Development and characterization of pioglitazone loaded liposphere for the effective treatment of diabetes mellitus type 2

Mithilesh Payasi*, Mithun Bhowmick, Girijesh Kumar Pandey, Amit Joshi and Balkrishna Dubey

TIT College of Pharmacy, Bhopal, India

* Correspondence Info:
Mithilesh Payasi
TIT College of Pharmacy, Bhopal, India
Email: mithileshpayasi04@gmail.com

Abstract
Lipospheres which represent novel drug delivery vehicles are water-insoluble lipid spheres forming a solid hydrophobic core, with a layer of phospholipids embedded on the surface of the core. Drugs or other biologically active agents may be contained in the hydrophobic core, adhered to the phospholipids, or a combination thereof. Lipid based carrier system (lipospheres) was adopted to eliminate the toxic effects associated with the use of polymers as carrier and entrapment of high amount of poorly bioavailable lipophilic compound. Due to its poor aqueous solubility of Pioglitazone HCl, was selected as drug candidate to develop lipospheres employing the melt dispersion technique. The Pioglitazone HCl - loaded lipospheres were found to be an effective natural carrier in terms of discrete particle size, encapsulation efficiency, and satisfactory in vitro release characteristics. In the presence of cetyl alcohol, the incorporated phospholipid could combine with drug by hydrophilic and hydrophobic interactions, consequently improved the drug entrapment efficiency and produced the sustained release rate.

Keywords: Lipospheres, Pioglitazone HCl, phospholipids, particle size, encapsulation efficiency, in vitro release

1. Introduction
Due to several limitations with polymeric delivery systems, extensive attempts are being made to develop alternate carriers. Lipids especially, are now being studied widely due to their attractive properties namely physicochemical diversity, ability to increase the oral bioavailability of poorly water soluble drug moieties, bio-compatible, and biodegradable that neither adheres to the gastrointestinal tract nor accumulates in the gut thus making them ideal candidates as carriers for problematic drugs. We wanted to use a technique that was rapid and economical and did not use organic solvents. Methods like solvent evaporation used in the preparation of liposomes and polyester microparticles leave organic solvent residues in the product which can result in severe acceptability and toxicity problems. Pioglitazone HCl (PZ), an oral hypoglycemic agent, is one of the most commonly prescribed drugs for the treatment of patients with diabetes mellitus type II. It is practically water insoluble. It belongs to class 2 of BCS (Biopharmaceutical classification system). Pioglitazone HCl having Short half-life (3-7 hr) and practically insoluble in water and potent drug, Pioglitazone hydrochloride to be administered in 2 to 3 doses of 15 to 45 mg per day So the strong clinical need of Pioglitazone HCl as develop prolonged and controlled release formulation of this drug for better control on type II diabetes and increase in bioavailability. The aim of the present study was to assess the feasibility of formulating lipospheres of Pioglitazone HCl by melt dispersion technique and obtaining a suitable release pattern.

2. Materials And Methods
2.1 Materials
Pioglitazone HCl was a generous gift sample from Zydus Cadila Healthcare Ltd. (India). Stearic acid, Tween 80,
cetyl alcohol, gelatin were purchased from S.D. Fine Chemicals Ltd. (India). All the other regents and chemicals used were of analytical grade.

2.2 Methods

2.2.1 Preformulation Studies

A) Organoleptic evaluation: It refers to the evaluation by sensory characters-taste, appearance, odor, feel of the drug, etc.

B) Solubility (at room temp): The spontaneous interaction of two or more substances to form a homogenous molecular dispersion is called as solubility in that solvent. For the qualitative or crude solubility study, a known amount of drug (10 mg) was suspended in a series of different solvents (10 mL) at room temperature in tightly closed test tubes and shaken on wrist action shaker for 24 hrs. The crude solubility was observed only by visual inspection.

C) Identification Test and compatibility study by FTIR Spectroscopy: Infra-red spectrum is an important record which gives sufficient information about the structure of a compound. This technique provides a spectrum containing a large number of absorption band from which a wealth of information can be derived about the structure of an organic compound.

D) Identification Test by Differential scanning calorimetry: Thermograms of Pioglitazone was recorded in a differential scanning calorimeter to characterize the solid state of the drug in the wax matrix. The samples were placed in flat bottomed aluminum pans and heated over a temperature range of 40–180 °C at a constant rate of 5 °C min⁻¹ with purging of nitrogen (50 ml min⁻¹), using alumina as a reference standard.

D) Loss on drying: Loss on drying directly measuring by IR moisture balance. Firstly calibrate the instrument by knob then take 5.000 gm sample (powder) and set the temp at 100°C to 105°C for 5 minutes and constant reading set the knob and check % moisture.

E) Determination of pH (1% w/v solution in water): 1gm of the Powder was taken and dissolved in 100ml of distilled water with sonication and filtered, pH of the filtrate was checked with standard glass electrode.

F) Melting point: It is one of the parameters to judge the purity of drugs. A small quantity of powder was placed into a fusion tube. That tube is placed in the melting point determining apparatus. The temperature was gradual increased automatically and read the temperature at which powder started to melt and the temperature when all the powder gets melted.

G) Bulk properties: Bulk density largely depends on particle shape, as the particles become more spherical in shape, bulk density increases. In addition as granules size increase, bulk density decrease. Bulk density was determined by measuring the volume of a known mass of powder sample that has been passed through a screen into a graduated cylinder or through a volumetric measuring apparatus into a cup. A known quantity of powder was poured into the measuring cylinder carefully level the powder without compacting, if necessary and read the unsettled apparent volume, V₀, to the nearest graduated unit.

The bulk density was calculated in gm per ml gm/cc, by the formula: Bulk density = Bulk Mass/ Bulk Volume

H) Compressibility index (Carr’s index): Compressibility index (C.I.) is an important measure that can be obtained from the bulk and tapped densities. Carr’s index a material having values of less than 20% to 30% is defined as the free flowing material.

It can be calculated as per given formula:

\[ C.I. = \frac{100 \times (V₀-V_f)}{V₀} \]

OR C.I. = \frac{Tapped density- Bulk density}{Tapped density} x100

I) Hausner ratio: It indicates the flow properties of the powder and is measured by the ratio of tapped density to bulk density.

\[ \text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Bulk density}} \]

J) Flow properties: The angle of repose is a relatively simple technique for estimating the flowability of a powder through a funnel and fall freely onto a surface. The height and diameter of the resulting cone is measured and using the following equation, the angle of repose can be calculated.

\[ \tan \theta = \frac{h}{r} \]

Where h, r is the relatively height and radius of the powder cone.
For most pharmaceutical powders, the angle of repose values range from 25 to 45, with lower values indicating better flow characteristics. Values of angle of repose ≤ 30 usually indicate a free flowing material and angle ≥40 suggest a poorly flowing material.

L) Moisture Content Determination: The titrimetric determination of water is based upon the quantitative reaction of water with an anhydrous solution of sulphur dioxide and iodine in the presence of a buffer that reacts with hydrogen ions.

In the original titrimetric solution, known as Karl Fisher Reagents, the sulfur dioxide and iodine was dissolved in pyridine and methanol. The test specimen may be titrated with the reagent directly, or the analysis may be carried out by a residual titration procedure. The titration of water is usually carried out with the use of anhydrous methanol as the solvent for the test specimen; however other suitable solvents may be used for special or unusual test specimens.

M) Determination of $\lambda_{\text{max}}$: Accurately weighed 10 mg of drug was dissolved in 10 ml of 0.1 N HCl in 10 ml of volumetric flask and prepare suitable dilution to make it to a concentration of 10 μg/ml make adequate of sample with concentration range of 10-30 μg/ml. The spectrum of this solution was run in 200-400 nm range in U.V spectrophotometer (Labindia-3000+).

N) Preparation of Standard Curve of Pioglitazone: 10 mg of Pioglitazone was accurately weighed and transferred to a 10 ml volumetric flask containing 10 ml of 0.1 N HCl and shaken to dissolve. The solution resulted is ≈1000 μg/ml. Then 0.1 ml of this solution is transferred to another 10ml volumetric flask to obtain solution of 10 μg/ml dilutions were done.

The absorbance was taken on double beam U.V. spectrophotometer using $\lambda_{\text{max}}$ at 272.0 nm. The absorbance values were plotted against concentration (μg/ml) to obtain the standard calibration curve.

2.2.2 Formulation Development

2.2.2.1 Method of Preparation

Drug encapsulated Liposphere were developed by melt dispersion technique. The formulation of different batches is depicted in Table 1. Briefly, the lipid core was melted on a water bath maintained at 70-72°C. Finely powdered drug was dispersed into the molten lipidic phase. The aqueous phase was prepared by heating a blend of water and surfactant to 70-72°C with a stabilizer. The molten lipidic phase was slowly transfered to the hot aqueous phase (o/w emulsion) and the emulsification was assisted by stirring the content on a sonicator continuously. The milky dispersion was then rapidly cooled to 20°C by immersing the formulation in an ice bath without stopping the agitation to yield a uniform dispersion of lipospheres. The obtained lipospheres were then washed with water and isolated by filtration.

Table No. 1: Formulation Design

| Formulation code | Drug (mg) | Lipid core (mg) | Tween 80 as Surfactant (ml) | Gelatin Stabilizer (mg) | Water (ml) |
|------------------|-----------|----------------|----------------------------|------------------------|------------|
| LP1              | 30        | 100            | 400                        | 2 ml                   | 2         | 98         |
| LP2              | 30        | 150            | 350                        | 2 ml                   | 2         | 98         |
| LP3              | 30        | 200            | 300                        | 2 ml                   | 2         | 98         |
| LP4              | 30        | 250            | 250                        | 2 ml                   | 2         | 98         |
| LP5              | 30        | 300            | 200                        | 2 ml                   | 2         | 98         |
| LP6              | 30        | 350            | 150                        | 2 ml                   | 2         | 98         |
| LP7              | 30        | 400            | 100                        | 2 ml                   | 2         | 98         |

2.2.2.2 Characterisation of Pioglitazone Encapsulated Lipospheres

i. Percentage Yield of Lipospheres

Yield of lipospheres percent w/w was calculated according to the following formula:

% Yield = Weight of lipospheres /Wt. of Drug + Wt. of Excipients

ii. Drug Entrapment efficiency

The amount of Pioglitazone present in lipospheres was determined by taking the known amount of lipospheres in
which 20 mg of drug should be present theoretically. Then the lipospheres were crushed and the powdered lipospheres was taken and dissolved in 10 ml of methanol and stirred for 15 minutes with an interval of 5 minutes and allowed to keep for 24 hours. Then the solution was filtered through whatman filter paper. Then the absorbance after appropriate dilution was measured spectrophotometrically at 272 nm by UV-visible spectrophotometer.

**Drug entrapment efficiency (%) = (Experimental drug content/Initial drug content in the formulation) × 100**

iii. Surface morphology

**Scanning electron microscopy:** Morphology and surface topography of the lipospheres were examined by scanning electron microscopy. The lipospheres from the optimized batch were mounted on the SEM sample stab using a double-sided sticking tape and coated with gold (~200 nm) under reduced pressure (0.133 Pa) for 5 min using an Ion sputtering device. The gold coated lipospheres were observed under the scanning electron microscope and photomicrographs of suitable magnifications were obtained.

iv. Flow property determination of the Lipospheres

Angle of repose (θ), Both loose bulk density (LBD), tapped bulk density (TBD), Carr’s Compressibility index, Hausners ratio of lipospheres were determined.

v. Drug Release Studies

The dissolution of Pioglitazone from the prepared lipospheres was monitored using USP XXV paddle II apparatus. The Amount of the lipospheres equivalent to 20 mg of Pioglitazone was dispersed into the dissolution medium. The dissolution media was 900 ml of 0.1 HCL (simulated gastric fluid without enzymes, pH 1.2) maintained at 37 ±0.5°C and rotating at 50 ±1 rpm. The 5ml aliquots were withdrawn at pre-determined time intervals and the withdrawn samples were replaced with fresh dissolution medium. The

3. Result and Discussion

3.1 Preformulation

3.1.1 Organoleptic evaluation

| Color | Odor     |
|-------|----------|
| White crystalline powder | Characteristic |

3.1.2. Solubility: Solubility study of Pioglitazone has been done in various solvent such as water, Phosphate buffer pH 6.8, Phosphate buffer pH 7.6 and 0.1N HCL solution. We were found that a solubility of Pioglitazone is good in a 0.1 N HCL solution.

| S.No. | Solvent   | Solubility     |
|-------|-----------|----------------|
| 1     | Water     | Poor soluble   |
| 2     | Ethanol   | Freely soluble |
| 3     | Methanol  | Freely soluble |
| 4     | Acetone   | Soluble        |
| 5     | 0.1 N HCL | Freely Soluble |
| 6     | PH 6.8 buffer | slightly soluble |
| 7     | PH 7.4 buffer | Slightly Soluble |

3.1.3. Identification test and compatibility study by FTIR: Identification of Pioglitazone by FTIR Spectroscopy with respect to marker compound.
3.1.4. Loss on Drying (LOD): The percentage of loss on drying was found to be 0.0285 %w/w.

3.1.5. Determination of pH (1% w/v solution in water): The pH determination of pioglitazone was done by Digital pH meter and found to be 2.11.

3.1.6. Melting Point: Melting point was determined by Melting point apparatus at 168-173°C.

3.2 FLOW PROPERTY OF PIOGLITAZONE POWDER

A. Bulk density:

Table no.4 Bulk Density of pioglitazone.

| S. No. | Density                     | Result     |
|-------|-----------------------------|------------|
| 1     | Untapped Density            | 0.344 g/cc |
| 2     | Tapped Density (after 50 tapping) | 0.476 g/cc |

B. Compressibility Index (%): The compressibility index of pioglitazone was found to be 27.73%.

C. Hausner ratio: The Hausner ration of pioglitazone was found to be 1.38.

D. Angle of Repose: The Angle of repose of pioglitazone was to be 35.4 Degree.

Partial size pass through 40# is 100 (%w/w).

E. Moisture by Karl-Fischer Apparatus (KF): The Moisture content of pioglitazone was to be 0.292 %.

3.3 Determination of $\lambda_{\text{max}}$ by UV-visible spectroscopy

The $\lambda_{\text{max}}$ was found to be found at 272 nm.

Table no.5 Calibration data of pioglitazone in 0.1 N HCl at $\lambda_{\text{max}}$ of 272 nm

| S. No. | Concentration (µg/ml) | Mean Absorbance at 272 nm (n=3) |
|--------|-----------------------|---------------------------------|
| 1      | 10                    | 0.171                           |
| 2      | 15                    | 0.279                           |
| 3      | 20                    | 0.404                           |
| 4      | 25                    | 0.504                           |
| 5      | 30                    | 0.585                           |
3.4 Characterisation of pioglitazone encapsulated lipospheres

3.4.1. Percentage Yield of Lipospheres: Yield of lipospheres percent w/w was calculated according to the following formula:

\[ \% \text{ Yield} = \frac{\text{Weight of lipospheres}}{\text{Wt. of Drug} + \text{Wt. of Excipients}} \]

Table No.7 Percentage Yield of Lipospheres

| Formulation Code | \% Yield |
|------------------|----------|
| LP1              | 72.64    |
| LP2              | 65.47    |
| LP3              | 63.39    |
| LP4              | 70.37    |
| LP5              | 68.30    |
| LP6              | 71.69    |
| LP7              | 78.86    |

3.4.2. Drug Entrapment efficiency

Table No.8 Drug Entrapment efficiency

| Formulation Code | \% Drug entrapment efficiency |
|------------------|-----------------------------|
| LP1              | 73.23                        |
| LP2              | 79.26                        |
| LP3              | 78.56                        |
| LP4              | 71.23                        |
| LP5              | 75.63                        |
| LP6              | 80.23                        |
| LP7              | 84.21                        |

3.4.3. Surface morphology by Scanning electron microscopy

Fig No.9 - SEM Image of Optimized Formulation No. LP7
3.4.4. Flow property determination of the Lipospheres

Table No.9 Flow Properties of Formulation No. LP1 TO LP7

| Formulation code | Parameters |  |  |  |  |
|------------------|------------|------------|------------|------------|------------|
|                  | Loose Bulk density (gm/ml) | Tapped bulk density (gm/ml) | Carr’s Index (%) | Hausner’s Ratio | Angle of Repose |
| LP1              | 0.55       | 0.61       | 16.66      | 1.10        | 31.50       |
| LP2              | 0.45       | 0.55       | 18.18      | 1.22        | 32.60       |
| LP3              | 0.51       | 0.68       | 25.00      | 1.33        | 31.50       |
| LP4              | 0.48       | 0.60       | 20.00      | 1.25        | 32.90       |
| LP5              | 0.47       | 0.55       | 14.54      | 1.17        | 33.60       |
| LP6              | 0.41       | 0.55       | 24.54      | 1.34        | 30.25       |
| LP7              | 0.51       | 0.61       | 16.66      | 1.19        | 30.89       |

3.4.5. Drug Release Studies: *In vitro* drug release study of Pioglitazone loaded Liposphere

Table No.10 Release study of Formulation LP-1

| Time  | Abs. Conc. (ug/ml) | Amt. in (mg) | DR | %DR | Drug in 5 ml | % CDR |
|-------|-------------------|--------------|----|-----|-------------|-------|
| 30    | 0.031             | 3.263        | 0.003 | 2.937 | 9.789 | 0.054 | 9.789 |
| 1     | 0.032             | 3.368        | 0.003 | 3.032 | 10.105 | 0.056 | 10.160 |
| 1.5   | 0.043             | 4.526        | 0.005 | 4.074 | 13.579 | 0.075 | 13.689 |
| 2     | 0.048             | 5.053        | 0.005 | 4.547 | 15.158 | 0.084 | 15.344 |
| 3     | 0.063             | 6.632        | 0.007 | 5.968 | 19.895 | 0.111 | 20.165 |
| 4     | 0.087             | 9.158        | 0.009 | 8.242 | 27.474 | 0.153 | 27.854 |
| 6     | 0.101             | 10.632       | 0.011 | 9.568 | 31.895 | 0.177 | 32.428 |
| 8     | 0.164             | 17.263       | 0.017 | 15.537 | 51.789 | 0.288 | 52.500 |

Table No.11 Release study of Formulation LP-2

| Time  | Abs. Conc. (ug/ml) | Amt. in (mg) | DR | %DR | Drug in 5 ml | % CDR |
|-------|-------------------|--------------|----|-----|-------------|-------|
| 30    | 0.038             | 4.000        | 0.004 | 3.600 | 12.000 | 0.067 | 12.000 |
| 1     | 0.036             | 3.789        | 0.004 | 3.411 | 11.368 | 0.063 | 11.435 |
| 1.5   | 0.042             | 4.421        | 0.004 | 3.979 | 13.263 | 0.074 | 13.393 |
| 2     | 0.048             | 5.053        | 0.005 | 4.547 | 15.158 | 0.084 | 15.361 |
| 3     | 0.075             | 7.895        | 0.008 | 7.105 | 23.684 | 0.132 | 23.972 |
| 4     | 0.099             | 10.421       | 0.010 | 9.379 | 31.263 | 0.174 | 31.682 |
| 6     | 0.112             | 11.789       | 0.012 | 10.611 | 35.368 | 0.196 | 35.961 |
| 8     | 0.145             | 15.263       | 0.015 | 13.737 | 45.789 | 0.254 | 46.579 |

Table No.12 Release study of Formulation LP-3

| Time  | Abs. Conc. (ug/ml) | Amt. in (mg) | DR | %DR | Drug in 5 ml | % CDR |
|-------|-------------------|--------------|----|-----|-------------|-------|
| 30    | 0.038             | 4.000        | 0.004 | 3.600 | 12.000 | 0.067 | 12.000 |
| 1     | 0.036             | 3.789        | 0.004 | 3.411 | 11.368 | 0.063 | 11.435 |
| 1.5   | 0.042             | 4.421        | 0.004 | 3.979 | 13.263 | 0.074 | 13.393 |
| 2     | 0.048             | 5.053        | 0.005 | 4.547 | 15.158 | 0.084 | 15.361 |
| 3     | 0.075             | 7.895        | 0.008 | 7.105 | 23.684 | 0.132 | 23.972 |
| 4     | 0.099             | 10.421       | 0.010 | 9.379 | 31.263 | 0.174 | 31.682 |
| 6     | 0.112             | 11.789       | 0.012 | 10.611 | 35.368 | 0.196 | 35.961 |
| 8     | 0.145             | 15.263       | 0.015 | 13.737 | 45.789 | 0.254 | 46.579 |
### Table No.13 Release study of Formulation LP-3

| Time | Abs. Conc. (ug/ml) | Amt. in (mg) | DR | %DR | Drug in 5 ml | % CDR |
|------|-------------------|--------------|----|-----|--------------|-------|
| 30   | 0.036             | 3.789        | 0.004 | 3.411 | 11.368       | 0.063 | 11.368 |
| 1    | 0.038             | 4.000        | 0.004 | 3.600 | 12.000       | 0.067 | 12.063 |
| 1.5  | 0.058             | 6.105        | 0.006 | 5.495 | 18.316       | 0.102 | 18.446 |
| 2    | 0.078             | 8.211        | 0.008 | 7.389 | 24.632       | 0.137 | 24.863 |
| 3    | 0.095             | 10.000       | 0.010 | 9.000 | 30.000       | 0.167 | 30.368 |
| 4    | 0.075             | 7.895        | 0.008 | 7.105 | 23.684       | 0.132 | 24.219 |
| 6    | 0.085             | 8.947        | 0.009 | 8.053 | 26.842       | 0.149 | 27.509 |
| 8    | 0.102             | 10.737       | 0.011 | 9.663 | 32.211       | 0.179 | 33.026 |

### Table No.14 Release study of Formulation LP-4

| Time | Abs. Conc. (ug/ml) | Amt. in (mg) | DR | %DR | Drug in 5 ml | % CDR |
|------|-------------------|--------------|----|-----|--------------|-------|
| 30   | 0.036             | 3.789        | 0.004 | 3.411 | 11.368       | 0.063 | 11.368 |
| 1    | 0.038             | 4.000        | 0.004 | 3.600 | 12.000       | 0.067 | 12.063 |
| 1.5  | 0.058             | 6.105        | 0.006 | 5.495 | 18.316       | 0.102 | 18.446 |
| 2    | 0.078             | 8.211        | 0.008 | 7.389 | 24.632       | 0.137 | 24.863 |
| 3    | 0.095             | 10.000       | 0.010 | 9.000 | 30.000       | 0.167 | 30.368 |
| 4    | 0.075             | 7.895        | 0.008 | 7.105 | 23.684       | 0.132 | 24.219 |
| 6    | 0.095             | 10.000       | 0.010 | 9.000 | 30.000       | 0.167 | 30.667 |
| 8    | 0.125             | 13.158       | 0.013 | 11.842 | 39.474       | 0.219 | 40.307 |

### Table No.15 Release study of Formulation LP-5

| Time | Abs. Conc. (ug/ml) | Amt. in (mg) | DR | %DR | Drug in 5 ml | % CDR |
|------|-------------------|--------------|----|-----|--------------|-------|
| 30   | 0.045             | 4.737        | 0.005 | 4.263 | 14.211       | 0.079 | 14.211 |
| 1    | 0.069             | 7.263        | 0.007 | 6.537 | 21.789       | 0.121 | 21.868 |
| 1.5  | 0.079             | 8.316        | 0.008 | 7.484 | 24.947       | 0.139 | 25.147 |
| 2    | 0.096             | 9.537        | 0.009 | 8.174 | 27.158       | 0.151 | 27.496 |
| 3    | 0.102             | 10.737       | 0.011 | 9.663 | 32.211       | 0.179 | 32.700 |
| 4    | 0.109             | 11.474       | 0.011 | 10.326 | 34.421       | 0.191 | 35.089 |
| 6    | 0.115             | 12.105       | 0.012 | 10.895 | 36.316       | 0.202 | 37.175 |
| 8    | 0.145             | 15.263       | 0.015 | 13.737 | 45.789       | 0.254 | 46.851 |

### Table No.16 Release study of Formulation LP-6

| Time | Abs. Conc. (ug/ml) | Amt. in (mg) | DR | %DR | Drug in 5 ml | % CDR |
|------|-------------------|--------------|----|-----|--------------|-------|
| 30   | 0.04              | 4.211        | 0.004 | 3.789 | 12.632       | 0.070 | 12.632 |
| 1    | 0.045             | 4.737        | 0.005 | 4.263 | 14.211       | 0.079 | 14.281 |
| 1.5  | 0.089             | 9.368        | 0.009 | 8.432 | 28.105       | 0.156 | 28.254 |
| 2    | 0.091             | 9.579        | 0.010 | 8.621 | 28.737       | 0.160 | 29.042 |
| 3    | 0.103             | 10.842       | 0.011 | 9.758 | 32.526       | 0.181 | 32.991 |
| 4    | 0.112             | 11.789       | 0.012 | 10.611 | 35.368       | 0.196 | 36.014 |
| 6    | 0.123             | 12.947       | 0.013 | 11.653 | 38.842       | 0.216 | 39.684 |
| 8    | 0.165             | 17.368       | 0.017 | 15.632 | 52.105       | 0.289 | 53.163 |
**Table No.17 Release study of Formulation LP-7**

| Time (hr) | Abs. Conc. (ug/ml) | Amt. in (mg) | DR | %DR | Drug in 5 ml | % CDR |
|----------|-------------------|-------------|----|-----|-------------|-------|
| 30       | 0.045             | 4.737       | 0.005 | 4.263 | 14.211 | 0.079 | 14.211 |
| 1        | 0.062             | 6.526       | 0.007 | 5.874 | 19.579 | 0.109 | 19.658 |
| 1.5      | 0.089             | 9.368       | 0.009 | 8.432 | 28.105 | 0.156 | 28.293 |
| 2        | 0.115             | 12.105      | 0.012 | 10.895 | 36.316 | 0.202 | 36.660 |
| 3        | 0.126             | 13.263      | 0.013 | 11.937 | 39.789 | 0.221 | 40.335 |
| 4        | 0.156             | 16.421      | 0.016 | 14.779 | 49.263 | 0.274 | 50.030 |
| 6        | 0.189             | 19.895      | 0.020 | 17.905 | 59.684 | 0.332 | 60.725 |
| 8        | 0.201             | 21.158      | 0.021 | 19.042 | 63.474 | 0.355 | 64.846 |

**Table No.18 Comparative Release Study data of formulations**

| Time (hr) | LP1 | LP2 | LP3 | LP4 | LP5 | LP6 | LP7 |
|-----------|-----|-----|-----|-----|-----|-----|-----|
| 0.5       | 9.789 | 12.000 | 12.000 | 11.368 | 14.211 | 12.632 | 14.211 |
| 1.0       | 10.160 | 11.435 | 11.435 | 12.063 | 21.868 | 14.281 | 19.658 |
| 1.5       | 13.689 | 13.393 | 13.393 | 18.446 | 25.147 | 28.254 | 28.293 |
| 2.0       | 15.344 | 15.361 | 15.361 | 24.863 | 27.496 | 29.042 | 36.660 |
| 3.0       | 20.165 | 23.972 | 23.972 | 30.368 | 32.700 | 32.991 | 40.335 |
| 4.0       | 27.854 | 31.682 | 31.682 | 24.219 | 35.089 | 36.014 | 50.030 |
| 6.0       | 32.428 | 35.961 | 35.961 | 30.667 | 37.175 | 39.684 | 60.725 |
| 8.0       | 52.500 | 46.579 | 46.579 | 40.307 | 46.851 | 53.163 | 64.846 |

**Table 19 Release Kinetics of Optimized Formulation LP-7**

| S. N. | Time (in Hrs.) | CUM%DRS | Time (in Hrs.) | LOG CUM %CDT | CUM%CDT | ROOT T | CUM%DRS | log time | log cum%DRs |
|-------|----------------|----------|----------------|---------------|----------|--------|----------|-----------|-------------|
| 1     | 0              | 0        | 0              | 2             | 100      | 0      | 0        | 0         | 0           |
| 2     | 0.5            | 14.211   | 0.5            | 1.93343161    | 85.789 | 0.7071068 | 14.211 | -0.30103 | 1.15262464 |
| 3     | 1              | 19.658   | 1              | 1.90494264    | 80.342 | 1      | 19.658 | 0         | 1.29353933 |
| 4     | 1.5            | 28.293   | 1.5            | 1.85556155    | 71.707 | 1.2247449 | 28.293 | 0.17609125 | 1.451679 |
| 5     | 2              | 36.660   | 2              | 1.80167806    | 63.34  | 1.4142136 | 36.66  | 0.301029996 | 1.56419246 |
| 6     | 3              | 40.335   | 3              | 1.77571964    | 59.665 | 1.7320508 | 40.335 | 0.47712125 | 1.60568206 |
| 7     | 4              | 50.030   | 4              | 1.69870935    | 49.97  | 2      | 50.03  | 0.602059591 | 1.6992305 |
| 8     | 6              | 60.725   | 6              | 1.59411619    | 39.275 | 2.4494897 | 60.725 | 0.77815125 | 1.78336752 |
| 9     | 8              | 64.846   | 8              | 1.54597475    | 35.154 | 2.8284271 | 64.846 | 0.903089987 | 1.81188319 |
The in vitro drug release data of the optimized formulation was subjected to goodness of fit test by linear regression analysis according to zero order, first order kinetic equation, Higuchi’s and Korsmeyer’s models in order to determine the mechanism of drug release. When the regression coefficient values of were compared, it was observed that ‘r’ values of Higuchi was maximum i.e 0.986 hence indicating drug.

4. Conclusion

Lipid based delivery systems like lipospheres offer new type of carrier system for lipophilic drugs. Easy availability of formulation ingredients and feasible, simple production techniques offer attractive option for formulation of lipospheres at industrial scale. Owing to the finer particle size of lipospheres and presence of a surface stabilized by emulsifier particles, bioavailability of several problematic drugs was found to increase. Recent works demonstrate sustained release of drugs entrapped in lipospheres. Hence lipospheres can be considered as new formulation approach for drug moieties.

The present study focused on the development of lipospheres of Pioglitazone by using stearic acid and Cetyl Alcohol as a release retarding polymer using melt dispersion technique. The in vitro data supports the retardation of lipid profile of single dose.

References

1. Simone EA, Dziubla TD, Muzykantov VR. Polymeric carriers:role of geometry in drug delivery. Expert Opin Drug Delivery.,2008;5(12):1283-1300.
2. Bennewitz MF, Saltzman WM. Nanotechnology for Delivery of Drugs to the Brain for Epilepsy. Neurotherapeutics.,2009;6(2):323-336.
3. Luxenhofer R, Schulz A, Roques C, Li S, Bronich TK, Batrakova EV, et al. Doubly amphiphilic poly (2-oxazoline) s as high-capacity delivery systems for hydrophobic drugs. *Biomaterials*, 2010;31(18):4972-4979.

4. Nakamura K, Morishita M, Ehara J, Onuki Y, Yamagata T, Kamei N, et al. Key functions in polymer carriers for intestinal absorption of insulin. *Int J Pharm.*, 2008;354(1-2):135-142.

5. Jang JH, Shea LD. Intramuscular delivery of DNA releasing microspheres: microsphere properties and transgene expression. *J Control Release.*, 2006;12(1):120-128.

6. Harris R, Lecumberri E, Heras A. Chitosan-genipin microspheres for the controlled release of drugs: clarithromycin, tramadol and heparin. *Mar Drugs.*, 2010;8(6):1750-1762.

7. Rawat A, Majumder QH, Ahsan F. Inhalable large porous microspheres of low molecular weight heparin: *in vitro* and *in vivo* evaluation. *J Control Release.*, 2008;12(3):224-232.

8. Hori Y, Winans AM, Irvine DJ. Modular injectable matrices based on alginate solution/microsphere mixtures that gel in situ and co-deliver immunomodulatory factors. *Acta biomater.*, 2009;5(4):969-982.

9. Yang L, Peng XH, Wang YA, Wang X, Cao Z, Ni C, et al. Receptor-targeted nanoparticles for *in vivo* imaging of breast cancer. *Clin Cancer Res.*, 2009;15(14):4722-4732.

10. Jain NK, Jain SK. Development and *in vitro* Characterization of Galactosylated Low Molecular Weight Chitosan Nanoparticles Bearing Doxorubicin. *AAPS PharmSciTech.*, 2010;11(2):686-697.

11. Liu J, Jiang Z, Zhang S, Saltzman WM. Poly ([omega]-pentadecalactone-co-butylene-co-succinate) nanoparticles as biodegradable carriers for camptothecin delivery. *Biomaterials* 2009;30(29):5707-5719.

12. Dey N, Majumdar S, Rao M. Multiparticulate drug delivery systems for controlled release. *Trop. J. pharm. Res.*, 2008;7(3):1067-1075.

13. Tseng YC, Tabata Y, Hyon SH, Ikada Y. *in vitro* toxicity test of 2 cyanoacrylate polymers by cell culture method. *J Biomed Mater Res A.*, 1999;24(10):1355-1367.

14. Lam K, Schakenraad J, Esselbrugge H, Feijen J, Nieuwenhuis P. The effect of phagocytosis of poly (L lactic acid) fragments on cellular morphology and viability. *J Biomed Mater Res A.*, 1993;27(12):1569-1577.

15. Mozfari MR. Liposomes: an overview of manufacturing techniques. *Cell Mol Biol Lett.*, 2005;10(4):711.

16. Fricker G, Kromp T, Wendel A, Blume A, Zirkel J, Rebmann H, et al. Phospholipids and Lipid-Based Formulations in Oral Drug Delivery. *Pharm Res.*, 2010:1-18.

17. Melander A. Influence of food on the bioavailability of drugs. *Clin Pharmacokinet.*, 1978;3(5):337.

18. Radtke M, Mülter RH. Nanostructured lipid drug carriers. *New Drugs*. 2001;2:48–52.

19. Sinha VR, Srivastava S, Goel H, Jindal V. Solid Lipid Nanoparticles (SLN’S)–Trends and Implications in Drug Targeting. *International Journal of Advances in Pharmaceutical Sciences*. 2011; 1(3):232-238.

20. Masters DB, Domb AJ. Liposphere local anesthetic timed-re-lease for perineural site application. Pharm Res., 1998;15(7):1038-1045.

21. Hersh E, Maniar M, Green M, Cooper S. Anesthetic activity of the lipospheres bupivacaaine delivery system in the rat. *Anesth Prog.*, 1992;39(6):197.

22. Domb AJ. Long acting injectable oxytetracycline-liposphere formulations. *Int J Pharm.*, 1995;124(2):271-278.

23. Amarji B, Raghuwanshi D, Vyas S, Kanaujia P. Lipid nano spheres (LNSs) for enhanced oral bioavailability of amphotericin B: development and characterization. *J Biomed Nanotechnol* 2007;3(3):264-269.
26. Bhatia A, Singh B, Rani V, Katare O. Formulation, characterization, and evaluation of benzocaine phospholipid-tagged lipospheres for topical application. *J Biomed Nanotechnol.*, 2007; 3(1):81-89.

27. Sznitowska M, Janicki S, Gajewska M, Kulik M. Investigation of diazepam lipospheres based on Witepsol and lecithin intended for oral or rectal delivery. *Acta Pol Pharm – Drug Res.*, 2000; 57:61-64.

28. Talukder R, Fassihi R. Gastroretentive Delivery Systems: A Mini Review. *Drug Dev Ind Pharm.*, 2004; 30(10): 1019-28.

29. Garg R, Gupta GD. Progress in Controlled Gastroretentive Delivery Systems. *Trop J Pharm Res.*, 2008 Sep; 7(3): 1055-66.

30. Patil JM, Hirlekar RS, Gide PS, Kadam VJ. Trends in floating drug delivery systems. *J Sci Ind Res.*, 2006 Jan; 65: 11-21.

31. Shaha SH, Patel JK, Pundarikakshudu K, Patel NV. An overview of a gastro-retentive floating drug delivery system. *Asian Journal of Pharmaceutical Sciences.* 2009 Jan; 4(1): 65-80.

32. http://www.pharmainfo.net/pharma-student-magazine/comprehensive-review-floating-tablets

33. Arora S, Ali J, Ahuja A, Khar RK, Baboota S. Floating Drug Delivery Systems: A review. *AAPS PharmSciTech.* 2005 Oct 19; 6(3): E372-E390.

34. Raza JA, Babb JD, Movahed A. Optimal management of hyperlipidemia in primary prevention of cardiovascular disease. *International Journal of Cardiology.* 2004 Dec; 97(3): 355-66.

35. Grundy SM. Atherogenic Dyslipidemia Associated with Metabolic Syndrome and Insulin Resistance. Clinical Cornerstone. 2006; 8(1): S21-S27.

36. Jain NK. Progress in controlled and Novel Drug Delivery Systems. 1st ed. Delhi: CBS Publishers and Distributors; 2004. P.76-95.

37. Vyas SP, Khar RK. Controlled drug delivery concepts and advances. 1st ed. Delhi: M.K. Jain for Vallabh Prakashan; 2002. P.196-215.

38. Mohan H. Textbook of Pathology. 5th ed. New Delhi: Jaypee; 2005. P. 250, 251, 280, 281, 282.