Scholars Research Library
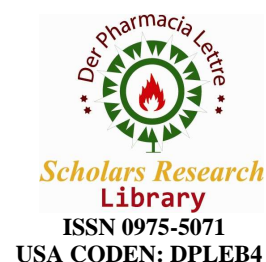
Development and evaluation of floating microspheres of Pioglitazone hydrochloride using ethyl cellulose

Satish V. Shirolkar, Mukund.G. Tawar, Nishant. S. Gandhi, Nilesh B Deore.

Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri Pune Maharashtra, India-411018

ABSTRACT

Various approaches have been used to retain the dosage form in the stomach as a way of increasing the gastric residence time (GRT), including floatation systems; high-density systems; mucoadhesive systems; magnetic systems; unfoldable, extendible, or swellable systems; and superporous hydrogel systems. The aim of this study was to prepare and evaluate floating microspheres of Pioglitazone hydrochloride for the prolongation of gastric residence time. The microspheres were prepared by emulsion solvent diffusion-evaporation method using ethyl cellulose and HPMC K100M. A full factorial design was applied to optimize the formulation. Preliminary studies revealed that the concentration of polymer and stirring speed significantly affected the characteristics of floating microspheres. The optimum batch of microsphere exhibited smooth surfaces with good flow and packing properties, prolonged sustained drug release, remained buoyant for more than 10 hrs, high entrapment efficiency upto 97%w/w. Scanning electron microscopy confirmed the hollow structure with particle size in the order of 190 µm. The studies revealed that increase in concentration of hydrophillic polymer (HPMC) increased the drug release from the floating mcirospheres. The results of 3^2 full factorial design revealed that the concentration of ethylcellulose 10 cps (X1) and stirring speed (X2) significantly affected drug entrapment efficiency, percentage release after 8 h and particle size of microspheres.

Key words: - Pioglitazone hydrochloride, ethyl cellulose, solvent diffusion evaporation, floating microspheres, factorial design.

___

INTRODUCTION

Drugs that are easily absorbed from the gastrointestinal tract (GIT) and having a short half-life are eliminated quickly from the blood circulation. To avoid this problem, the oral controlled-release (CR) formulations have been developed as these will release the drug slowly into the GIT and maintain a constant drug concentration in the serum for a longer period of time. Such oral drug delivery devices have a restriction due to the gastric retention time (GRT), a physiological limitation.

An incomplete release of the drug and shorter residence time of the dosage forms in the upper GIT, a prominent site for the absorption of many drugs, will lead to lower bioavailability [1]. Therefore, prolonged gastric retention is important in achieving control over the GRT because this helps to retain the CR system in the stomach for a longer and predicted time. In addition, this improves the bioavailability of the basic drugs that have poor solubility in higher pH [2].

Several approaches have been developed to prolong the residence time of dosage forms in the stomach [3]. Various approaches have been used to retain the dosage form in the stomach as a way of increasing the gastric residence time (GRT), including floatation systems; high-density systems; mucoadhesive systems; magnetic systems; unfoldable, extendible, or swellable systems; and superporous hydrogel systems [4]. Floating drug delivery systems (FDDS) or hydrodynamically balanced systems (HBS) are among the several approaches that have been developed to increase the GRT of dosage forms. Both single and multiple unit systems have been developed. The single- unit floating systems are more popular but have a disadvantage owing to their 'all-or-nothing' emptying process, leading to high variability of the gastrointestinal transit time [5,6]. In contrast, multiple-unit particulate dosage forms (e.g., microspheres) have the advantages that they pass uniformly through the gastrointestinal tract (GIT) to avoid the vagaries of gastric emptying and provide an adjustable release, thereby reducing the intersubject variability in absorption and risk of local irritation. Recently, hollow microspheres with a lower density than that of the GI fluids were adopted [7]. The floating microspheres were prepared by the emulsion solvent diffusion– evaporation technique using different polymer solution systems [8,9].

Pioglitazone hydrochloride(PH) acts as an agonist at peroxisome proliferator-activated receptors (PPARs) in target tissues for insulin action, such as adipose tissue, skeletal muscle, and liver. Activation of the PPAR-g regulates the transcription of insulin-responsive genes involved in the control of glucose production, transport, and utilization. In this manner, PH enhances tissue sensitivity to insulin [10]. PH has all the requisite characteristics suitable for developing an FDDS, which includes the decrease in solubility of PH with increase in pH and the half life of PH is 3-5 hrs so is eliminated quickly with conventional dosage form. Hence, floating microspheres were prepared to improve the bioavailability and achieve steady-state plasma concentration of the drug.

MATERIALS AND METHODS

Materials:-
Pioglitazone hydrochloride was obtained as gift sample from Aurobindo Pharma, Hyderabad. Ethyl cellulose N10 was obtained from Colorcon Asia pvt. Ltd, Goa. Dichloromethane (DCM), Ethanol (ETN) and Tween 80 were purchased from Research labs, Pune.

Preparation of Floating Microspheres:-
The floating microspheres were prepared by using the emulsion solvent diffusion-evaporation method similar to one reported by kawashima et al [8]. In the preliminary trials, weighed amount of drug (Pioglitazone hydrochloride), EC N10 and HPMC K100M were dissolved in a mixture of Dichloromethane (DCM): Ethanol (ETN) (1:1) at room temperature. This solution was poured into 100ml distilled water containing 0.1% Tween 80 maintained at a temperature of 30^0-40^0C. The resultant emulsion was stirred with a propeller type agitator at 1200 rpm for 45 min to allow volatile solvent to evaporate. The resultant microspheres were filtered and dried. G1 to G10 were the preliminary batches prepared using different levels of ethyl cellulose, HPMC conc., stirring speed and temperature as shown in Table 1.

Design of Experiments:-
 A 3^2 full factorial was applied to design the experiments. On the basis of Preliminary trials concentration of EC N10 and stirring speed were used as independent variables, whereas % drug release after 8hrs, % floating capacity, particle size and % entrapment efficiency were kept as dependent variables. Formulations FG1 to FG9 were prepared using three different levels of EC concentration and stirring speed. The summary of the formulations is shown in Table 2. Microspheres thus obtained were filtered, washed with water, and dried overnight at room temperature. The polynomial equations were generated for each responses using Design expert software (8.0.2) and intensive grid search was performed over the experimental   domain   to locate five optimum formulations (S1 – S_5). These five formulations were then formulated and used to validate the obtained polynomial equation model.

Table No 1:- Optimization of process and formulation of Pioglitazone ethyl cellulose Microspheres (Preliminary batches)

Batch Code	Ethyl Cellulose concentration %	Speed (rpm)	Temperature ^{0}C	HPMC K100 M concentration %
G 1	11	1200	30	1
G 2	14	1200	30	1
G 3	17	1200	30	1
G 4	20	1200	30	1
G 5	11	800	30	1
G 6	11	1600	30	1
G 7	11	1200	45	1
G 8	11	1200	60	1
G 9	11	1200	30	1.5
G 10	11	1200	30	0.5

* For all batches inner phase was dichloromethane : ethanol (1:1) and
*Inner phase(ethanol : dichloromethane) to external phase (water) solvent   ratio was 1:10 (20:200)

Table No 2:- 3^2 full factorial design for optimization of Ethyl cellulose Pioglitazone floating microspheres

Formulation	X1(EC conc.) %		X2 (RPM)	
	Actual value	Code value	Actual value	Code value
FG 1	11	-1	900	-1
FG 2	11	-1	1200	0
FG 3	11	-1	1500	1
FG 4	12.5	0	900	-1
FG 5	12.5	0	1200	0
FG 6	12.5	0	1500	1
FG 7	14	1	900	-1
FG 8	14	1	1200	0
FG 9	14	1	1500	1

* For all the formulations FG1 to FG9, 0.1% v/v span 80 was used.
*For all batches inner phase was dicholoromethane : ethanol (1:1)
*Drug weight of 300 mg was taken for all batches

Independent variables	Dependent variables
X_1 = Ethyl cellulose concentration	Y_1 = % drug release after 8 hrs
X_2 = RPM	Y_2 = % Floating capacity
	Y_3 = Microsphere size
	Y_4 = % Entrapment efficiency

Evaluation of formulations subjected to optimization:-

In Vitro Drug Release Studies:-
In vitro drug release studies were carried out using the rotating basket method specified in USP XXIII dissolution apparatus (Apparatus I) with 100 rpm speed at 37 ± 0.5^0C). Dissolution was carried out in 0.1 N HCl. The weighed amount of microspheres were wrapped in muslin cloth and kept in baskets. The drug release studies were carried out in 900 ml of 0.1N HCl as dissolution media. 5 ml Samples were withdrawn at predetermined time interval (1 h) from each dissolution vessel, filtered using Whatman filter paper, samples were analyzed for drug at 270 nm using a UV visible double beam spectrophotometer (Model-UV1701, Shimadzu, Japan).

In vitro evaluation of floating ability:-
Floating behavior of hollow microspheres was studied using a USP dissolution test apparatus II by spreading the microspheres (50 mg) on 900 ml of 0.1 N HCl containing 0.02% Tween 80 as surfactant. The medium was agitated with a paddle rotating at 100 rpm and maintained at 37°C.

After 10 hours, both the floating and the settled portions of microspheres were collected separately. The microspheres were dried and weighed. The percentage of floating microspheres was calculated using the following equation [11]:

$$\% \text{ Floating Microspheres} = \frac{\text{Weight of Floating Microspheres}}{\text{Total weight of microspheres}} \times 100$$

Microsphere size:-
The size was measured using an optical microscope and the mean microsphere diameter was calculated by measuring 100 particles with the help of a calibrated ocular micrometer [11].

Percentage yield of microspheres:-
Percentage yield of microspheres was calculated using the following formula-

$$\text{Percent Yield} = \frac{\text{The amount of microspheres obtained}}{\text{The amount (g) of Non-volatile material taken.}} \times 100$$

% Entrapment efficiency:-
Accurately weighed 10 mg of crushed microspheres were dissolved in 0.1N HCl, and then transferred to 100 ml volumetric flask. The volume was made up to 100mL with 0.1N HCl. The solution was filtered using Whatman filter paper no. 41. The samples were assayed for drug content using UV spectrophotometry at 270 nm.

Entrapment efficiency of microspheres were calculated using the following formula-

$$\text{Entrapment Efficiency} = \frac{\text{The amount of Drug Encapsulated.}}{\text{Theoretical amount of Drug.}} \times 100$$

Morphology analysis:-
The external and internal morphology of the microspheres were studied by scanning electron microscopy (SEM). The samples for SEM were prepared by lightly sprinkling microspheres on a double adhesive tape stuck to an aluminum stub. The stubs were then coated with platinum to a thickness of about 10 Å under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. Afterwards, the stubs containing the coated samples were placed in the scanning electron microscope (JSM-6360A, JEOL and Tokyo, Japan) chamber. The samples were then randomly scanned and photomicrographs were taken at the acceleration voltage of 20 kV and original magnification for 30 to 500. To investigate the further internal morphology, microspheres were cut into two pieces.

Differential Scanning Calorimetry:-
The possibility of any interaction between Pioglitazone and polymers and other excipients during the microencapsulation process was assessed by carrying out the thermal analysis on microspheres using Differential Scanning Calorimetry. Samples were accurately weighed and put into aluminum pans and then sealed with aluminum lids. The thermograms of the samples

were obtained at a scanning rate of 10^0C/min. The obtained thermographs were used to decide any interaction between Pioglitazone and polymers.

X-Ray Diffraction Studies:-

X-ray diffraction study was performed in Philips PW3710 Analytical XRD. X-ray diffractometer using Cu K 2α rays with a voltage of 40 kV and a current of 25 mA. Samples were scanned for 2θ from 5 to 50^0. Diffraction pattern for Pioglitazone and Pioglitazone – Ethyl Cellulose microspheres were obtained.

Stability Studies [12]:-

During the storage if one performs studies at normal temp it will take a longer time and hence it would be convenient to carry out the accelerated stability studies where the product is stored under extreme conditions of temperature.

Optimized formulation sealed in aluminum packaging coated inside with polyethylene, and various samples were kept in the humidity chamber maintained at 40°C and 75% RH for 2 months. At the end of studies, samples were analyzed for the physical appearance, drug content and drug release.

RESULT AND DISCUSSION

Table No. 3:- Results of Preliminary Trial Batches of Pioglitazone loaded Ethyl Cellulose Microspheres

Sr. No.	Batch Code	Sphericity	Percentage Yield (% w/w)	Mean microsphere Size (µm)	Encapsulation Efficiency (%w/w) *	% Floating capacity*	% Cumulative Drug release after 8 hrs*
1	G 1	Spherical	79.2	125.4	85.4±1.6	77.6± 2.8	80.33±1.2
2	G 2	Spherical	94.9	168.8	82.5±1.0	90.3±2.3	67.46±2.1
3	G 3	Spherical	71.2	207.5	64.6±0.9	92.6±2.6	57.53±0.9
4	G 4	Less Spherical	65.6	245.8	61.5±2.4	96.5±1.0	46.73±2.1
5	G 5	Less Spherical	77.8	235.2	78.0±3.0	94.2±1.6	90.9±1.5
6	G 6	Less Spherical	83.1	97.1	89.4±1.2	63.3±1.6	77.7±1.2
7	G 7	Non Spherical	45.6	170.4	63.6±1.2	65.8±0.8	66.5±2.2
8	G 8	Non Spherical	37.6	195.3	52.5±1.1	73.5±2.8	60.6±0.7
9	G 9	Less Spherical	59.4	110.4	81.4±0.7	65.6±0.8	94.5±1.2
10	G 10	Less Spherical	73.5	153.5	88.4±1.7	83.5±1.9	70.5±0.7

*All values are expressed as Mean ±SD, n = 3.

Preliminary trial batches:-

As the EC concentration was increased from 11 to 14 %, encapsulation efficiency decreased from 85.4 %w/w to 61.5 %w/w because at 11% and 14% concentration, enough quantity was available to form the microspheres. As the EC concentration was increased there was agglomeration of polymer resulting in lesser % encapsulation efficiency. At 20% concentration due to agglomeration yield was less i.e. only 65.6%. Increase in polymer concentration from 11 to 20% resulted in increased diameter of microspheres from 125.4 µm to 245.8µm. With increase in EC concentration, sphericity was found to be decreased due to improper coating of the polymer. For optimization EC concentration was chosen as independent variable (X_1) at three levels 11%, 12.5% and 14% for further studies.

The yield of resulting microspheres increased with increase in stirring speed. Thus when stirring speed was increased from 800 to 1600 rpm (G 5, G 1 and G 6 refer Table no.1), percentage yield increased from 77.8 to 83.1 %w/w as shown in Table no.3. At low stirring speed, polymer aggregated around the propeller shaft and the resultant yield of microsphere was less. Microsphere size was found to be decreased from 235.3 µm to 97.1 µm and size distribution was found to be uneven when the stirring speed was increased till 1600. This is because smaller emulsion droplets were produced through stronger shear forces and increased turbulence. Encapsulation efficiency was increased from 78.0 to 89.4 % w/w with an increase in the stirring speed. For optimization speed was chosen as the independent variable (X_2) at three levels i.e. 900, 1200 and 1500 rpm.

Temperature showed significant effect on % yield of microspheres. As temperature was increased from 30^0C to 45^0C (G1 and G7 Table no. 1), yield decreased from 79.21 to 45.6 % w/w as shown in Table no. 3. at 45^0C, due to rapid evaporation of solvent system did not get enough time to stabilize emulsion resulting into non-spherical microspheres. At 60^0C, there is a decrease in % yield. At 30^0C, 85.4% w/w encapsulation efficiency was observed, it decreased to 63.6 and 52.5 % at 45^0C and 60^0C .This is because as the temperature is increased solvent evaporated rapidly providing insufficient mixing time resulting into large placebo microspheres. Considering sphericity, stirring time and encapsulation efficiency, 30^0C was chosen as the temperature for further study.

As the HPMC concentration increased (0.5 to 1.5%) the % drug release increases from 70.5 % to 94.5% as HPMC is a hydrophilic polymer which leads to better drug release. Increase in HPMC concentration also leads to decrease in microspheres size and the % floating capacity. 1 % was selected as the fixed HPMC concentration for further studies.

Evaluation of formulations subjected to optimization

This floating microparticulate system was developed by emulsion solvent diffusion–evaporation method by using DCM and ETN as solvents. Finely dispersed droplets of the solution of drug and polymer solidify in the aqueous phase due to the diffusion of ETN. Evaporation of DCM from the solidified droplets leaves the cavity in the microspheres filled with water. During the drying procedure, the cavity inside each microsphere becomes filled with air, generating the microballoon.

Total nine batches of Pioglitazone loaded Ethyl Cellulose microspheres were prepared and these batches were evaluated for % yield, microsphere size, % entrapment efficiency , % floating capacity, % drug release$_{8h}$ and sphericity as shown in Table 4

Table No. 4:- Results of Batches of Pioglitazone loaded Ethyl Cellulose Microspheres

Sr. No.	Batch Code	Percentage Yield (% w/w)	Mean microsphere Size (µm)	Entrapment Efficiency (%w/w)	% Floating	Sphericity
1	FG 1	71.6	151.3	88.95±0.9	80.30±1.4	Spherical
2	FG 2	77.2	125.3	85.76±1.2	77.53±2.0	Less-Spherical
3	FG 3	83.5	107.7	79.58±1.5	72.53±1.2	Less-Spherical
4	FG 4	79.3	171.4	92.93±2.5	84.76±2.8	Less-Spherical
5	FG 5	85.7	161.3	89.53±1.8	82.46±1.4	Spherical
6	FG 6	89.7	145.5	82.75±2.9	79.16±2.9	Less Spherical
7	FG 7	89.2	189.7	97.53±1.1	89.66±1.1	Spherical
8	FG 8	94.3	178.4	94.55±1.2	85.56±1.8	Spherical
9	FG 9	96.7	160.8	90.09±1.8	83.49±0.9	Non-Spherical

% Yield of Microspheres: - The effect of EC conc. and speed was determined. The % yield increased from 71.6% to 83.5 for 11% EC conc. with increase in speed from 900 to 1500 rpm. Similarly the %yield increases for the rest of EC conc. with increase in speed. When the speed is constant at 900 rpm, the % yield increase from 71.6% to 89.2% indicating that as the EC conc. increase the % yield increases, as more amount of polymer is available so yield is improved.

Particle size: - The particle size decreased from 151.3 to 107.7 µm with increase in speed from 900 to 1500 rpm at 11 % EC conc. The same effect is observed for the EC conc 12.5% and 14% with increase in speed.

%Entrapment Efficiency: - Formulations showed good % entrapment efficiency with maximum upto 97.53% as shown in Table 4. The % entrapment efficiency decreases from 88.95 to 79.58%, 92.93 to 82.75 and 97.53 to 90.09% for 11, 12.5 and 14 % EC conc. with increase in speed from 900 to 1500 rpm. With increase in EC conc. from 11 to 14% the % entrapment efficiency increased from 88.95 to 97.53 at 900 rpm, this may be because as more amount of polymer is available to entrap the drug.

% Floating capacity:- The purpose of preparing floating microspheres was to extend the GRT of the drug. The microspheres containing EC showed good floating ability for more than 10 hrs (Table 4) due to insolubility of EC polymer in SGF (pH 1.2). With increase in speed as the particle size decreases the % floating capacity also decreases, which indicates that larger the particle size, the longer the floating time.

% Drug release:- At different EC concentration, when RPM was increased, the drug release after 8 hours from microsphere is increased for each levels of EC conc.It can also be seen that for different levels -1(11%), 0(12.5%) and 1(14%) of EC concentration the % drug release decreased (85.38% for FG1, 79.33% for FG4 and 74.73% for FG7) at fixed RPM . As the RPM increased the particle size increased which leads to decrease in drug release because release of drug from smaller particle is faster than larger particle as the area available is more for drug release. Fig 1 shows the plot of cumulative % drug release vs time graph for the 9 formulations.
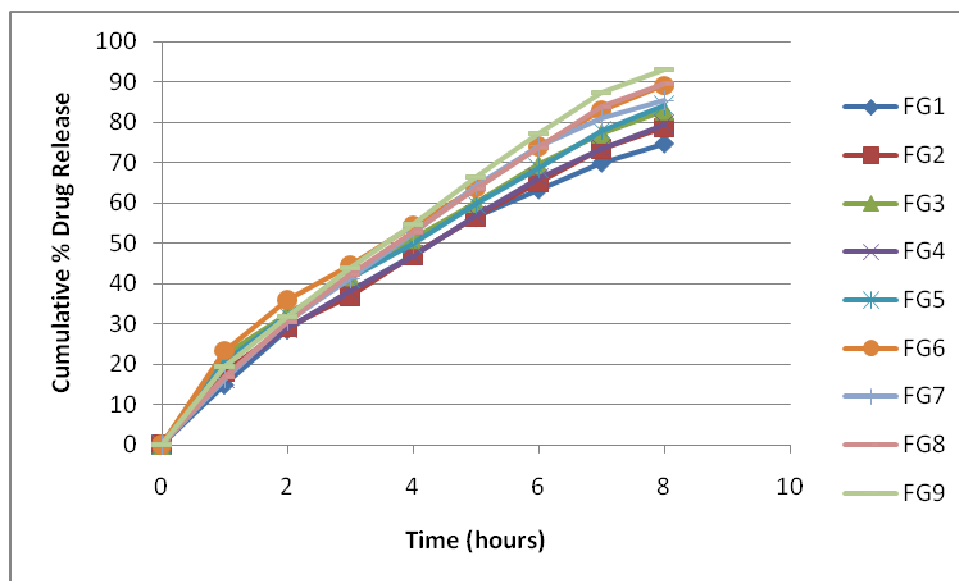
Figure 1:- Comparative cumulative % drug release profiles of formulations FG1 to FG9

Data analysis:-
On the basis of the data obtained from the formulations subjected to optimization, a general statistical model can be depicted with respect to the above data. The model developed can be characterized by using the polynomial equation representing the respective response data. This can be given as follows:

Drug Release after 8 hrs (Y1) $= 84.13 - 5.29 X_1 + 4.32 X_2$ 1

%Floating capacity (Y2) $= 81.72 + 4.73 X1 - 3.26 X2$ 2

Microsphere Size (Y3) $= 154.74 + 24.32 X_1 - 16.23 X2$ …......3

% Entrapment Efficiency (Y4) $= 82.68 + 4.64 X_1 - 4.49 X_2$ 4

From the above polynomial equations, response surface graphs and contour plots of the respective responses were generated, which were then used to predict the responses of dependent variables at the intermediate levels of independent variables.

The response surface plot (Fig. 2 a) and contour plot (Fig. 2 b) indicated the relative effect of increasing EC concentration (X_1) and RPM (X_2) on % drug release of microspheres (Y_1). With increase in RPM (X_2), % drug release was increased and with increase in EC conc. % drug release is decreased this can be confirmed from equation 1.

269

At different levels (-1, 0, 1) of EC concentration, when RPM was increased, the drug release after 8 hours from microsphere is increased for each levels of EC conc(85.38% to 93.18 for FG1 to FG3 , 79.33% to 89.14 for FG4 to FG6 and 74.73 to 83.03% for FG7 to FG9) It can also be seen that for different levels -1, 0 and 1 of EC concentration the % drug release decreases (85.38% for FG1, 79.33% for FG4 and 74.73% for FG7) at fixed RPM . As the RPM increases the particle size increases which causes the decrease in drug release because release of drug from smaller particle is faster than larger particle as the area available is more for drug release.

(a)   (b)
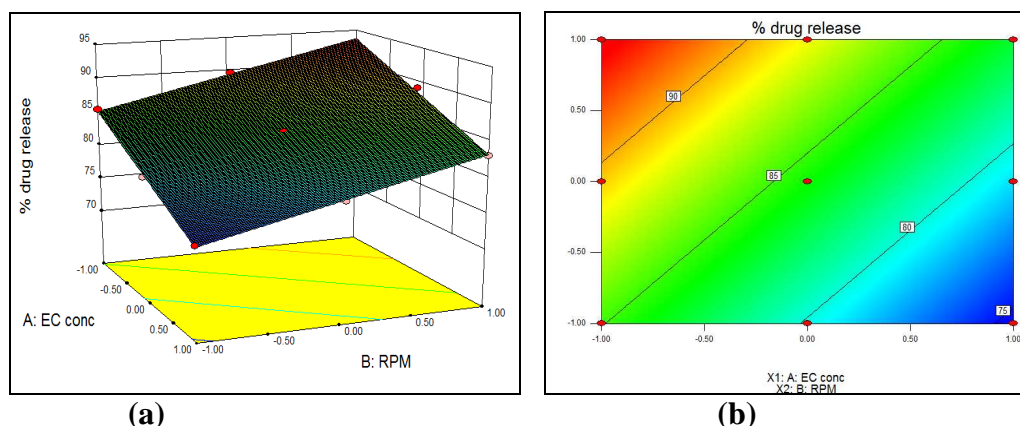
Figure 2. Various plots showing influence of RPM and EC conc. on the % drug release of microspheres a) Response surface plot and b) Contour plot.

The surface response plot (Fig. 3 a) and contour plot (Fig. 3 b) showed decrease in response Y_2 (% floating capacity) as X_2 (RPM) was increased from lower level to higher level. At lower level (-1) of RPM % floating capacity increased with increase in EC concentration i. e. at 900 RPM when polymer to drug ratio was increased from 11 to 14 % floating capacity was increased from 80.30% to 89.66%, this is because with increase in polymer there is an increase in particle size which leads to better floating capacity at same RPM. For other two levels same effect was observed. This can be further confirmed by equation 6 which showed negative sign for variable X_2 and positive sign for variable X_1 which indicated that increase in X_2(rpm) gives decrease in response and and on inrease of X_1(EC conc.) there is an increase in response.

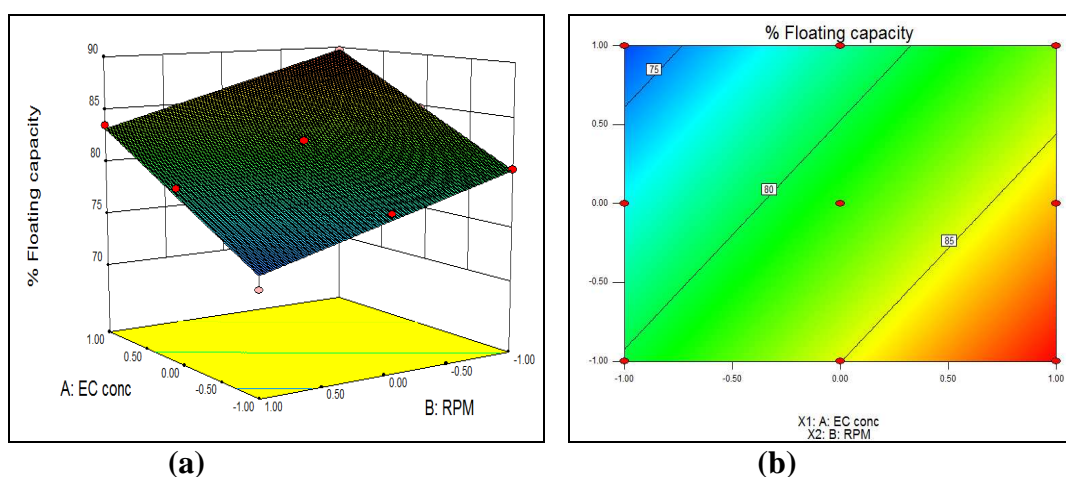(a)   (b)

Figure 3 Various plots showing influence of RPM and EC concentration on %floating capacity of microspheres a) Response surface plot and b) Contour plot

270

Effect of variable X_1 & X_2 on Microsphere size (Y_3) can be explained with response curve (Fig. 4a) and contour plot (Fig. 4b). Increase in EC concentration (X_1) results in increased particle size (Y_3) at same rpm due to availability of increased amount of polymer to form microspheres. Increase in RPM (X_2) showed the decrease in response (Y_3) this is because at higher level (1) of RPM 1600 due to greater speed the particle size is reduced.
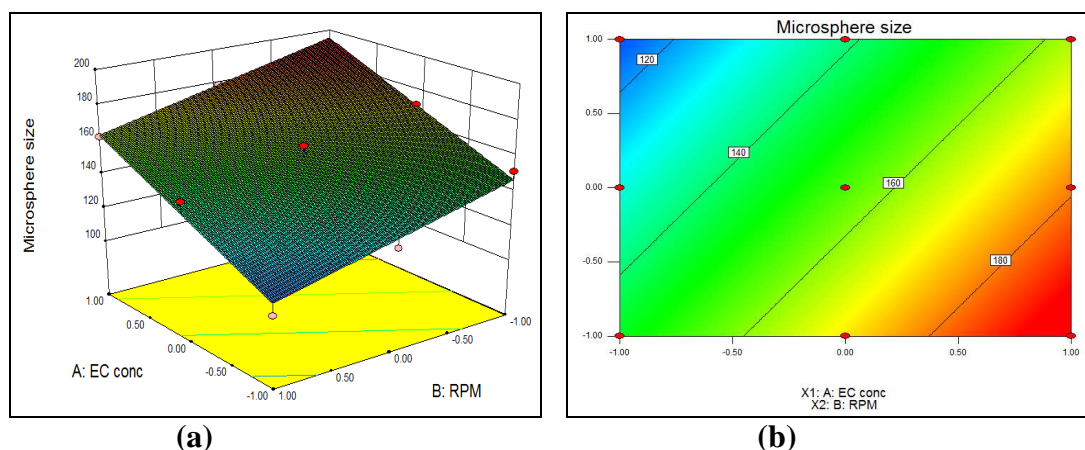

(a)   **(b)**

Figure 4 Various plots showing influence of RPM and EC Concentration on the size of microspheres a) response surface plot and b) contour plot.

Response surface plot (Fig. 5 a) and contour plot (Fig. 5 b) showed a linear effect of X_1 & X_2 on %entrapment efficiency (Y_4) and there is no interaction between two variables. At all three levels of RPM (X_2), when EC concentration was increased from lower level (-1) to higher level (1), there was increase in % entrapment efficiency from 88.95% to 97.53% at 900 rpm .This is because as there is an increase in polymer concentration the sufficient amount of polymer was present to entrap the drug thus the entrapment efficiency was increased. This can be further explained from the model equation 4.

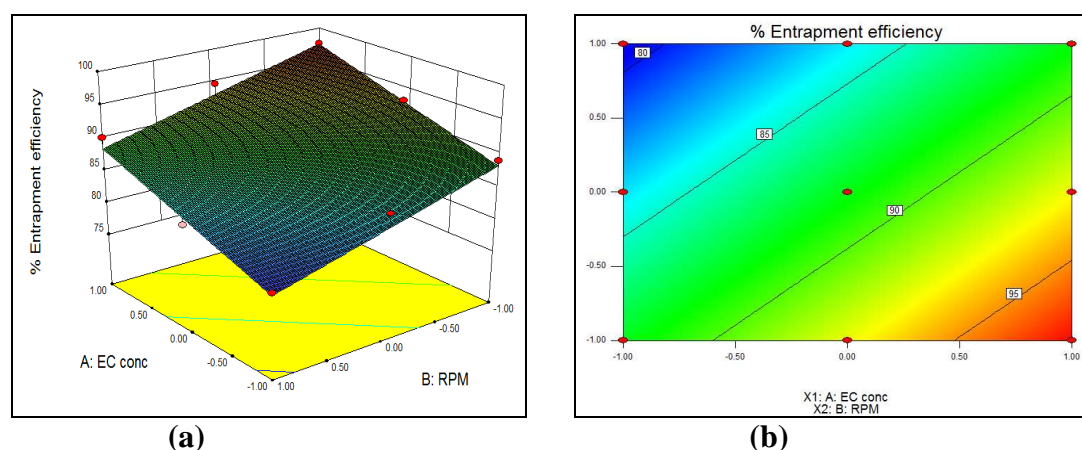**(a)**   **(b)**

Figure 5 Various plots showing influence of RPM and EC concentration on % entrapment efficiency of microspheres a) response surface plot and b) contour plot.

Validation of Optimum Microspheres Formulations:-
For all 5 checkpoint formulations shown in Table 5 the results of the physical evaluation and microspheres drug content and percentage cumulative drug release were carried out.

271

Table 5 lists the composition of the checkpoints, the predicted and experimental values of all the response variables, and the percentage error in prognosis. Fig. 6 show linear correlation plots between the observed and predicted values of % drug encapsulation efficiency, Drug Rel$_{8h}$, % floating capacity and microsphere size. The linear correlation plots drawn between the predicted and observed responses demonstrated higher values of R^2 (ranging between 0.94 and 0.98), indicating excellent fitting of model.

Table No. 5 Comparison of experimental results with predicted responses of Microsphere formulations

Batch Code	Composition		Response	Predicted Value	Experimental Value	Percentage Error
	X_1 (EC) conc%	X_2 RPM				
S1	12.5	924	Drug release (%)	74.86	73.65	-1.6
			(%) Floating	89.42	90.16	0.82
			Microsphere size µm	193.09	195.38	1.11
			EE (%)	97.04	97.34	0.30
S2	12.35	912	Drug release (%)	75.1	73.75	-1.7
			(%) Floating	89.22	89.89	0.75
			Microsphere size µm	192.17	194.8	1.3
			EE (%)	96.91	97.23	0.33
S3	12.0	900	Drug release (%)	75.15	74.74	-1.3
			(%) Floating	89.12	89.56	0.5
			Microsphere size µm	192.36	196.7	2.2
			EE (%)	96.86	97.14	0.28
S4	14.1	942	Drug release (%)	75.46	76.34	1.1
			(%) Floating	88.85	89.35	0.56
			Microsphere size µm	191.62	194.86	1.6
			EE (%)	96.54	96.23	-0.32
S5	13.5	996	Drug release (%)	76.42	78.95	3.3
			(%) Floating	88.14	88.56	0.47
			Microsphere size µm	187.52	179.5	-4.2
			EE (%)	96.51	96.12	-0.4
Mean (± S.E.M.) of Percentage Error					0.255±0.35	

Upon comparison of the observed responses with that of the anticipated responses, the Mean(± S.E.M.) of Percentage Error was found to be 0.255±0.35. Thus, the low magnitudes of error as

well as the significant values of R^2 in the current study indicated a high prognostic ability of floating microspheres formulations of Pioglitazone using RSM optimization.

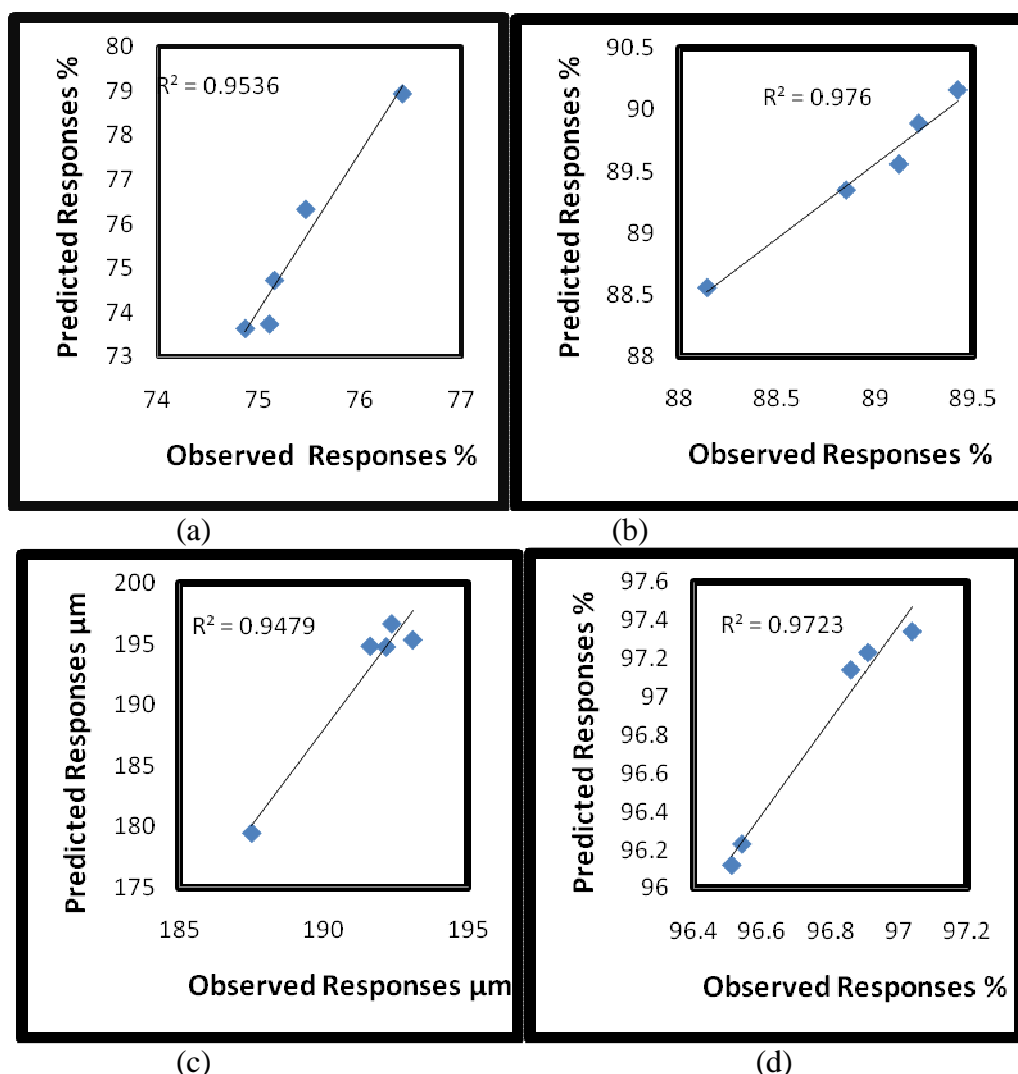(a)   (b)

(c)   (d)

Figure 6 Linear plots between observed and predicted values of (a) % Drug Release (b) % floating capacity (c) Microsphere Size (d) %Entrapment Efficiency

Morphology:-
Size and Shape
Morphology of microspheres batch (S3) was examined by scanning electron microscopy. The Fig 7a shows the top view of hollowness of microsphere and the complete microsphere, the top view of the microspheres showed a spherical structure with a smooth surface morphology shown in Fig. 7c. and 7(b) exhibited a range of microspheres within each batch.
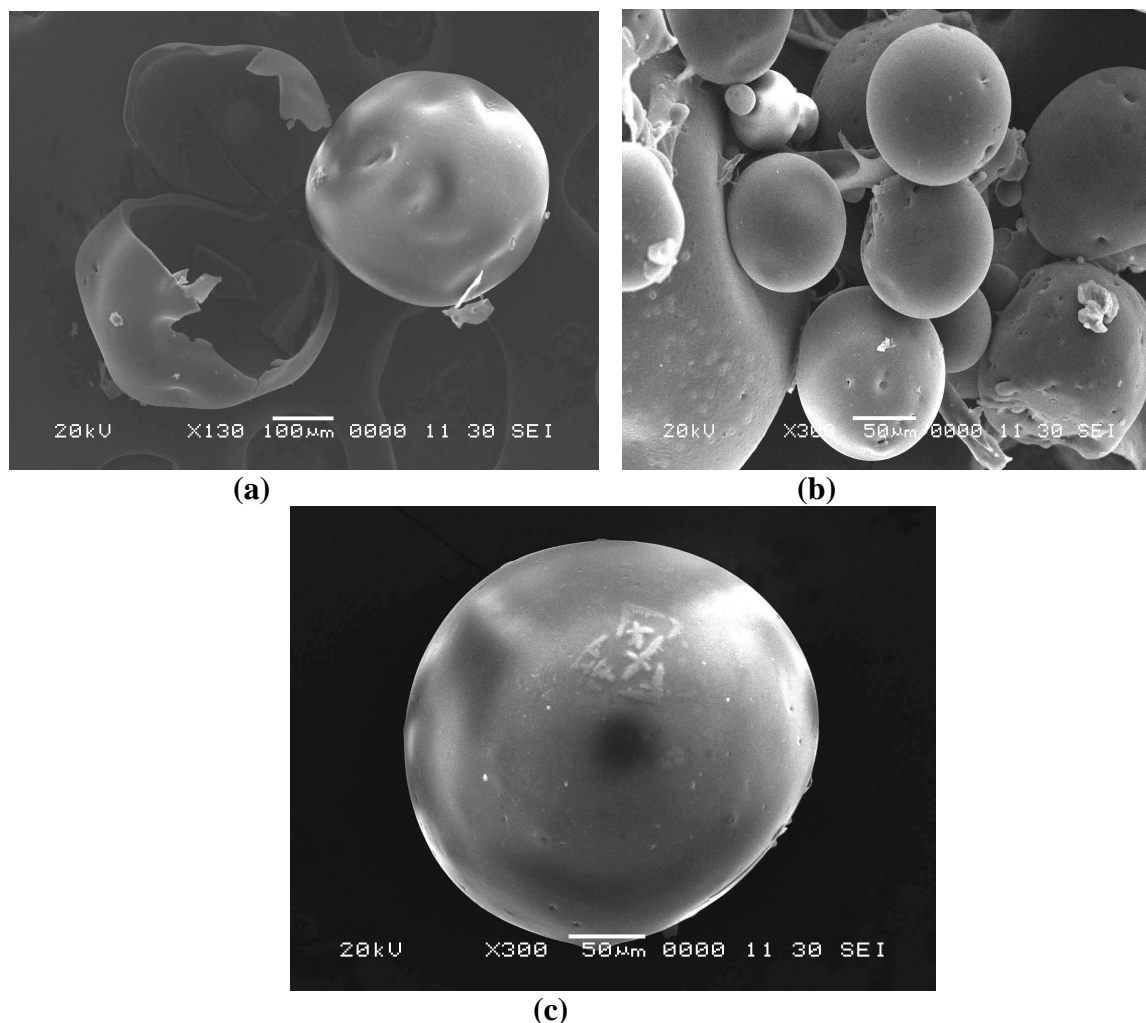
(a)

(b)

(c)

Figure 7 Scanning Electron Microscopy (SEM) of Pioglitazone loaded Ethyl Cellulose Microspheres. (a) view showing hollow microsphere (b) smooth surface of the microspheres (c) top view of microspheres showing sphericity.

Differential scanning Calorimetry:-
The differential scanning Calorimetry was carried out for the Pioglitazone Ethyl Cellulose microspheres, to study the compatibility or any interaction of drug and polymer after the formation of microspheres and is shown in Fig. 8, peak of pure drug was found at 192-193°C. The peak obtained for the Pioglitazone Ethyl Cellulose microspheres is at 193-194°C for the drug. Thus there was no significant change in the position of peak of the drug in the Pioglitazone Ethyl Cellulose microspheres. But there is change in the relative intensities of the peak of the drug as there is less amount of drug in the microspheres. This indicates that the drug is only physically entrapped in the polymer matrix and there is no interaction between drug and polymers. It confirmed the fact that during the microencapsulation process, Pioglitazone remained suspended in the matrix of Ethyl Cellulose even after evaporation of solvent during the process.
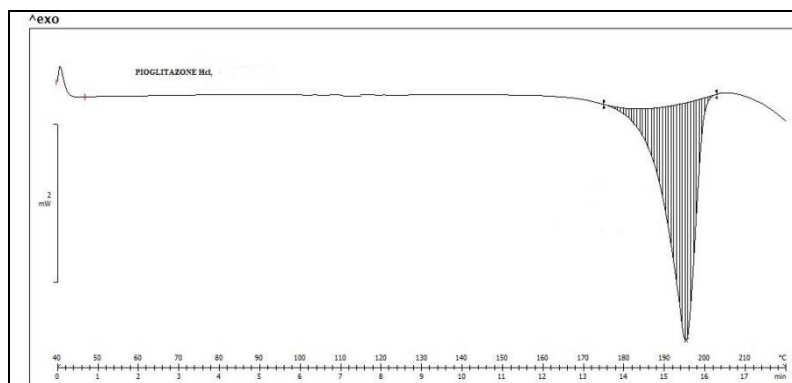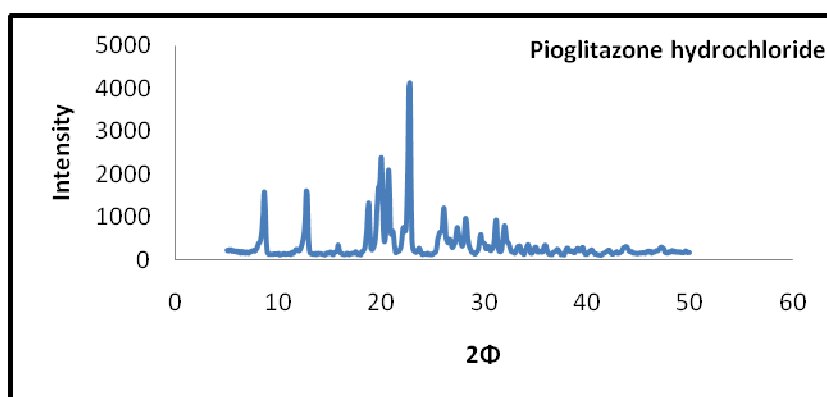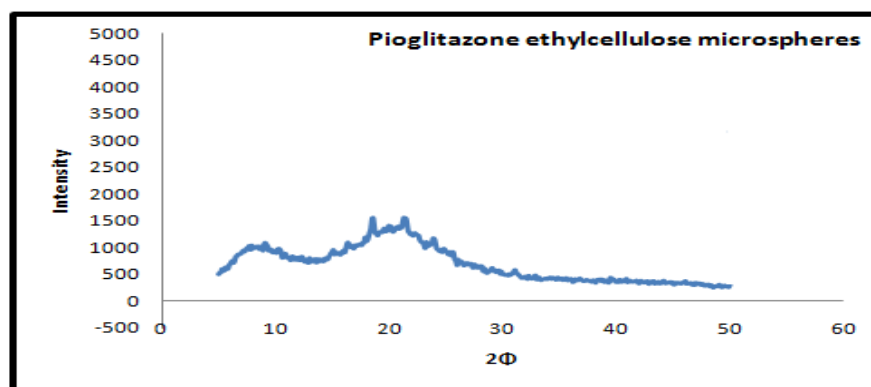
(a)
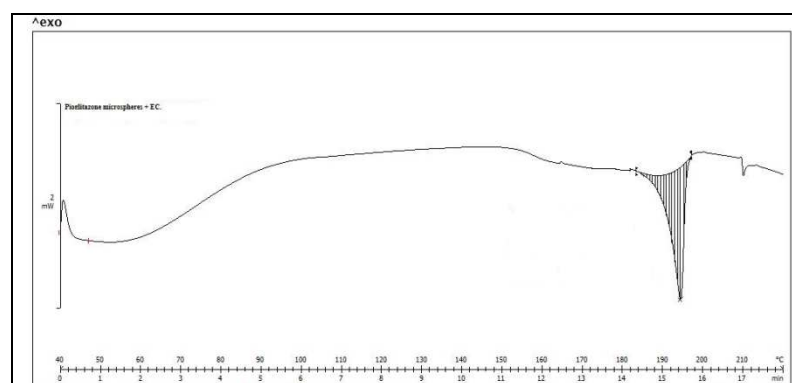
(b)

Figure 8 DSC thermograph of a) Pioglitazone hydrochloride pure drug b) Pioglitazone Ethyl Cellulose microspheres.

X-Ray Diffraction Study:-

X-ray diffraction pattern of Pioglitazone (pure drug), Pioglitazone Ethyl Cellulose microspheres are shown in Fig.9 (a) and (b). Fig 9 (b) did not contain any peaks associated with crystalline nature of Pioglitazone hydrochloride, suggesting that drug might have changed into amorphous state during microencapsulation process due to solubilisation in internal solvent and early re precipitation.

(a)

(b)

Figure 9 X-ray diffraction pattern of a) Pioglitazone (pure drug) b) Pioglitazone Ethyl cellulose microspheres.

Stability Study:-

The stability studies were carried out on optimized formulation S_3. The formulation was stored at 40 ± 2^0C/75 ± 5 % RH (Climatic zone IV condition for accelerated testing) for 2 months to assess its stability. After 15, 30 and 60 days, samples were withdrawn and retested for physical appearance, % drug content, % drug release and %floating capacity studies as shown in Table 6. The results indicated that the formulation was able to retain its stability for 2 months.

Table 6. Drug Content of optimized batch S_3 after 2 month storage

Days	Drug content %	Floating capacity %	% Drug release after 8hrs.
Before Storage			
0 day	97.2	89.6	74.8
After Storage*			
15	97.0±2.4	89.5±1.4	74.6±2.1
30	96.3±1.4	89.2±2.5	74.3±1.5
60	95.8±2.1	87.9±2.2	73.7±1.3

*Storage at 40°C and 75% RH for two months (n = 3).

CONCLUSION

The floating microspheres of Pioglitazone hydrochloride were prepared and optimized using the 3^2 factorial design. The concentration of Ethyl cellulose (EC) had significant impact on drug entrapment efficiency and particle size. HPMC K100M was selected in combination with ethylcellulose to increase the drug release from microspheres but at the same time drug entrapment efficiency and yield of microsphere decreases with increase in concentration of HPMC K100M. The results of a 3^2 full factorial design revealed that the concentration of EC N10 (X_1) and stirring speed (X_2) significantly affected the dependent variables such as drug entrapment efficiency, drug rel_{8h} and particle size of microspheres. Evaluation of five formulations, chosen as optimal from grid searches, indicated that the formulation **S_3 (EC: 12% and stirring speed: 900rpm)** fulfilled maximum requisites because of better drug entrapment efficiency, sustained release of the drug and optimum particle size.

REFERENCES

[1] Willing, P. G. and Dobrinska, M. R. Robinson, J. R. and Lee, V. H. L. (eds) (**1987**) *Controlled Drug Delivery: Fundamentals and Applications* **6** , pp. 253-291. Marcel Dekker, Inc., New York

[2] Moes, A. J. (**1993**) *Current Status on Targeted Drug Delivery to the Gastrointestinal Tract* , Capsugel Symposia Series . pp. 97-112.

[3] Singh NB, Kim KH. (**2000**). *J Control Release*, 63:235–59.

[4] Arora S, Ali J, Ahuja A, Khar RK, Baboota S. (**2005**). *AAPS PharmSciTech*, 6(3), E372–90.

[5] Deshpande AA, Rhodes CT, Shah NH, Malick AW. (**1996**). *Drug Dev Ind Pharm*, 22:531–39.

[6] Srivastava AK, Ridhurkar DN, Wadhwa S. (**2005**). *Acta Pharma*, 55:277–85.

[7] Chaurasia H, Jaina K, Prajapatis K, Chaurasia D, Gupta R, Arya R, Bhardwaj P. (**2007**). *Indian Pharm*, 6(65):101–03.

[8] Kawashima Y, Niwa T, Takeuchi H, Hino T, Ito Y. (**1991**). *J Control Release*, 16:279–90.

[9] Jain SK, Awasthi AM, Jain NK, Agrawal GP. (**2005**). *J Control Release*, 107:300–09.

[10] Naggar VF, E-Kamel AH, Sokar MS, Al-Gamal SS. (**2001**). *Int J Pharm*, 220:13–21.

[11] Martin A, Bustamante P, Chun AHC. (**1996**). Physical Pharmacy. New Delhi, India: B I Waverly Pvt. Ltd., 423–52.

[12] Singh B, Ahuja N. *Drug Dev Ind Pharm* **2002**; 28: 431-442.