FORMULATION AND IN – VITRO EVALUATION OF HOLLOW MICROSPHERES OF PIOGLITAZONE HYDROCHLORIDE – A GASTRORETENTIVE CONTROLLED DRUG DELIVERY SYSTEM

P.Naga Haritha¹, S.K.Umadevi¹, P.Sunil K Chaitanya²
¹Department of Pharmaceutics, St.Pauls College of Pharmacy, Hyderabad, A.P., India.
²Department of Pharmaceutical Analysis, St.Pauls College of Pharmacy, Hyderabad, A.P., India.

ABSTRACT

Most of the floating systems have inherent drawbacks of high variability in the gastrointestinal transit time, invariably affecting the bioavailability of drug. To overcome it, a multiple unit floating system with extended GI transit time, capable of distributing widely throughout the gastrointestinal tract for effective enteric release of the drug has sought. Microballoons loaded with drug in their outer polymer shells were prepared by novel emulsion solvent diffusion method. The ethanol: dichloromethane solution of drug and polymers (HPMC, Eudragit S100, Carbapol 934) each with a ratio of 1:1, 1:2 and 1:3 respectively were poured into the solution of heavy liquid paraffin containing 1.5% span 80. The flowability of resulting microballoons improved when compared to that of the pure drug. The formulations were evaluated for percentage practical yield, particle size analysis, percentage drug loading, drug entrapment efficiency, floating behavior, Scanning Electron Micrography, FTIR, DSC, dissolution study and the drug release kinetics. The enhanced floatability of the formulations and their retention in GIT may attribute for the increased bioavailability and decrease in frequency of administration. Of all the three polymers used it was observed that HPMC to be a suitable candidate for sustained release of the drug from that of the floating microspheres (microballoons). Among the three formulations prepared with HPMC, formulation F1 (drug: polymer 1:1) was found to be an optimized one with particle size of 32.96µm, 40.46% drug entrapment efficiency, 91.03 percenta...
INTRODUCTION

Diabetes is one of the major causes of death and disability in the world. World Health Organization estimate for the number of people with diabetes worldwide in 2000 is 171 million, which is likely to be at least 366 million by 2030[1]. Non insulin dependent (Type 2) diabetes mellitus is a heterogenous disorder characterized by an underlying insufficiency of insulin. This insufficiency results from defective insulin utilization and can be corrected by administration of one or more of the currently available oral hypoglycemic agents [2]. Type 2 diabetes necessitates prolonged action as the chronic patients are characterized by deteriorated insulin sensitivity. Therefore any attempt that prolongs the half life is therapeutically beneficial.

Pioglitazone Hydrochloride is an oral anti diabetic drug belongs to thiazolidinedione class that can precisely control the blood glucose level in the type 2 diabetes mellitus [3].Its primary action is enhancement of insulin sensitivity in adipose tissue, skeletal muscle and the liver. Clinically Pioglitazone Hydrochloride decreases plasma glucose concentrations and glucosylated hemoglobin. Additional favorable metabolic effects include decreased hepatic glucose output, lower free fatty acid concentrations and improved lipid profiles [4].

The Pioglitazone Hydrochloride is a highly selective and potent agonist for the peroxisome proliferator activator receptor (PPAR-γ) that regulates the transcription of number of insulin responsive genes. Activation of PPAR – γ receptor enhances insulin sensitivity through several mechanisms. Furthermore, thiazolidinediones – mediated receptor activation promotes adipogenesis and the differentiation of adipocytes causing a favorable redistribution of fat from visceral to subcutaneous stores. Subcutaneous adipocytes tend to be less lipolytic and more insulin sensitivity. These effects contribute to the overall improved metabolic effects associated with thiazolidinediones use including insulin sensitivity peripherally [4].

The biological half life of Pioglitazone hydrochloride is 3 – 4 hrs [5] so it requires control release owing to its short biological half life. To reduce the dosing frequency of drug, it is necessary to develop a newer and safer formulation which release drug to the body for complete and prolonged duration. Moreover the site of absorption of Pioglitazone Hydrochloride is in stomach [3]. Hollow microspheres that are retained in the stomach would increase the absorption, improve drug efficiency and decrease dose requirements.

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to achieve promptly and then maintain the desired drug concentration [6]. The most convenient and commonly employed route of administration is oral route. Drugs that are easily absorbed from the gastrointestinal tract and having a short half life are eliminated quickly from the blood circulation. To avoid these problems oral controlled drug delivery systems have been developed since they release the drug slowly into the GIT and maintain a constant drug concentration in the serum for longer periods of time. Bioavailability of the dosage forms is influenced by various factors. One of the important factors is the Gastric residence time (GRT) of the dosage forms [7]. Thus, gastro retentive dosage forms, which prolong the residence time of drugs in the stomach and improve their bioavailability, have been explored and deployed [8].

APPROACHES TO GASTRIC RETENTION[9, 10]

A number of approaches have been used to increase gastric retention time of a dosage form in stomach by employing a variety of concepts. These includes in fig 1.

---

**Fig.1. Illustration of types of gastro retentive drug delivery systems.**

www.iajpr.com
Hollow microspheres:
Hollow microspheres are in strict sense, spherical empty particles without core. These microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers, ideally having a size less than 200µm. These are also called as micro balloons due to its characteristic internal hollow structure and excellent in vitro buoyancy. These are low density systems that have sufficient buoyancy to float over gastric contents and remain in stomach for prolonged period. As the system floats over gastric contents, the drug is released slowly at desired rate resulting in increased gastric retention with reduced fluctuations in plasma drug concentration [11, 12].

MECHANISM OF FLOATING MICROSPHERES
When microspheres come in contact with gastric fluid the gel formers, polysaccharides, and polymers hydrate to form a colloidal gel barrier that controls the rate of fluid penetration into the device and consequent drug release. As the exterior surface of the dosage form dissolves, the gel layer is maintained by the hydration of the adjacent hydrocolloid layer. The air trapped by the swollen polymer lowers the density and confers buoyancy to the microspheres. However a minimal gastric content needed to allow proper achievement of buoyancy. Hollow microspheres of acrylic resins, eudragit, polyethylene oxide, and cellulose acetate; polystyrene floatable shells; polycarbonate floating balloons and gelucire floating granules are the recent developments [13].
The main objective of the present work is to formulate and explore a new formulation as floating hollow microspheres (microballoons) of Pioglitazone Hydrochloride.

LITERATURE SURVEY
Literature survey revealed that no work has been reported on floating microspheres of Pioglitazone hydrochloride gastroretentive drug delivery system with three different polymers Hydroxy Propyl Methyl Cellulose (HPMC), Carbapol 934, Eudragit S100. So an attempt has been made to formulate and evaluate the same in the pursuit of a better formulation that serves the intended purpose.

MATERIALS AND METHODS
Pioglitazone Hcl was obtained as a gift sample from MSN Pharmaceuticals Ltd., Hyderabad, India. EudragitS100, HPMC, Carbapol934 were obtained as a gift samples from SIPRA pharmaceuticals, Hyderabad, India. Heavy liquid paraffin, dichloromethane, ethanol, span 80 were purchased from Merk Specialties Pvt Ltd., Hyderabad, India. All other chemicals were of analytical reagent grade and were used as received.

EXPERIMENTAL
Preformulation studies:
Preformulation testing is the first step in rational development of dosage forms of a drug substance. Preformulation study is the process of optimizing the delivery of drug through determination of physicochemical properties of new compound that could affect drug performance and development of an efficacious, stable and safe dosage form, it gives the information needed to define the nature of the drug substance and provide a framework for the drug combination with pharmaceutical excipients in the dosage form. Hence Preformulation studies were performed for the obtained sample of the drug for identification and compatibility studies. The following Preformulation studies were performed for Pioglitazone hydrochloride and polymers.
1. Determination of melting point of Pioglitazone
2. Drug – polymer compatibility studies.

Determination of melting point: [14]
Melting point was determined by taking small amount of Pioglitazone in a capillary tube closed at one end. The capillary tube was placed in an electrically plated melting point apparatus and the temperature at which the drug melts was recorded. This was performed thrice and average value was calculated.

Drug excipient compatibility studies: [15]
Excipients were integral components of almost all pharmaceutical dosage forms. The successful formulation of a stable and effective solid dosage forms depends on the selection of excipients which are added to facilitate administration of the drug and protect it from degradation.

FT – IR:
Drug polymer compatibility studies were carried out using Fourier Transform Infra Red spectroscopy to establish any possible interaction of Pioglitazone hydrochloride with the polymers used in the formulation. The FT – IR spectra of the formulations was compared with that of the pure drug.

Differential Scanning Calorimetry:
Differential scanning calorimetry (DSC) experiments were carried out to characterize the physical state of Pioglitazone in microspheres as well as to find out the presence of any interaction among drug and the excipients. The differential thermal analyzer was used for this purpose.
PREPARATION OF DRUG LOADED FLOATING MICROSPHERES

The floating microspheres of Pioglitazone hydrochloride were prepared by emulsion solvent diffusion method. Nine batches of microspheres were prepared by taking drug polymer ratio as 1:1, 1:2, and 1:3 with same drug and three different polymers (table 1). Drug and polymer were weighed and codissolved at room temperature into a mixture of ethanol and dichloromethane (1:1%v/v) with vigorous agitation to form uniform drug polymer dispersion. This was slowly poured into the dispersion medium consisting of heavy liquid paraffin (50ml) containing 1.5% span 80. The system was stirred using over head propeller agitator at a speed of 700 – 800rpm at room temperature over a period of 4 – 5 hrs, to ensure complete evaporation of the solvent. Liquid paraffin was decanted and the microspheres were separated by filtration through a watmann filter paper, washed thrice with 180 ml of n- hexane and air dried for 24 hrs.

### Table 1: Formulation batches of hollow microspheres of Pioglitazone Hydrochloride.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Ingredients</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pioglitazone HCl</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>HPMC</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Carbopol 934</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Eudragit S100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>Heavy liquid paraffin</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>Dichloromethane</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>Ethanol</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>Span 80</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>9</td>
<td>n–hexane</td>
<td>180</td>
<td>180</td>
<td>180</td>
<td>180</td>
<td>180</td>
<td>180</td>
<td>180</td>
<td>180</td>
<td>180</td>
</tr>
</tbody>
</table>

All the proportions mentioned are drug: polymer ratios.

### Characterization of hollow microspheres:

Floating microspheres are characterized by their micromeritic properties such as particle size, tapped density, compressibility index, true density and flow properties [16].

Particle size of PIO was determined by using a microscope. Standard stage micrometer is used to calibrate the eye piece micrometer. Stage micrometer is a glass slide (7.5cm X 2.5cm) which has the scale engraved on it. The scale is usually 1mm in length. One mm is divided into 100 divisions (0.1 and 0.01 parts). Thus the smallest division (least count) of the stage micrometer represents 0.01mm (10µm) length. Mean particle size was calculated by measuring 200 – 300 particles with the help of a calibrated ocular micrometer.

The tapping method was used to determine the tapped density and percent compressibility index [14] as follows:

\[
\% \text{compressibility index} = \left\{1 - \frac{V}{V_0}\right\} \times 100
\]

Here V and \(V_0\) are the volumes of the sample after and before standard tapping, respectively.

True density was determined using a liquid displacement method. Porosity [17] (\(\varepsilon\)) was calculated using the equation:

\[
\varepsilon = \left\{1 - \frac{P_t}{P_p}\right\} \times 100
\]

Where \(P_t\) and \(P_p\) are tapped density and true density, respectively.

Angle of repose \(\theta\) of the microspheres, which measures the resistance to particle flow, was determined by a fixed funnel method [14] and calculated as

\[
\tan \theta = \frac{2H}{D}
\]

Where \(2H/D\) is the surface area of the free standard height of the microspheres heap that is formed on a graph paper after making the microspheres flow from the glass funnel.

### Percentage yield: [18, 19]

The percentage yield was calculated by calculating the weight of dried microspheres and then the actual weight of the drug and polymer used to prepare the microspheres. The percentage yield was calculated by the following equation

\[
\frac{\text{Weight of dried microspheres}}{\text{Weight of drug + Weight of polymer}} \times 100
\]

### Percentage drug entrapment efficiency: [20]

It was calculated by taking 50mg of microspheres. The drug was extracted from microspheres by digesting for 24hrs with 10ml of simulated gastric fluid (PH 1.2). During this period the suspension was agitated. After 24hrs, the solution was filtered and the filtrate was analyzed for drug content. The drug entrapment efficiency was calculated by using the following formula:
Floating behavior: [21]

50mg of microspheres were placed in 100ml of simulated gastric fluid (pH 2.0) containing 0.02% w/v tween 20. The mixture was stirred at 100 RPM with a magnetic stirrer. After 8hrs the layer of buoyant microspheres was pipette and separated by filtration. Particles in the sinking particulate layer were separated by filtration. Particles of both types were dried in a dessicator until constant weight was achieved. Both the fractions of microspheres were weighed and buoyancy was determined by the weight ratio of floating particles to the sum of floating and sinking particles.

\[
\text{% Buoyancy} = \frac{W_f}{W_f + W_s} \times 100
\]

Where \(W_f\) = weight of floating particles. 
\(W_s\) = weight of settled particles.

Drug loading:

The microspheres were evaluated for Pioglitazone content. The dried microspheres were crushed in mortar with pestle and the homogenous solution thus formed was sonicated for 2min at 60MHz of frequency. About 20ml of methanol was added to precipitate the polymers. Pioglitazone was analyzed by UV – Visible spectrophotometer at \(\lambda_{max}\) value of 238nm.

Scanning Electron Micrography (SEM): [22]

The surface morphology and structure were visualized by scanning electron microscopy (SEM). The samples were prepared by lightly sprinkling the microspheres powder on a double side adhesive tape which already shucked to aluminum stubs. The stubs were then placed into fine coat ion sputter for gold coating. After gold coating the samples were randomly scanned for particle size and surface morphology.

In Vitro drug release: [23]

\text{In vitro} drug release studies was carried out in USPXXI paddle type dissolution test apparatus using simulated gastric fluid (pH 1.2) as dissolution medium. Volume of dissolution medium was 900ml and bath temperature was maintained at (37±1)c throughout the study. Paddle speed was adjusted to 50rpm. At an interval of 1hr 10 ml of sample was withdrawn with replacement of 10ml fresh medium and analyzed for drug content by UV – Visible spectrophotometer at 238nm. The study was conducted for 12hrs. Cumulative percentage drug release was calculated using an equation obtained from the standard curve.

Release kinetics: [24]

To analyze the mechanism for the release and release rate kinetics of the dosage form, the data obtained was fitted into zero order, first order, Higuchi Matrix and Korsmeyer. Comparing the R values obtained the best fit model was selected.

RESULTS AND DISCUSSION

The hollow microspheres of Pioglitazone Hydrochloride were prepared by emulsion solvent diffusion method (Table 1). In this process, the diffusion of ethanol precedes the evaporation of dichloromethane from the droplet into the aqueous medium drastically reducing the solubility of polymer in the droplet and forming a gel like film on the surface. The mechanically strong solidified film produced at the surface of droplet with further depletion of ethanol prevented rupture and shrinkage of microspheres during the evaporation of dichloromethane from the droplets. The cavity produced by gas phase was gradually filled with water due to the reduced pressure inside the droplet that was caused by evaporation of dichloromethane. The hollow microspheres were prepared by removing water from the cavities of the microsphere with air drying. The presence of span 80 prevents aggregation of droplets as it acts as an emulsifying agent. It was assumed that it gets adsorbed at the interface between droplets and aqueous medium.

Preformulation studies

Identification

Melting point of Pioglitazone was found to be 189\(^0\)c which is in the range of 188 – 192\(^0\)c as reported in the literature, thus indicating the purity of the drug sample. Any impurity, if present will cause variation in the melting point of a given drug substance.

Solubility:

On performing the solubility studies the Pioglitazone was found to be soluble in dimethylformamide, slightly soluble in anhydrous ethanol, very slightly soluble in acetone and acetonitrile, practically insoluble in water and ether.
FT-IR:

The IR spectra of pure Pioglitazone hydrochloride and polymers used in the formulations showed their characteristic absorption bands in the IR region as follows.

Table: 2 FT – IR results of pure drug and the formulations.

<table>
<thead>
<tr>
<th>Functional groups</th>
<th>Pioglitazone hydrochloride</th>
<th>HPMC</th>
<th>Carbopol 934</th>
<th>Eudragit S100</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-H stretching</td>
<td>3362.28</td>
<td>3361.28</td>
<td>3361.70</td>
<td>3416.98</td>
</tr>
<tr>
<td>C = O</td>
<td>1680.74</td>
<td>1681.81</td>
<td>1688.76</td>
<td>1694.33</td>
</tr>
<tr>
<td>C – S – C</td>
<td>738.67</td>
<td>738.63</td>
<td>738.96</td>
<td>739.25</td>
</tr>
<tr>
<td>C – O – C</td>
<td>1149.22</td>
<td>1148.64</td>
<td>1153.76</td>
<td>1148.90</td>
</tr>
<tr>
<td>C = N</td>
<td>1617.93</td>
<td>1616.89</td>
<td>1614.77</td>
<td>1619.61</td>
</tr>
<tr>
<td>Aromatic C – H</td>
<td>3084.62</td>
<td>3082.87</td>
<td>3084.76</td>
<td>3084.24</td>
</tr>
<tr>
<td>Aliphatic C – H</td>
<td>2736.06</td>
<td>2742.94</td>
<td>2612.81</td>
<td>2616.53</td>
</tr>
<tr>
<td>C – N</td>
<td>1460.88</td>
<td>1462.86</td>
<td>1460.74</td>
<td>1460.78</td>
</tr>
</tbody>
</table>

The overlapping of the spectra of the drug and polymers is also many times used to ascertain the change in the position characteristic absorption bands for various groups and bonds present in the drug molecule.

In the present study, the IR spectrum of pure drug and various polymers used in the formulations are compared as described in the table. The study reveals that, there is no change in the positions of characteristic absorption bands of the pure drug in its pure form and also in with different polymers and excipients. This fact is also clear from the overlapping spectra of drug and polymers. [Figure 1]

Therefore, finally it can be concluded that, there is no interaction of the drug with different polymers and other excipients used during the present investigation. The results of the FT-IR are shown in [Figure 1]
In addition to IR spectral studies, DSC thermogram study of pure drug and its various optimized formulations is also used to establish their physical characteristics.

The DSC thermogram of pure drug (Pioglitazone hydrochloride) showed characteristic endothermic peak at 189°C indicating the melting point of the pure drug.

The melting point as observed from the thermogram of the drug is in conformation with the reported literature value. The DSC thermogram for different formulations containing HPMC, Carbopol 934 and Eudragit S100 are also taken. The comparative study of thermograms of polymers clearly reveals that the drug has an endothermic peak corresponding to melting point 191.96°C, 187.48°C and 192.79°C respectively with negligible change.

It is very much clear from the nature of thermogram that there is a negligible change in the melting point of drug with the various polymers.

The above fact is clear from the Figure [2 - 5] of the formulation containing drug with different polymers which resemble in their appearance without much variation in the position of the endothermic peaks. The DSC thermogram which generally indicates the thermal behavior of the drug suggests that there is no change in the physical characteristics including melting point of the drug in its pure form and also in its various formulations.

This suggests that there is no interaction of the drug with the polymers and other excipients used during the present study.
Characterization of microspheres:

Particle size:

Particle size analysis of all the nine formulations was done by optical microscopy method. The average particle size was found to be in the range of 30.53 ± 1.22 (F1) to 56.26 ± 3.20 µm (F3) as shown in the figure 6. There was a significant increase in the mean particle size and this may be due to high viscosity of polymer solution. Since high viscosity of polymer solution requires high energy for breaking off droplets of the emulsion. Particle size was decreased with increased stirring speed and time due to the fact that increase in stirring speed produces high energy which leads to further decrease in droplet size.
The tapped density values ranged from 0.41(F1) – 0.64g/cm³ (F4) while their true densities ranged between 1.52(F1) – 1.82g/cm³ (F4) of all the formulations which may be due to the presence of low density particles in the microspheres. The porosity of all the formulations was found to be in the range of 67(F4) – 75% (F1). The compressibility index ranged between 21(F6) – 27% (F1). All formulations showed excellent flowability in terms of angle of repose in the range 23°0.1(F2) – 34°0.7(F4). The better flow property indicates that the hollow microspheres produced are non-aggregated.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>True density* (g/cm³)</th>
<th>Tapped density* (g/cm³)</th>
<th>Compressibility index* (%)</th>
<th>Porosity * (%)</th>
<th>Angle of repose</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1.52±0.1</td>
<td>0.41±1.03</td>
<td>26.8±1.1</td>
<td>74.6±0.7</td>
<td>26°0.4±2.1</td>
</tr>
<tr>
<td>F2</td>
<td>1.54±0.1</td>
<td>0.45±1.12</td>
<td>24.3±2.0</td>
<td>72.56±1.2</td>
<td>23°9.0±1.2</td>
</tr>
<tr>
<td>F3</td>
<td>1.60±0.2</td>
<td>0.50±0.9</td>
<td>26.1±0.9</td>
<td>70.58±0.9</td>
<td>32°1.4±1.4</td>
</tr>
<tr>
<td>F4</td>
<td>1.82±0.3</td>
<td>0.64±1.3</td>
<td>24.3±1.5</td>
<td>66.66±2.2</td>
<td>34°7.2±2.3</td>
</tr>
<tr>
<td>F5</td>
<td>1.71±0.01</td>
<td>0.52±0.7</td>
<td>26.3±1.1</td>
<td>71.27±1.4</td>
<td>26°1.1±2.1</td>
</tr>
<tr>
<td>F6</td>
<td>1.57±0.2</td>
<td>0.54±1.3</td>
<td>21.0±1.3</td>
<td>67.66±1.1</td>
<td>34°2.2±2.4</td>
</tr>
<tr>
<td>F7</td>
<td>1.65±0.1</td>
<td>0.44±1.3</td>
<td>22.8±2.4</td>
<td>74.85±2.0</td>
<td>29°1.0±3.1</td>
</tr>
<tr>
<td>F8</td>
<td>1.71±0.3</td>
<td>0.49±1.0</td>
<td>20.7±1.7</td>
<td>72.92±1.5</td>
<td>31°7.4±1.2</td>
</tr>
<tr>
<td>F9</td>
<td>1.66±0.2</td>
<td>0.57±0.9</td>
<td>21.2±0.7</td>
<td>70.28±1.1</td>
<td>29°5.5±1.7</td>
</tr>
</tbody>
</table>

*= mean ± S.D (n = 3)

**Percentage yield:**

The percentage yield of microspheres calculated from theoretical and practical yield were found to be in the range of 87.1 ± 0.20 (F7) to 100.0 ± 0.9% (F3) as shown in the figure 7. The maximum yield was shown by the formulation F3 where as minimum yield was shown by the formulation F7 containing drug and eudragit in the ratio of 1: 1.

**Drug loading & drug entrapment efficiency:**

Drug loading percentage was found to be in the range of 19.12 ± 1.61(F4) to 40.17 ± 1.12 (F9). maximum drug loading was shown by F9 where as minimum by F4. Figure 8.

Drug entrapment efficiency was found to be in the range of 24.2 ± 0.50% (F4) to 91.96 ± 0.82% (F3). Formulation F3 containing drug and HPMC in the ratio of 1: 3 showed maximum drug entrapment efficiency of about 92% whereas formulation F4 containing drug and cabopol in the ratio 1: 1 showed minimum percentage drug entrapment efficiency of about 24%. Microspheres obtained from carbopol are irregular in shape. Therefore drug loss from the surface also leads to less drug entrapment efficiency of microspheres obtained from formulation F2. Rank order of percentage drug entrapment efficiency of various formulations was found to be as follows.

F3 > F6 > F9 > F5 > F2 > F8 > F1 > F7 > F4
Percentage buoyancy:
Buoyancy of microspheres was found to be in the range of 61.65 ± 0.38 (F7) to 85.27 ± 0.72 (F3) which indicates that most of the microspheres were still floating after 12hrs because of their lower density and internal voids as shown in Figure 9. Maximum buoyancy was shown by F3 containing HPMC where as minimum by that of the formulation containing Eudragit S100 (F7).

SCANNING ELECTRON MICROGRAPHY:
The surface morphology and structure were visualized by SEM. SEM analysis of microspheres revealed that all microspheres prepared were spherical in shape and have porous outer skins. [Figure 10]. The hollow nature was responsible for the microspheres flotability. The texture of microsphere was clearly seen by magnifying the single microsphere. [Figure 11]. The release of the drug from microspheres can be attributed to diffusion and erosion mechanism.
In vitro drug release:

Solubility of Pioglitazone depends on pH. Pioglitazone hydrochloride is a drug that gets easily absorbed in the stomach. Maximum absorption may be expected with increasing solubility in acidic environment. Hence floating microspheres were developed. It was assumed that better solubility of Pioglitazone hydrochloride in acidic environment of stomach may result in a greater amount of drug absorbed and its greater concentration in plasma. It is known that microspheres constitute multiple unit dosage forms which have many advantages as compared to tablets. They spread more evenly in the stomach which leads to a decreased risk of high local concentration and adverse effects. Moreover these forms are characterized by a high reproducibility of drug release due to relatively large surface area and short diffusion way of the drug. Drug release study of all the formulations was performed in simulated gastric fluid (pH 1.2) at 37°C ± 1 for 12hrs. The drug release rate of all the formulations was shown in the [Figure 12].

Drug release from these microspheres was slow, extended and dependent on the type and concentration of the polymer used. Formulations F1, F2, F4 and F5 had shown initial burst release. This is attributed to the release of the drug from the surface of microspheres as the drug might have migrated to the surface along with water during the drying process or presence of uncovered crystals on the surface of the microspheres. In case of other formulations burst release was not observed due to the drug was sufficiently encapsulated in the shell. At the end of 12th hr F1 showed maximum release of 96.42%. The rates of dissolution of HPMC batches were much better than carbopol and eudragit batches due to hydrophilic nature of the polymer. With the increase in polymer concentration there was decrease in the drug release rate in all the batches.
Release kinetics mechanism:

The in vitro release study data was fitted into various mathematical models to determine the best fit model. [Figure13 & 14]

![Zero and first order kinetics of F1](image)

**Figure 13: Zero and first order kinetics of F1**

From the literature review we can knew that for the low water soluble drug the self erosion of the matrix will be the principle model. The optimized formulation F1 had shown highest regression value of zero order kinetics (0.997).

![Higuchi and Korsemeyer models of F1](image)

**Figure 14: Higuchi and Korsemeyer models of F1**

The release exponent value n is found to be 0.88 indicating that it exhibited anomalous diffusion (non fickian transport) mechanism. Thus the drug release from the microspheres was by both diffusion and erosion.

CONCLUSIONS

It has been found that the drugs like Pioglitazone hydrochloride could be formulated as hollow microspheres, (microballoons) using the polymers like HPMC, carbopol934P and eudragitS100 by emulsion solvent diffusion method. All the formulations were evaluated for micromeritic properties and other evaluation parameters like percentage yield, entrapment efficiency, floating behavior, particle size analysis etc. With the increase in polymer concentration there is an increase in all these parameters. Upon evaluation of all the necessary parameters the formulation F1 (PIO: HPMC 1:1) was found to be an appropriate formulation. Spectral (I.R) studies and thermal (DSC) studies have established the compatibility between the drug and the polymers used. The type and concentration of polymers are the major factors affecting the drug release. Increase in the polymer concentration decreased the drug release. The formulation F1 has been proven as the best formulation with 96.42% of drug release at 12th hr. It exhibited anomalous (non fickian transport) diffusion mechanism and followed zero order kinetics. Thus by formulating Pioglitazone Hydrochloride as hollow microspheres it showed prolonged action by increase in its half life. Hence these types of dosage forms can be used in the treatment of type 2 diabetes where the prolonged action of the drug is required.

REFERENCES