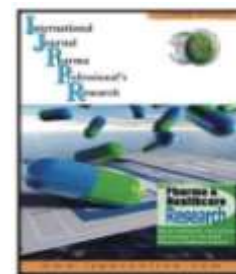




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**DEVELOPMENT AND CHARACTERIZATION OF ROSIGLITAZONE LOADED  
PLGA NANOPARTICLES USING TWO STEP DESOLVATION METHOD**

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**Keywords:**

Nanoparticles, drug delivery, TEM, Rosiglitazone maleate, two steps desolvation.

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**ABSTRACT:**

Application of nanotechnology in drug delivery system has opened up new areas of research in sustained release of drugs. The nanoparticles have the advantages of reaching otherwise less accessible sites in the body by escaping phagocytosis and entering tiny capillaries. Sustained release of the drug from the nanoparticles could maintain the therapeutic concentration for long durations. Rosiglitazone loaded PLGA nanoparticles were prepared by two step desolvation method. The nanoparticles were characterized for various parameters. Drug encapsulation efficiency was found to be between 82-90%. In vitro release study was conducted across a spectrapore membrane (cut off 3500 Da) precluding PLGA. Nanoparticle formulation released the drug at a sustained rate for prolonged duration (80% drug released at the end of 32 h). The release pattern followed the Korsmeyer Peppas equation. PLGA nanoparticles also exhibited excellent redispersibility and minimal increase in particle size. The results indicated that two step desolvation method is well suited to prepare PLGA nanoparticles and the process variables of the procedure can be fine-tuned depending on the clinical applications.

**Introduction:**

Colloidal drug carriers, which mainly involve submicron emulsions, nanoparticles, liposomes and lipid complexes, have been attracting increasing interest in recent years, mainly as vehicles of lipophilic drugs for i.v administration and also as improved delivery systems for drug targeting. The fact that most

drugs have not only pharmacological effects, but that they also exhibit side effects, makes the concept of drug targeting very attractive. Nanoparticles are solid colloidal particles ranging in size from 10 nm to 1000 nm. They consist of macromolecular materials in which the active principle is dissolved, entrapped, or encapsulated

Nanoparticles exhibit attractive properties like high stability and the ability to modify their surface characteristics easily. Nanoparticles are expected to be promising carriers for the transport of the drugs. Moreover, since the drug targeting at the cellular or tissue level is size dependent, small particles are taken up to a higher degree than larger ones. Rosiglitazone maleate is an antidiabetic drug used in Type 2 diabetes.

It has short half life of 3-4 h. Although the drug is highly insulin sensitive, it suffers major drawbacks such as hepatic toxicity, anemia, g.i.t disturbances and oedema. PLGA is one of the basic materials that can be used for the production of nanoparticles. It is a natural macromolecule that is readily available, possesses a relatively low antigenicity and is widely used in parenteral formulations. The preparation of PLGA nanoparticles (NP) by desolvation method was first described in 1978. This method was rather tedious and the resulting particles frequently encounter stability problems; these particles not only formed irreversible aggregates during cross linking, but additionally tended to aggregate. This aggregation process was highly undesirable. To overcome these problems two step desolvation method was opted to prepare nanoparticles that resulted in reduced tendency for aggregation<sup>4</sup>. The primary goal of this study was to formulate and characterize rosiglitazone maleate loaded nanoparticles.

## **MATERIALS AND METHODS**

Rosiglitazone was a generous gift from GLPL Pvt.Ltd, Baroda. Glutaraldehyde 25% LR was procured from S.D. fine Chem. Ltd Mumbai and PLGA was procured from CDH New Delhi. All other materials used were of analytical grade.

### **Preparation of rosiglitazone loaded PLGA nanoparticles<sup>5</sup>:**

PLGA nanoparticles were prepared by two step desolvation method<sup>5</sup>. PLGA type B (60mg) was dissolved in 1.2 ml water at 350-370C temperature. In order to achieve the desolvation and rapid sedimentation of the PLGA, 1.2 ml of acetone was added to this solution. The supernatant consisted of some desolvated PLGA as well as PLGA in solution and was discarded. The remaining sediment was redissolved in 1.2 ml of water under 350-370C temperature. The PLGA was then desolvated again by

drop wise addition of 1.2 ml acetone. 2% Tween 80 (2 ml) and drug 60mg were added in the PLGA solution. After 10 min of stirring, glutaraldehyde 100 µl/ml (25%) was added to cross link the particles. After stirring for another 8 hrs, the dispersion was centrifuged at 10,000 g for 30 min. The particles were purified by three fold centrifugation and redispersion in 5ml of acetone: water (30:70 ratio) and sonicate it. After the last redispersion, the acetone was evaporated on a water bath at 35°C.

### **Encapsulation efficiency<sup>6</sup>**

For determination of drug entrapment, the amount of drug present in the clear supernatant after centrifugation was determined (w) by UV spectrophotometry at 243.5nm. 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 (W). Effectively, (W-w) will give the amount of drug entrapped in the pellet. Then percentage entrapment is obtained by  $(W-w) \times 100 / W$ .

### **Transmission electron microscopy (TEM)<sup>7,8</sup>**

Particle morphology was analyzed by a transmission electron microscope (Philips Netherlands) using an acceleration voltage of 200 kV. Specimens were prepared by dropping the sample solution on to an upper grid, and then a drop of 2% uranyl acetate was added to give negative stain. The grid was then allowed to stand for 1 min and excess staining solution was removed by draining. The specimens were air dried and examined using TEM.

### **Size analysis and Zeta potential<sup>9</sup>**

The mean particle size of the formulation was determined by photo correlation spectroscopy with a zeta master (Malvern Instruments, UK) equipped with the Malvern PCS software. Every sample was diluted with phosphate buffered saline pH 7.4. The surface charge (Zeta potential) was determined by measuring the electrophoretic mobility of the nanoparticles using a Malvern zeta sizer (Malvern Instruments, UK). Samples were prepared by dilution with phosphate buffer saline pH 7.4. The zeta potential value was calculated by the software using Smoluchowski's equation.

### **Study of release kinetics<sup>6,10</sup>**

The release of Rosiglitazone from the NPs was studied using a United States Pharmacopoeia (USP) XXXIII dissolution test apparatus with a rotating paddle stirrer at 50 rpm and  $(37.0 \pm 0.5)^\circ\text{C}$  in phosphate buffer (pH 7.4). 5 ml sample of NPs equivalent to 8 mg of Rosiglitazone was used in each test. Samples of dissolution fluid were withdrawn through a filter (0.45  $\mu\text{m}$ , Millipore Millex-HN) at different time intervals and were assayed at 243.5 nm for Rosiglitazone content using a double-beam UV spectrophotometer. The drug release experiments were conducted in triplicate ( $n = 3$ )

## RESULTS AND DISCUSSION

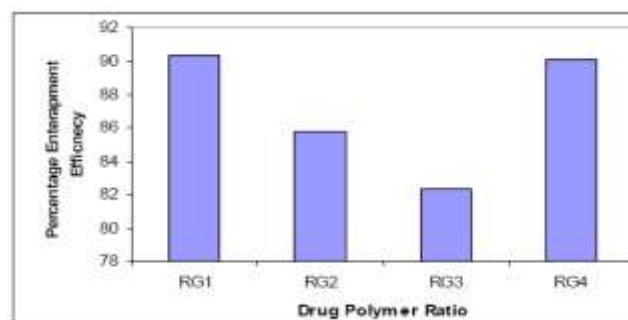
### Encapsulation efficiency

The effect of formulation variables as rosiglitazone maleate PLGA ratio on the encapsulation efficiency of nanoparticles is shown in Table 1. The entrapment efficiency of nanoparticles decreased with increase in the rosiglitazone PLGA ratio (Figure 1). It is probably due to presence of desolvating agent acetone. Literature showed the evidence that an increase of acetone volume in the formulations decrease the drug loading due to solubility of rosiglitazone maleate in acetone. The highest encapsulation efficiency was showed by the formulation ratio 1:1.

**Table 1:** Preparative Variables and Results of Rosiglitazone Loaded PLGA Nanoparticles entrapment efficiency

| Sample No. | Batch code | Drug : polymer ratio | Entrapment Efficiency (%) |
|------------|------------|----------------------|---------------------------|
| 1.         | RG 1       | 1:1                  | 90.40                     |
| 2.         | RG 2       | 1:2                  | 85.81                     |
| 3.         | RG 3       | 1:3                  | 82.33                     |
| 4.         | RG 4       | 1:4                  | 90.14                     |

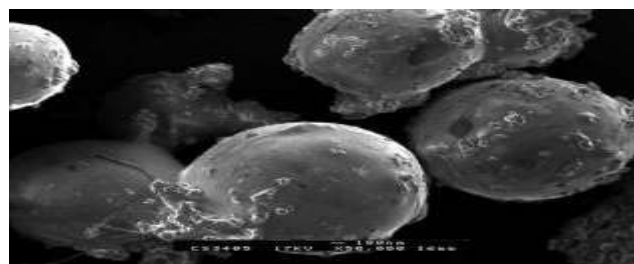
**Figure 1:** Entrapment efficiency of rosiglitazone loaded PLGA nanoparticles



### Transmission electron microscopy (TEM)

TEM of drug loaded nanoparticles (sample2) as shown in figure 2 revealed that the nanoparticles were of uniform and definite shape. The nanoparticles were found in between the size range of 22 nm to 76 nm, whereas the mean particle size by zeta sizer was 280 nm. The larger size measured by zeta sizer was probably due to the formation of aggregates.

**Figure 2:** Transmission electron microscopy (TEM) picture of the rosiglitazone loaded PLGA nanoparticles



### Size analysis and Zeta potential

Optimum stability of zeta potential was obtained at around pH 7.4. Sample 2 exhibited negative zeta potential value (-34.6 mV) with polydispersity index 1 as shown in figure 3. This negative value may be due to the quick agglomeration. Literature Size analysis and Zeta potential

Optimum stability of zeta potential was obtained at around pH 7.4. Sample 2 exhibited negative zeta potential value (-34.6 mV) with polydispersity index 1 as shown in figure 3. This negative value may be due to the quick agglomeration. Literatures showed that zeta potential and particle size is not the primary consideration in enabling the drug loading process even under iso-osmotic conditions. The relative consistency of the zeta potential value upon storage indicated that there was no significant drug diffusion out from the nanoparticles into the aqueous phase. After 3 months storage, the PLGA nanoparticles size difference was observed as shown in Table 2.

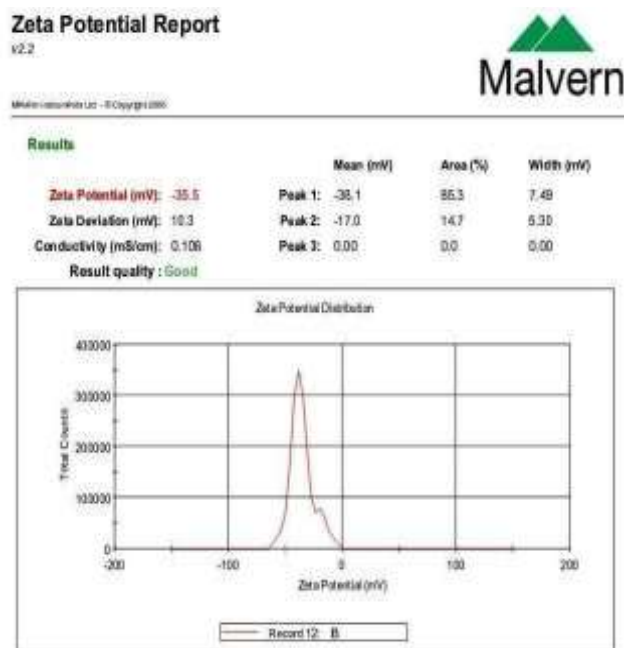
Table 2: Malvern Zeta Sizer and Transmission

Electron Microscopy results of the Rosiglitazone loaded PLGA nanoparticles.atures showed that zeta potential and particle size is not the primary consideration in enabling the drug loading process even under iso-osmotic conditions<sup>3</sup>. 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 PLGA nanoparticles size difference was observed as shown in Table 2.

**Table 2:** Malvern Zeta Sizer and Transmission Electron Microscopy results of the Rosiglitazone loaded PLGA nanoparticles.

| Size of the PLGA nanoparticles | After preparation | After 3 months |
|--------------------------------|-------------------|----------------|
| TEM                            | 49 nm             | 73 nm          |
| Malvern zeta sizer             | 230 nm            | 3230 nm        |
| Polydispersity                 | 1                 | 1              |

**Figure 3:** Zeta Potential of Rosiglitazone loaded PLGA nanoparticles

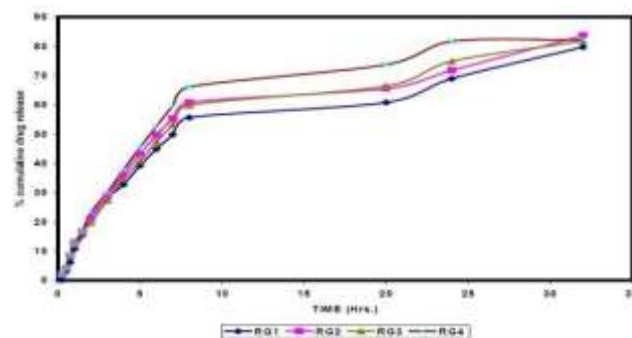


**In vitro release kinetics**

Rosiglitazone loaded PLGA nanoparticles obtained by two step desolvation method released the drug in a sustained and prolonged manner and drug release at prefixed time intervals was calculated and plotted in terms of percentage entrapment. From figure 4 it can also be seen that the nanoparticles formulations exhibited a biphasic pattern of drug release, an initial

burst effect due to immediate release of the surface associated drug and prolonged release in the later stage due to the slow diffusion of drug from the matrix. The fraction released during the first 8 h could correspond to the fractions of the free drug that was released without control from the carrier. However the drug which was covalently bound to the nanoparticles continued to release in a sustained and prolonged manner and the total amount of drug in terms of percentage entrapment was 80% at the end of 32 h (figure 4).

**Figure 4:** In vitro release kinetics of rosiglitazone loaded PLGA nanopartcles. The drug is released in a sustained manner slowly over 24 h. Total release at the end of 32 h was almost 80%.



In practical situation it would mean reduced toxicity, lesser frequent doses of application & therapeutic coverage for prolonged duration. There is a need for a sustained release formulation avoiding pulse entry, side effects due to prolonged use & frequent dosing. Our study showed that rosiglitazone loaded nanoparticles can be useful in these situations because they are of small size 22nm to 76nm which makes them suitable for targeted drug delivery & they are capable of releasing the drug in slow sustained manner over days.

**CONCLUSION**

Rosiglitazone loaded PLGA nanoparticles of sizes 22 nm to 76 nm were prepared by varying the rosiglitazone PLGA ratio. These particles showed high encapsulation efficiency of 82 % to 90 %. In vitro release kinetics study showed that the rosiglitazone loaded PLGA nanoparticles are capable of releasing the drug in a sustained manner (80 % at the end of 32 h). PLGA nanoparticles also showed excellent redispersibility and minimal increase in particle size. From the above results it can conclude that two step dissolution methods are suited to formulate rosiglitazone loaded PLGA nanoparticles. These

nanoparticles can be promising agents for rational drug delivery in diabetes.

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