 600 RPM at constant temp. i.e. 40°C. The pH of solution was adjusted to 2.5 for gelatin type A. In situ gelatin nanoparticles were formed during a second desolvation step by dropwise addition of ethanol under constant stirring until permanent turbidity was observed in solution. After 10 min, 30 µl of Glutaraldehyde (25% w/v) was added to the reaction vessel to crosslink the nanoparticles & it was further stirred for 6 hrs. at 600 RPM. The resulting solution was centrifuged for 20 min. at 10,000 rpm & the supernatant was removed to determine unloaded drug. The settled particles were washed & re-suspended in highly purified water.

The surface modification was done on zaltoprofen loaded gelatin nanoparticles by adding oleic acid in (mole ratio of gelatin to oleic acid in 1:6) for preparing nanoparticulate suspension & were incubated for 2 hrs. for topical drug delivery. To the above prepared nanogel, the pluronic F-127 was added in the incubated solution of the surface coated nanoparticles at cooling condition (4°C) in conc. of 25% of total solution under cont. stirring for 1 hr for homogenization & kept the solution overnight in cold condition for dissolution of pluronic. The diagrammatic representation is shown in Figure 1.

**Zalto-loaded gelatin nanoparticles characterization**

**Particle size**

Particle size and polydispersity index was determined using a photon correlation spectrometer (Zetasizer 3000 HAS, Malvern Ltd., UK) based on the laser light scattering phenomenon. Diluted samples were directly placed into the module and measurements were made in triplicate after 2-min stirring. Z-average particle sizes and polydispersity index (PI) were measured.

**Entrapment efficiency**

Loading of zaltoprofen in gelatin nanoparticles was carried out by calculating the amount of unentrapped drug. To calculate the same, the nanoparticulate suspension was centrifuged at 14,000 rpm for 20 min. and supernatant was collected & analyzed by a UV-Spectrophotometer at 227 nm as a λ_{max}. From the amount of drug added in the formulation. Drug entrapment was calculated by using the formula,

$$\text{Entrapment efficiency (\%)} = \frac{\text{(actual drug content in nanoparticles / theoretical drug content)}}{\times 100}$$

Samples were characterized by DSC at different ranges at a heating rate 10°C per minute.

**Fourier transform-infrared spectroscopy (FTIR)**

Fourier-transform infrared (FTIR) spectra were obtained using an FTIR spectrometer (Shimadzu 8400S, Japan). The inclusion complex was mixed with KBr. The KBr discs were prepared by compressing the powders at a pressure of 5 tons for 5 min in a hydraulic press. All the spectra acquired scans between 400 to 4000 cm\textsuperscript{-1}.

**Scanning electron microscopy (SEM)**

The morphological features of zalto-GNPs were examined by scanning electron microscopy (Q). The samples were sprinkled onto a double sided tape and sputter coated with a 5 nm thick gold layer. The inner structure of nanoparticles was observed after fracturing by a razor blade.
**Texture characterization**

**Texture Profile Analysis (TPA):** Texture profile analysis (TPA) was performed using a CT3 Texture Analyzer in TPA mode. Sensory properties included measures such as consistency, firmness, cohesiveness, (attractive forces within the formulation) & work of adhesion (attraction between the formulation & substrate). A conical shape sample holder was filled evenly with the nanogel & testing surface which was as flat as possible to avoid early triggering of the test. The probe (TA3/100) was programmed to descend into the sample at a speed of 0.5 mm/s with a target value of 20 mm and then ascend back at the same speed to its original position the force encountered by the probe to break away from the gel when the point it began to ascend (the point of maximum force) was measured.

**Spreadability:** The spreadability of the formulations is a characteristic derived from its more basic property i.e. viscosity. The greater the viscosity the longer will be the time taken for spreading. The spreadability also depends on the polymer in formulation, possessing typical physicochemical properties which create surface tension between slide and product. Spreadability test was performed by using CT3 Texture Analyzer in Compression mode. A cone analytical probe (60°C) was forced down into each sample at a defined rate (0.5 mm/s) and to a defined depth (12 mm). The test was performed and results were observed.

**Viscosity**

A Brookfield digital viscometer, cone and plate type of viscometer was used to determine viscosity (cp) of the formulations. The viscosity was measured at 5 rpm after 30 seconds, by using the spindle no. 7.

**Drug content**

The accurately weighed 0.50gm zaltoprofen nanogel was added in100ml volumetric flask which contains100 ml of PBS 6.8. The resultant solution was kept for sonication for 30 mins for complete solubility of the drug, then the resultant solution was filtered. The absorbance of 50 µg/ml solution was checked at 227 nm and compared with pure drug absorbance at the same wavelength and concentration. Thus % assay was calculated this procedure was carried out in triplicate.

**pH**

The accurately weighed 2.5 gms of gel was dispersed in 25 ml of distilled water. The pH of dispersion was measured by using digital pH meter.

**In vitro drug release**

The *in vitro* drug release study of zalto nanogel was performed to investigate the amount of drug released from a gel. A porous membrane of mol. wt. cut off 12,000- 14,000 Da (HIMEDIA) was used. The membrane was mounted between the donor & receiver compartment of Franz diffusion cells. The surface coated (oleic acid) & uncoated zalto–nanogel were then applied evenly on the surface of the membrane in the donor compartment. The receiver compartments were filled with PBS (pH 6.8), stirred at 300 rpm, and maintained at 32°C using a circulating water bath. At the predetermined time intervals (1, 2, 4, 6, 8, 12, 24, 36 and 48 h), 0.5 ml samples were collected from the receiver compartment and replace with fresh buffer solution. The samples collected from the receiver compartment were analyzed for the drug content using UV spectrometric method at 227 nm wavelength.

**Ex-vivo study**

Rat abdominal skin (0.22μ) was used as diffusion membrane. Membrane was soaked in 6.8 phosphate buffer for 10 minutes before subjecting to diffusion study. The receiver/receptor compartment contains 25ml of phosphate buffer. In the upper donor compartment 0.5 gm of formulation was spread evenly on the membrane. The receptor phase (phosphate buffer) was continuously stirred with help of magnetic stirrer during the experiments. The 3 ml of the sample was withdrawn from the receiver compartment at time intervals 1, 2, 3, 4, 5, 6, 12, 24 up to 48 hours and the same amount of fresh buffer solution was added to maintain the sink condition in the receiver compartment. Care was taken to ensure that no air bubbles were lodged underneath the diffusion membrane during the experiments. The samples were analyzed spectrophotometrically at a wavelength of 227.0 nm. Percentage of zaltoprofen in sample was determined by referring to the previously prepared standard curve.

**Drug release kinetics:** To find the kinetics of the drug release from the test formulation, the following models were used such as, zero order, first order, Higuchi and Ritger-peppas models.

**In vivo study**

To carry out *in vivo* anti-inflammatory studies, the approval was obtained from the local animal ethical committee, (RCPIPER, NMU Jalgaon) and their guidelines were followed for the studies. The sustained anti-inflammatory effect of the nanogel was evaluated by carrageenan induced hind paw edema method. Young male wistar rats weighing (200 – 250 gm) were randomly divided into three groups i.e. Control, test, Standard. In each group six animals were placed. Topical dose of the nanogel was calculated based on the weight of the rats according to the surface area ratio. The dorsal side of the rat skin was shaved 12 h before nanogel and marketed gel formulation application on the dorsal region of all animals except in control group. The plantar injection of carrageenan was given before half an hour in the right paw. Paw edema was induced by injecting 0.2 ml of 1% dispersion of carrageenan in distilled water. Certain amount of Nanogel (100 mg) was gently rubbed topically on the right hind paw of the rats. The area of application was occluded with bandages and it was left as it is for two hours. The dressing was then removed and nanogel remaining on the surface of the skin was wiped off with
Oleic acid coated nanogel for sustained delivery of zaltoprofen

a piece of cotton. The animals were then injected with 0.2 ml of 1% freshly prepared saline carrageenan solution in in plantar region on the right hind paw. The right hind paw thickness was measured with plethesmometer before and 0 and 1, 3, 6 and 12 hrs. after the sub-plantar injection. The amount of paw swelling was determined time to time and expressed as percent edema. Percent inhibition calculated against the control group and graphs can be obtained by using Graph pad prism 5 software. Results of these studies were compared using the dunnett test of one way analysis of variance.29,30

Skin irritation study
Male wistar rats (Animal ethical committee) weighing 200-250 g; were used in this study. The animals were housed in propylene cages, with free access to standard laboratory diet and water. Animals were acclimatized for at least seven days before experimentation, the dorsal abdominal skin rats were shaved 24 hrs before study. Then zaltoprofen nanogel was applied onto the dorsal skin to an area of 1” × 1” sq. on wistar rats.31

Stability study
In the present work, stability study was carried out for the optimized formulation for condition and time period. Selected formulations (10gm) were filled in lacquered aluminum tubes and were stored at an accelerated temperature (45°C ± 1°C and 75% ± 5% RH). The samples were tested initially and then at 15 days intervals for a total period of 3 months. These samples were evaluated for its appearance, pH and drug content.

Results and Discussion
Experiments of 3-level factorial design
Experimental design of different batches of zaltoprofen loaded GNP was prepared based on the $3^2$ factorial designs. The independent variables were Glutaraldehyde (X1) and Agitation speed (X2). The independent variables and their levels are shown in Table 1. Particle size of the GNP (Y1) and Entrapment efficiency (Y2) were taken as response parameters as the dependent variables given in Table 2.

Mathematical modeling
Mathematical relationship was formulated between the factors (independent variables) and responses (dependent variables) using the statistical package Design-Expert. First step in mathematical modeling was fitting the experimental data to an appropriate model. A suitable model was selected by software on the basis of different parameter obtained from regression analysis such as p-value, adjusted R2, predicted R2 and Predicted Residual Sum of Square (PRESS) value (Table 3). ANOVA was applied for estimating the significance of model, at 5% significance level given in Table 4. Focussed on maximizing the value of adjusted R2 and predicted R2. Low PRESS value indicated adequate fitting of model. General quadratic equation for two independent variables is as follow:

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_{12}X_1X_2 + \beta_1^2X_1^2 + \beta_2^2X_2^2$$

Where: $\beta_0$ is the intercept representing the arithmetic averages of all the quantitative outcomes of 9 runs. $\beta_{12}$, $\beta_1$ and $\beta_2$ are all coefficients calculated from the observed experimental values of Y, X1 and X2 are the coded levels of factors. The terms X1, X2 and Xi (i є {1, 2}) represent the interaction and quadratic terms, respectively. Coefficients with one factor represent the effect of that particular factor while the coefficients with more than one factor and those with second order terms represent the interaction between those factors and the quadratic nature of the phenomena, respectively. Synergistic effect and antagonistic effect of factor were indicated by a positive sign and negative sign in front of that factor term, respectively.

<table>
<thead>
<tr>
<th>Source</th>
<th>Linear vs Mean</th>
<th>Quadratic vs 2FI</th>
<th>Linear</th>
<th>Quadratic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>f-value</td>
<td>p-value</td>
<td>f-value</td>
<td>p-value</td>
</tr>
<tr>
<td>Y1</td>
<td>2.635</td>
<td>0.1516</td>
<td>1.23</td>
<td>0.033</td>
</tr>
<tr>
<td>Y2</td>
<td>4.33</td>
<td>0.068</td>
<td>2.21</td>
<td>0.030</td>
</tr>
</tbody>
</table>

Zaltoprofen loaded gelatin nanoparticles characterization
Particle size
The particle sizes of all 9 batches were ranges from 754.1 - 247 nm. The optimized size of zaltro loaded GNP was 247.1 nm. Average particle size for optimized batch of zaltro- GNP was found 247.1 nm with PDI value is 0.175.

From the p-values presented in Table 3, linear model and quadratic model was found to be significant for particle size. Quadratic model was selected on the basis of maximum value of adj.R2 and low PRESS value.
indicating adequate fitting of model (Table 3). Quadratic model was significant with model f-value of 10.55 (p-value < 0.0001). The quadratic equation generated by software is as follows:

\[ Y_1 = 257.08 - 113.80X_1 - 67.00X_1^2 + 30.0X_1X_2 + 227.83X_2 + 12.93X_2^2 \]

Equation reveals that both factors (X1 and X2) affect particle size characteristics significantly. It also indicated that the effect of the change in agitation speed seems to be more pronounced in comparison with that of the change in conc. of Glutaraldehyde. The combined effect of factors X1 and X2 can further be elucidated with the help of response surface plots Figure 2A, which demonstrated that Y1 varies in a linear fashion with the effect of both parameter. However, the steeper ascent in the response surface with agitation speed (X2) – instead of Glutaraldehyde conc. (X1) – is clearly discernible from response surface plots, indicating that the effect of agitation speed is comparatively more pronounced than that of Glutaraldehyde conc. From this discussion, one can conclude that the particle size may be changed by appropriate selection of the levels of X1 and X2. Figure 2B shows a linear relationship between observed response values and predicted values indicating correctness of model.

![Figure 2](image)

**Figure 2.** (A) Response surface plot showing the effect of glutaraldehyde conc. and agitation speed on particle size (Y1); (B) Linear plot between observed and predicted value of Y1.

**Entrapment efficiency**

The entrapment efficiency of zaltoprofen for various batches is given in the Table 2. The highest drug loading was 76.21% and the particle size for this batch is 247.1 nm. As the particle size decreases the surface area increases and ultimately there is more space to bind a drug hence entrapment efficiency increases.

From the p-values presented in Table 3, quadratic model was found to be significant for entrapment efficiency. Quadratic model was selected on the basis of maximum value of adj. R2 and low PRESS value indicating adequate fitting of the model (Table 3). Quadratic model was significant with model f-value of 16.53 (p-value < 0.0003). The quadratic equation generated by the software is as follows:

\[ Y_2 = 70.78 + 9.36X_1 + 6.52X_2 - 3.96X_1X_2 - 14.68X_1^2 - 0.55X_2^2 \]

Equation reveals that both factors (X1 and X2) affect entrapment efficiency characteristics of particle size significantly. Equations also indicated that the effect of the change in glutaraldehyde concentration seems to be more pronounced in comparison with that of agitation speed. The combined effect of factors X1 and X2 can further be elucidated with the help of response surface plots Figure 3A, which demonstrated that Y2 varies in a linear fashion with the effect of both factors. However, the steeper ascent in the response surface with glutaraldehyde (X1) – instead of agitation speed (X2) – is clearly discernible from response surface plots, indicating that the effect of glutaraldehyde is comparatively more pronounced than that of agitation speed. From this discussion, one can conclude that the entrapment efficiency may be changed by appropriate selection of the levels of X1 and X2. Figure 3B shows a linear relationship between observed response values and the predicted values indicating correctness of the model.

**Qualitative determination of crosslinking efficiency**

The gelatin was cross-linked with glutaraldehyde and qualitative crosslinking of gelatin nanoparticles was determined potentiometrically. The gelatin solution of conc. 20%, 40%, and 60% w/v were titrated against 0.1 M HCl. Addition of 30 µl of 0.1 M HCl, the entire 3 curve crossover at point pH 6 which is isoelectric point of gelatin type A. The gelatin nanoparticles solution of conc. 20%, 40%, and 60% w/v were titrated against 0.1 M HCl. Addition of 30 µl of 0.1 M HCl, the entire 3 curve shows crossover at point near to 4.8-5 which was less than that of the pure isoelectric point of gelatin. This difference between crossover points is indication of crosslinking of gelatin used to prepare nanoparticles. The crosslinking process involves the modification of amines group converting them into aminals. Hence in case of nanoparticles dispersion, less amine group was
present for protonation than in parent gelatin, resulting in lower IEP. Hence from the resulting potentiometric graphs indicate that gelatin got crosslinked with the glutaraldehyde.

**Differential scanning calorimetry (DSC)**
DSC is a highly useful means of detecting drug-excipient incompatibility in the formulation. Zaltoprofen alone and in formulation was studied using DSC. For the bulk material of zaltoprofen, the melting process took place with maximum peak at 138.62°C (Figure 4-I). Also DSC of pluronic f 127 polymer (Figure 4-II) shows intense peak at 58°C. DSC thermogram of freeze dried GNP showed an endotherm at 146.46°C (Figure 4-III), the shift of DSC peak is associated with the formation of crosslinked GNP in which the drug is entrapped. Hence from the above data it is confirmed that there was no interaction between drug – polymers and drug were loaded in the gelatin polymers.

**Fourier transform-infrared spectroscopy (FTIR)**
The FT-IR spectrum of the Gelatin are characterized by intense bands at 3150–3650 cm\(^{-1}\) due to O–H stretching vibrations, 2889.27 cm\(^{-1}\) due to stretching vibration of the –CH and CH\(_2\) groups, 1737.86 cm\(^{-1}\) due to C=O stretching and peak at 1671 cm\(^{-1}\) further conform the aryl C-C ester group of zaltoprofen. The FT-IR spectrums of the HP-β-CD (Figure 5 C) contains broad peak at 3200-3500cm\(^{-1}\) region due to O-H stretching, 2875-2925 cm\(^{-1}\) region due to C-H and C-H\(_2\) vibrations. Any sign of interaction would be reflected by changes in the characteristic peaks of zalto, depending on the extent of interaction. The FTIR spectra (Figure 5) of the investigated physical mixtures did not show any significant shifts in principal peak with respect to the FTIR spectra of the zalto, only the intensity of the peaks were decreased. In the FTIR spectrum of nanogel, all characteristics stretching bands disappeared along with the reduced intensity of the other band. This might indicate the inclusion of zalto in the hydrophobic cavity of the carrier.

**Scanning electron microscopy (SEM)**
The shape and surface morphology of optimized Batch GNP 4 was studied by SEM. The SEM micrograph observed formation of spherical nanoparticles with smooth surface and little wide size distribution, (Figure 6). From the figure, we can see that some particles are as small as100 nm. Also, There is clogging of some particles with other particles and it may be due to some charges developed on the surface of polymeric nanoparticles because gelatin is one of the charge sensitive polymer.

**Zaltoprofen nanogel characterization**

**Texture Characterization**

**Texture Profile Analysis (TPA):** TPA is a method to determine mechanical properties of the gel in which an analytical probe is twice suspended into the sample at a defined rate to a desired depth, allowing a predefined necessary period between the end of the first and the beginning of second compression (Figure 7).
The peak / maximum force is taken as a measurement of firmness; higher the value the thicker is the consistency of the sample. The negative region of the graph produced on probe return is an a result of the weight of sample which is lifted primarily on the upper surface of the disc on return i.e. due to the back extrusion and hence gives again an indication of consistency or resistance to flow off the disc. The maximum negative force is taken as an indication of the stickiness / cohesiveness of the sample. The more negative the value the more stuff the sample.

Results were obtained; deformation at Hardness was 5.12 mm. Adhesiveness, cohesiveness and gumminess was found to be 0.7 mJ, 0.60, 1.4 gm respectively.

Spreadability: The spreadability of zaltoprofen loaded nanogel can be evaluated with the test parameters. The graph obtained from test was uniform in nature i.e. the positive peak of graph (Figure 7) having same area as that of negative peak area hence, spradability of given test sample was good.
**Viscosity**
The values of viscosity measurements of zaltoprofen loaded nanogel can be carried out. The nanogel being made up of Gelatin nanoparticles dispersed in poloxamer gel show moderate viscosities and hence better acceptability the viscosity of optimized nanogel was found to be 734 cp.

**Drug content**
The drug content of optimized formulations was carried out in triplicate and average drug content was found to be of 90.076%. Hence uniformity of drug content was found satisfactory.

**pH**
The pH value of optimized formulations was carried out in triplicate and average pH was found to be 6.6. Hence the formulation was satisfactorily complying with pH values needed for topical application.

**In-vitro drug release**
The in-vitro drug release from zaltoprofen loaded nanogel was evaluated by in-vitro diffusion study from Franz diffusion cell, dialysis membrane used as a semi permeable membrane and at pH 6.8 temperature was about 32°C. The nanogel containing gelatin nanoparticles and nanogel containing oleic acid decorated gelatin nanoparticles were used for in-vitro drug release. The Figure 8 shows Cumulative percentage drug release from uncoated nanogel in 48 hrs, which was found to be nanogel 51 and 69 % respectively. Result indicates that, oleic acid coated gelatin nanoparticles containing nanogel shows high permeation and hence high drug release.

In-vitro drug release study from surface decorated and plane nanogel by using franz diffusion cell.

**Ex-vivo study**
The Ex-vivo drug release study of the surface decorated nanogel, where rat abdominal skin was used as diffusion membrane. The diffusion study was performed for 48 hrs, the cumulative percentage drug release shown in Figure 8 from plane nanogel was found to be 69.42 % and surface coated nanogel 78.58 % respectively. Result indicates that, oleic acid coated gelatin nanoparticles containing nanogel show high permeation and hence high drug release.

Figure 8. In-vitro(A) and Ex-vivo(B) drug release study from surface decorated (Oleic acid) and plane nanogel by using franz diffusion cell.
Drug release kinetics: To determine kinetics of drug release from the test formulation, there are models such as, zero order, first order, Higuchi and Ritger-peppas models. Zero order indicates the system where the drug release rate is independent of time and drug conc. of dissolved substances. This release is particularly important for drug having narrow therapeutic index and release of drug within 24 hrs. in this therapeutic index. First order release system indicates the release of drug totally depends upon its concentration. The Higuchi equation describes that, release of drug is controlled by diffusion. Verification of experimental data using the Ritger-peppas equation and interpretation of the release exponent (n) provides better understanding of the mechanism of controlled release. The release exponent of coated nanogel was found to be 0.9513 means, n > 0.43 then it shows anomalous transport (Non Fickian) controlled release. Also, release exponent of uncoated nanogel was found to be 0.6604 means, n > 0.43 then it shows anomalous transport (Non Fickian) controlled Release. The selection of model on the basis of goodness of fit test Regression coefficient (R) obtained by fitting experimental release data to distinct model are given in Table 5. For the zalto-GNP both coated with oleic acid and uncoated gelatin nanoparticles, it is observed that the R value is largest, when fitted to the Ritger-peppas equation as opposed to the other equation, which indicates a Ritger- peppas release from prepared nanogel.

Table 5. The Regression equation of coated zaltoprofen nanogel and uncoated zaltoprofen nanogel

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Model</th>
<th>Coated nanogel (R)</th>
<th>Uncoated nanogel (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Zero order kinetics</td>
<td>0.9053</td>
<td>0.8129</td>
</tr>
<tr>
<td>2</td>
<td>First order Kinetics</td>
<td>0.914</td>
<td>0.9153</td>
</tr>
<tr>
<td>3</td>
<td>Higuchi equation</td>
<td>0.9416</td>
<td>0.9211</td>
</tr>
<tr>
<td>4</td>
<td>Ritger - peppas</td>
<td>0.9532</td>
<td>0.9424</td>
</tr>
</tbody>
</table>

R = Regression Coefficient

Skin irritation study
For the skin irritation test the nanogel applied on dorsal area of skin. Absence of skin irritation gel formulation is acceptable by patient skin irritation test was no erythema, edema or reddening of skin. All gel formulation and marketed gel were found to be free from irritation. Thus observations indicate acceptability of these gels for topical use.

In-vivo study
Carrageenan induced paw edema inflammation test: The anti-inflammatory effect of developed Nanogel formulation (GN 5) was compared with control group treated with gel base without any active drug and marketed volini gel was used as standard shown in Figure 8. The results of study showed that % rise in paw edema of animals treated with developed nanogel formulation (GN 5) was 23%, 37%, 55% after 1 hr, 3 hr, and 6 hrs respectively shown in (Figure 9). While % rise in the animals treated with standard marketed formulation was 20%, 27%, and 44% respectively. The formulated nanogel showed statistically significant (p < 0.05) decrease in rat paw edema from 1 hr to 6 hrs after carrageenan injection. The anti-inflammatory activity of zaltoprofen drug after 1 hr of carrageenan injection may be due to inhibition of histamine or serotonin. Similarly the activity of drug even up to 6 hrs may be due to inhibition of prostaglandins.

The results of study showed that developed formulation containing zaltoprofen has anti-inflammatory activity which last up to 6 hrs after carrageenan injection. The retention of activity for longer duration may be due to bioadhesive characteristic of the formulation. The results are also comparable with standard volini gel. Nanogel formulation indicates that it can be used for local as well as for transdermal drug delivery system. An increase in the systemic anti-inflammatory effect of zaltoprofen can leads to inhibition of the inflammation process by blockining B2- type bradykinin receptor.32,31

Accelerated Stability study
The accelerated stability studies were done for the developed formulations, during stability study the formulations were analysed for pH, and drug content. After a 3 month study, it was revealed that there was no change observed in homogeneity. Formulation showed slight changes in pH, but they were in acceptable limits (±0.5). Study of drug content i.e. in the range of 89.06% to 86.23% within 3 months, revealed that there was no definite change observed for the drug degradation.

Conclusion
The present approach gives us promising results regarding sustained analgesic activity, stability of zaltoprofen within the GNP and its acceptable texture characteristics for the sake of better patient compliance. The retention of activity up to longer duration may be due to bioadhesive characteristic of the formulation. In vivo studies of our formulation gives significant results when compared with marketed nanogel. Stability data revealed stability of formulation up to 3 months.
Oleic acid coated nanogel for sustained delivery of zaltoprofen

**Figure 9.** Effect of nanogel on carrageenan induced rat paw edema (1 hr, 3 hr, 6 hr) and Effect of nanogel on carrageenan induced rat paw edema.

**Acknowledgments**
The authors are grateful to the managements of HRPIPER, Shirpur and SCPER Kopargaon, for providing necessary facilities to carry out research work.

**Ethical Issues**
Not applicable.

**Conflict of Interests**
The authors report no conflict of interests.

**References**