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Formulation and *in vitro* evaluation of mucoadhesive microspheres of pioglitazone hydrochloride

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ABSTRACT

The work was aim to formulate and evaluate mucoadhesive microspheres of Pioglitazone Hydrochloride for treatment of diabetes type-2 by combining the potential advantages of mucoadhesion with controlled drug delivery using various ratio of polymers. Mucoadhesive microspheres were prepared by emulsion diffusion solvent evaporation technique. Microspheres were found to be discrete, spherical and free flowing. They ranged in particle size from 73-125 µm , drug release shown was 73.39-91% and mucoadhesiveness was 68-84%. Mainly in this Stirring speed affects particle size, Concentration of HPMC K100 affects drug release and concentration of Carbopol 934 affects mucoadhesiveness The microspheres exhibits good mucoadhesive property in in-vitro wash off test and showed high drug entrapment efficiency. Pioglitazone Hydrochloride release from these microspheres was slowed, extended and depended on the type of polymer used. The work has demonstrated that among all the formulations of microspheres, particularly those of formulation F8 are promising candidates for the sustained release of Pioglitazone Hydrochloride .

Keywords: Mucoadhesive microspheres, Pioglitazone hydrochloride, Emulsion solvent diffusion evaporation method.

INTRODUCTION

Primary objectives of Controlled drug delivery system are to ensure the safety and to improve efficiency of drug as well as patient compliance. This is achieved by better control over plasma drug level and less frequent dosing. Controlled drug delivery occurs when a polymer, whether natural or synthetic, is judiciously combined with a drug or other active agent in such a way that the active agent is released from the material in a predesigned manner. Microspheres in general, have the potential to be used for targeted and controlled release drug delivery; but coupling of mucoadhesive properties to microspheres has additional advantages, e.g. efficient absorption and enhanced bioavailability of drug due to a high surface to volume ratio and much more intimate contact with the mucus layer .

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Mucoadhesive microspheres have gained considerable attention due to their ability to adhere to the mucus layer and release the loaded drug in a sustained manner. Mucoadhesive microspheres widely used because they release the drug for prolonged period, reduce frequency of drug administration and improve patient compliance[1,2,7].Diabetes is a syndrome characterized by disordered metabolism and inappropriately high blood sugar (hyperglycemia) resulting from either low levels of insulin effects coupled with inadequate levels of insulin secretion to complex state. Type II diabetes remains a difficult clinical challenge characterized by progressive insulin deficiency and frequent cardiovascular events requiring multiple therapeutic decisions. Over 30 million have now been diagnosed with diabetes in India. [3]

A thiazolidinedione anti diabetic agent was selected as a drug candidate in the present study. Pioglitazone HCl has the pKa value of 4.9, so it is largely present in unionized form and well absorbed from the acidic pH

of the stomach than the basic pH condition of intestine. Also the drug is having short biological half life of 3 to 7 h and hence is suitable for the sustained release dosage, control release (CR) products are needed for pioglitazone to prolong its duration of action and to improve patience compliance And localize the drug in the stomach for an effective The site of absorption of Pioglitazone is in the stomach. Dosage forms that are retained in the stomach would increase the absorption, improve drug efficiency, and decrease dose requirements. Controlled release formulation is also needed for pioglitazone for better control of blood glucose levels to prevent hypoglycemia and enhance clinical efficacy, to reduce g.i. disturbances and to enhance patient compliance. Mucoadhesion has been a topic of interest in the design of drug delivery system to prolong the residence time of the dosage form at the site of application or the absorption and to facilitate intimate contact dosage form with the underlying absorption surface. Therefore, mucoadhesive microspheres of Pioglitazone Hydrochloride are promising candidate for delivery of for treatment of diabetes type-II.[4] .Thus present study was envisaged to formulate and in vitro evaluate the microspheres of Pioglitazone hydrochloride which release the drug in a sustained release manner. No controlled release formulations of Pioglitazone are available commercially.

MATERIALS AND METHODS

Pioglitazone Hydrochloride was a gift sample from Micro Labs Ltd, Bangalore. Carbopol 934 was from Ana lab Fine chemicals Mumbai, HPMC K 100 was from Ana lab Fine chemicals Mumbai, Dichloromethane from Loba Chemie, Mumbai, Ethanol from Reserach Lab Fine Chem, Mumbai, Tween80 from Loba Chemie,Mumbai and n-hexane from Research Lab Fine Chem,Mumbai .All the material and reagents are of analytical grade (A.R)

Preparation of Mucoadhesive Microspheres [5]

The mucoadhesive microspheres were prepared by using the emulsion solvent diffusion-evaporation method similar to one reported by kawashima et al . In the preliminary trials, weighed amount of drug (Pioglitazone hydrochloride), Carbapol 934 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 30-40ºC. The resultant emulsion was stirred with a propeller type agitator at variable speed for 2-3 hours to allow volatile solvent to evaporate. The resultant microspheres were filtered and dried. F1 toF15 were the preliminary batches prepared using different levels of Polymers.

Table No.1: Formulation batches of Pioglitazone Hydrochloride microsphere [13,17]

Batch code	Variable Level in Coded Form		
	X1	X2	X3
F1	-1	-1	-1
F2	1	-1	-1
F3	-1	1	-1
F4	1	1	-1
F5	-1	-1	1
F6	1	-1	1
F7	-1	1	1
F8	1	1	1
F9	-1.682	0	0
F10	1.682	0	0
F11	0	-1.682	0
F12	0	1.682	0
F13	0	0	-1.682
F14	0	0	1.682
F15	0	0	0

Coded values	Actual values		
	X1	X2	X3
-1	600	100	100
0	950	200	200
1	1300	300	300
-1.682	361.37	31.82	31.82
1.682	1538.63	368.18	368.18

Where, X1= Stirring Speed (RPM), X2= Conc. Of Carbapol 934(MG), X3= Conc. Of HPMC K100 (MG)

Evaluation [4- 11]

Infrared spectroscopic study (IR)

IR absorption spectrum of Pioglitazone hydrochloride and its formulation evaluated by using Shimadzu FTIR spectrophotometer wherein 2 - 4 mg of drug sample was used. The resultant spectrum of the drug was compared with reference spectrum of Pioglitazone hydrochloride.

Differential scanning calorimetry (DSC)

The thermal behavior of Pioglitazone hydrochloride and its formulation was examined by DSC,using a Mettler differential scanning calorimeter. The system was calibrated with a high purity sample of Indium. PZ were scanned at the heating rate of 20˚C/min over a temperature range of 70 to 300˚C (Figure no). Peak transitions and enthalpy of fusion were determined for the samples using TA60 integration software

Micromeritic properties of microspheres

Flow properties: The flow properties of microsphere were studied by determining various parameters like the angle of repose, Carr's index, and bulk density and tapped density**.**

1) Production Yield (%)

The production yield of microsphere was calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of microsphere and % production yield was calculated as per the formula mentioned below**.**

Production yield**:** Wo / WT X100

Where, WO = Practical mass (microspheres);

WT = Theoretic mass (Polymer + Drug).

2) Particle size analysis

Particle size of different batches of microspheres was determined by optical microscopy. The projected diameter of microspheres from each batch was determined using ocular micrometer and stage micrometer equipped with optical microscope. Analysis observing the slide containing microspheres under the microscope.

3) Drug entrapment efficacy

50 mg of microsphere were taken and drug was extracted from microspheres by digesting for 24 h with 10 ml of 0.1 N HCl. During this period the suspension was agitated. After 24 h the suspension was centrifuged at 2000 rpm for about 3 min. The supernatant obtained was assayed spectrophotometrically for drug contents. Entrapment efficiency was calculated according to equation.

Entrapment efficiency = (Practical drug content / theoretical drug content) × 100

4) **Surface morphology**

The surface morphology and shape 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 on aluminum stubs. The stubs were then placed into fine coat ion sputter for gold coating. After gold coating samples were randomly scanned for particle size and surface morphology.

***In-vitro* mucoadhesivenes [10,12- 16]**

The mucoadhesive property of prepared microspheres was evaluated by in-vitro wash off method. A rat stomach mucosa was tied on the glass slide using a thread. About 100 microspheres were spread on to wet rinsed tissue specimen and prepared slide was hung on to one of the grooves of a USP tablet disintegration apparatus. By operating the disintegrating test apparatus the tissue specimen was given a slow regular up and down movement in the test fluid at 37±0.50C. At every 1 h interval the equipment was stopped and the number of particles still adhering to tissue was counted. Percent mucoadhesion was given by the following formula.

% mucoadhesion = (no. of particle remains on mucosa/ no. of applied microsphere)×100

In-vitro drug release

The release rate of Pioglitazone Hydrochloride from microspheres was determined using United States Pharmacopeia (USP*)* dissolution testing apparatus 2 (paddle type). The dissolution test was performed using 900 ml of 0.1N HCl, at 37 ± 0.5°C and 50 rpm. A sample (10 ml) of the solution was withdrawn from the dissolution apparatus hourly for 12 h, and the samples were replaced with fresh dissolution medium to maintain the sick condition. The samples were filtered through a membrane filter and diluted to a suitable concentration with 0.1N HCl solution. Absorbance of these solutions was measured at 269 nm using a model 1700-E Shimadzu, double-beam UV spectrophotometer.

RESULTS AND DISCUSSION

Infrared spectroscopic study

The spectrum of Pioglitazone HCl, Formulation , Pioglitazone HCl& Carbapol 934 and Pioglitazone HCl& HPMC K100 was recorded in Shimadzu FT-IR is shown in Fig. no.1,2&3.

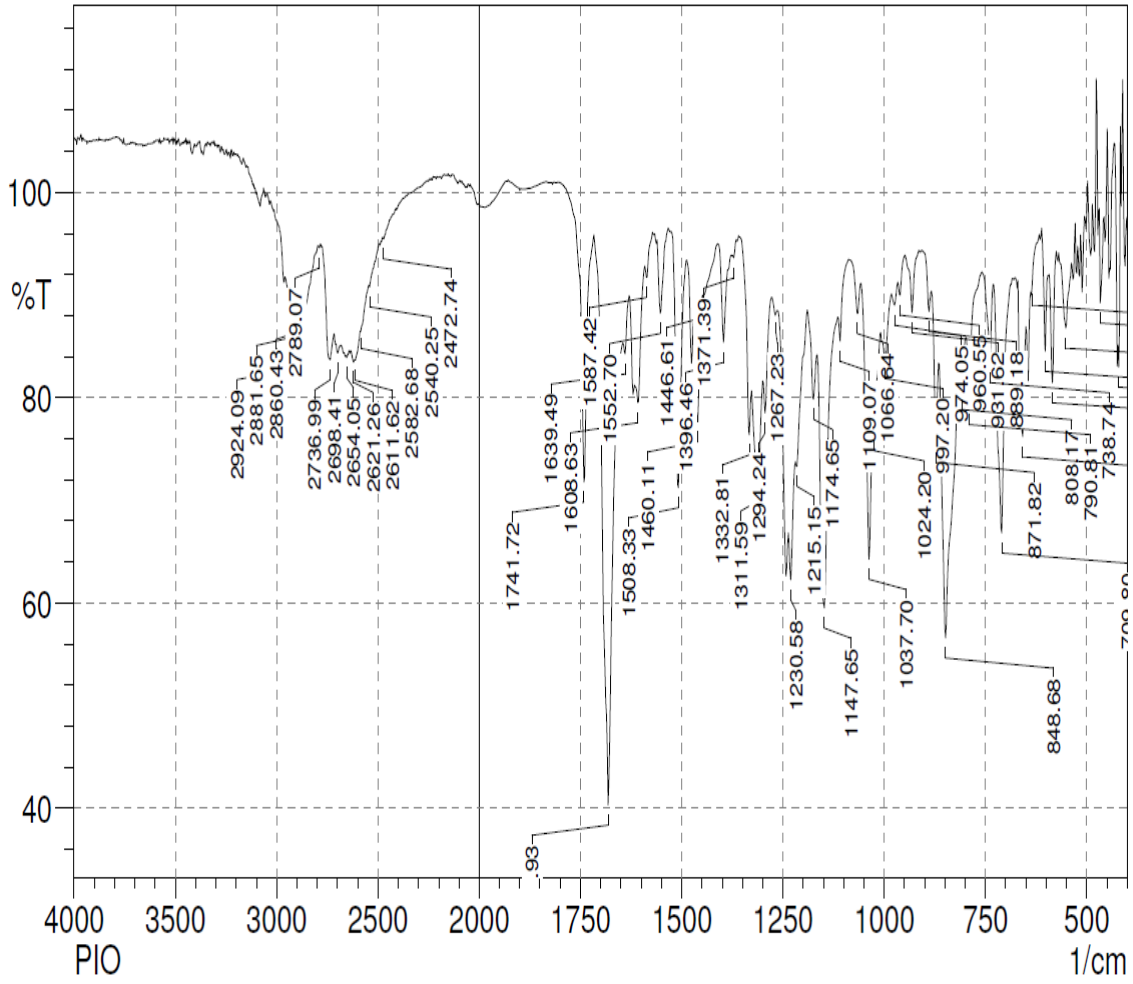


Fig No.1: FTIR Spectra of Pioglitazone Hydrochloride

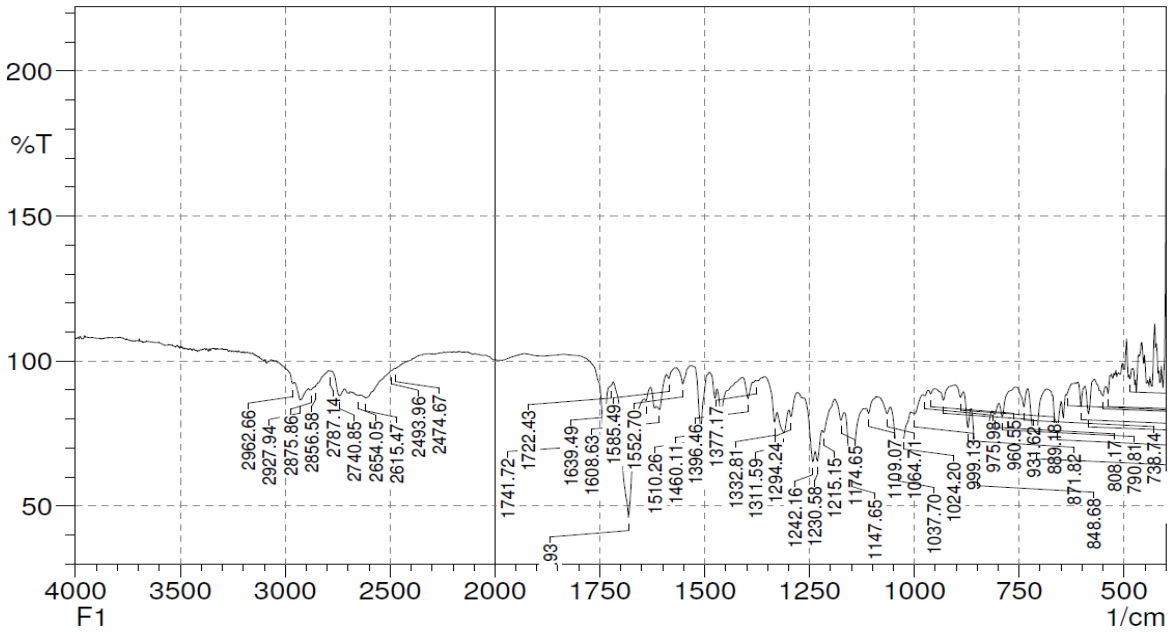
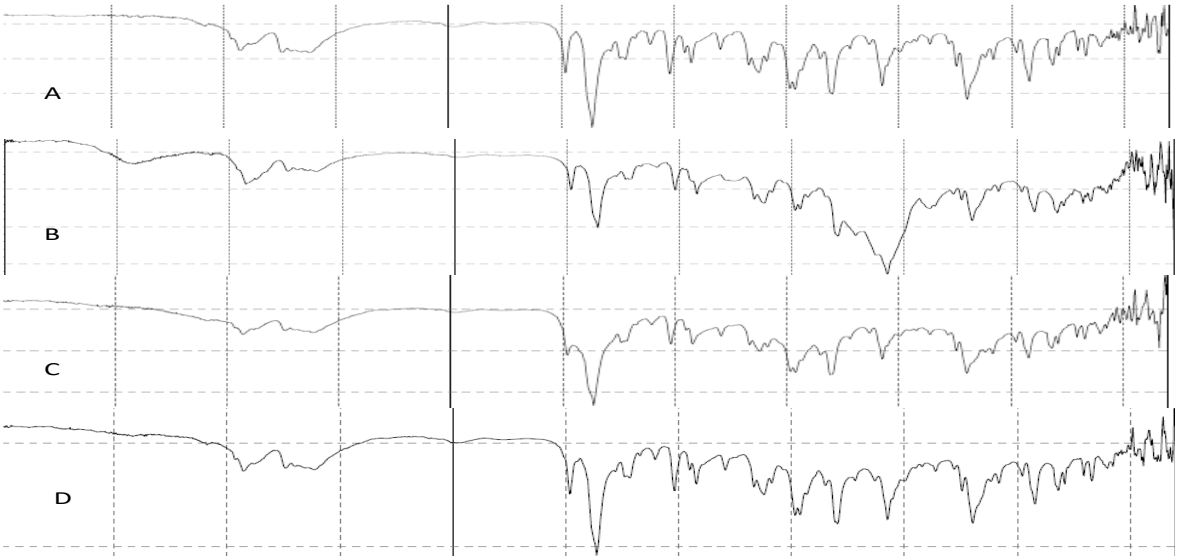


Fig No.2: IR Spectra of Formulation



A=Pioglitazone, B=Pioglitazone+Carbapol934, C=Pioglitazone+HPMCK100, D=Final formulation.

Fig No.3: Drug-excipients compatibility studies

Differential scanning calorimetry (DSC)

The thermal behavior of PZ was examined by DSC, using a Mettler differential scanning calorimeter. The DSC thermogram of Pioglitazone HCl featured a single sharp melting endotherm, having a peak temperature of 200.13°C, and Formulation having a peak temp. of 199.44 ºC .Figure no.4 shows the DSC Thermogram of Pioglitazone HCl.

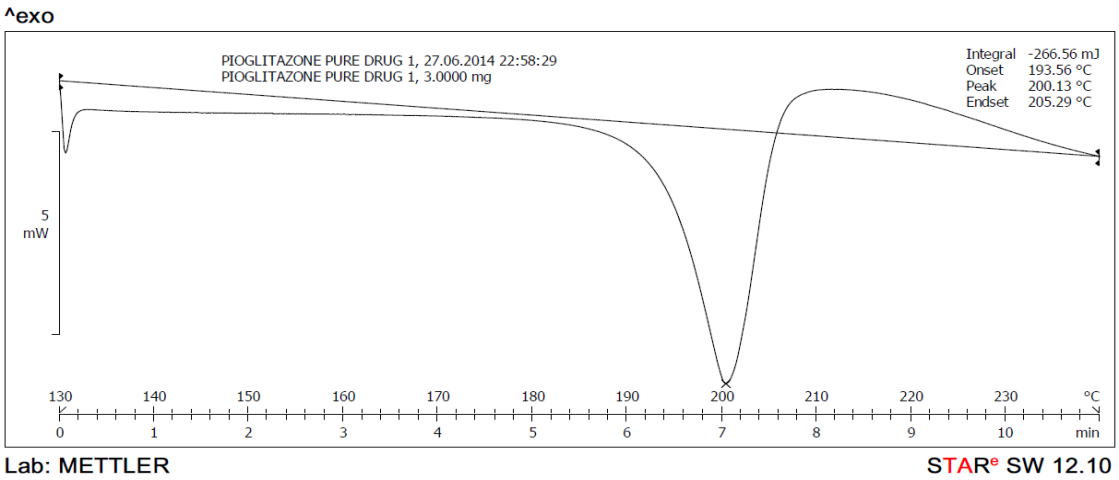


Fig No.4: DSC thermogram of Pioglitazone HCl

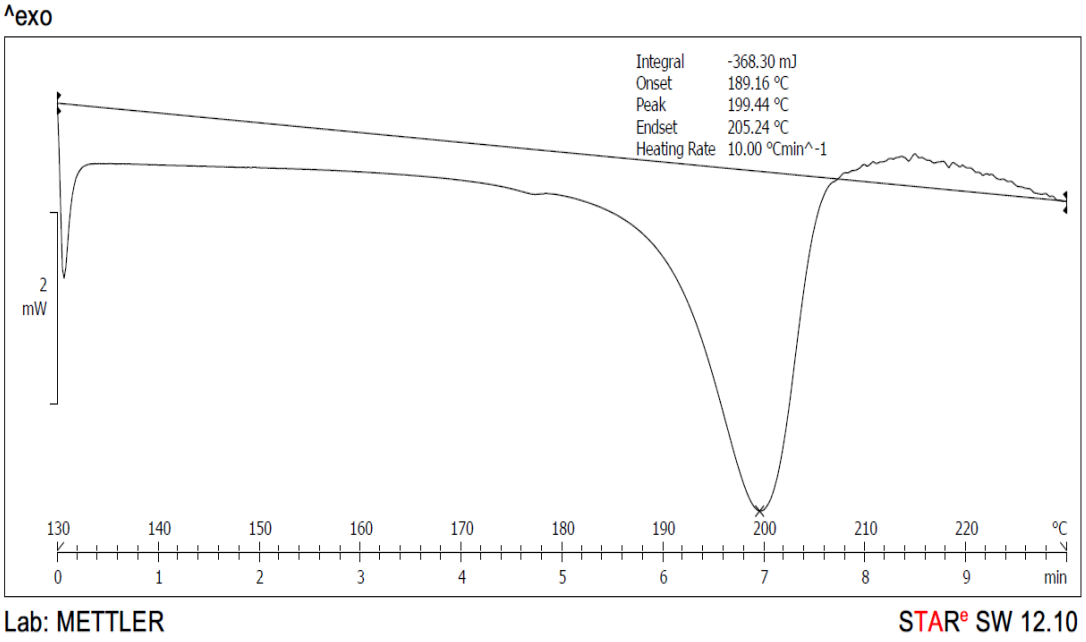


Fig No.5 : DSC thermogram of Formulation

1) Evaluation of micromeritic properties

The prepared formulations were evaluated for angle of repose, bulk density, tapped density, Carr's index and hausner's ratio. The results are shown in table no. 2

Table No. 2: Micromeritic properties of microspheres

Formulation Code	Angle of repose (θ)	Bulk Density (gm/cm^3)	Tapped Density (gm/cm^3)	Carr's Index (%)	Hausner's ratio
F1	28.25±0.92	0.583±0.03	0.657±0.02	11.25±0.56	1.12±0.007
F2	27.08±0.32	0.584±0.01	0.669±0.04	12.72±1.15	1.14±0.015
F3	29.23±1.16	0.577±0.05	0.666±0.02	13.35±1.04	1.15±0.013
F4	29.47±0.35	0.585±0.02	0.697±0.04	16.12±0.83	1.19±0.011
F5	29.23±1.16	0.601±0.01	0.703±0.01	14.49±1.38	1.16±0.019
F6	28.22±1.32	0.615±0,03	0.720±0.02	14.54±1.41	1.17±0.019
F7	26.68±1.22	0.610±0.04	0.712±0.01	14.26±0.28	1.16±0.003
F8	29.93±0.63	0.601±0.04	0.704±0.00	14.63±0.96	1.17±0.013
F9	28.25±0.91	0.609±0.02	0.714±0.02	14.67±0.84	1.17±0.011
F10	27.61±0.36	0.579±0.04	0.686±0.01	15.52±0.56	1.18±0.007
F11	29.04±0.68	0.582±0.02	0.682±0.03	14.70±0.19	1.17±0.002
F12	30.08±0.33	0.624±0.02	0.714±0.04	12.64±1.19	1.14±0.015
F13	29.45±0.33	0.603±0.03	0.686±0.01	12.07±0.49	1.13±0.006
F14	29.26±0.62	0.573±0.04	0.678±0.03	15.45±0.61	1.18±0.008
F15	30.68±0.31	0.614±0.01	0.705±0.03	12.84±1.44	1.14±0.018

Mean ± S.D., n=3

From the table the angle of repose, bulk density, tapped density; Carr's index and hausner's ratio were found to be within the limit as per reported literature. The Carr's index and hausner's ratio shows good flow properties for all the formulation. Also lower values for hausner's ratio indicates good flow properties of the formulation

Evaluation parameters of microspheres:-

The prepared microspheres were evaluated for Percentage yield, drug entrapment efficiency, and average particle size. The results are shown in the table no.3

Table No.3 : Evaluation parameters of microspheres

Formulation Code	**Yield (%)**	**Entrapment (%)**	**Particle size (µm)**
F1	75.83±2.81	64.43±1.94	94±2.51
F2	65.23±2.80	70.70±1.01	75±2.30
F3	70.36±2.70	76.36±2.15	105±2.08
F4	78.53± 2.85	80.53±0.86	83±3.60

F5	64.86±2.73	65.37±1.67	100±3.21
F6	75.73±2.30	71.03±0.75	77±2.08
F7	80.26±2.66	81.76±1.55	100±2.51
F8	78.63±2.01	80.63±1.16	80±3.0
F9	73.33±2.90	68.26±1.20	125±3.05
F10	79.36± 2.69	76.93±1.35	73±2.88
F11	73.43±2.75	78.03±1.40	94±3.21
F12	68.70±3.02	86±2.83	98±3.0
F13	63.43±2.71	82.70±1.93	93±2.64
F14	76.40±2.85	78.93±1.1083	97±2.0
F15	67.80±3.03	76.76±1.81	96±3.05

From the table no 3, the percent yields of the all formulations were found to be in the range of 64.86±2.73 to 84.43±2.71.

The low percentage yield in some formulations may be also due to microspheres lost during the washing process.

The drug entrapment efficiency was found to be in the range of 64.43±1.94 to 86 ±2. 6.

The mean particle sizes of the drug loaded microspheres were carried out by optical microscopy. The mean particle size might be affected by mainly Stirring speed &different types of polymers which are used in preparation of microspheres.

The average particle size of microspheres was found to be in the range of73±2.88 to 125±3.05 µm.

The particle size decreases as the stirring speed increases and particle size increases as the polymer concentration increases formation of the large droplets during addition of polymers.

From the result, it was observed that the drug entrapment efficiencies increased progressively with increasing the concentration of copolymer resulting in the formation of larger microspheres entrapping the greater amount of the drug, this may be attributed to the greater availability of carbopol coating on the microsphere.

***In Vitro* drug release**

The dissolution studies were performed in a fully calibrated eight station dissolution test apparatus (37 ± 0.5ºC, 50 rpm) using the USP rotating basket method in 0.1 N HCl buffer media (pH 1.2, 900 ml).

Microsphere formulation equivalent to 30 mg Pioglitazone for each formulation was employed in all dissolution studies.

The samples of 5 ml each were withdrawn at predetermined time intervals and replenished immediately with the same volume of fresh pre-warmed 0.1N HCl buffer maintaining sink condition throughout the experiment. The aliquots, following suitable dilution, were analyzed spectrophotometrically at 270 nm.

The concentrations of PZ in the test samples were calculated using a regression equation of the calibration curve in 0.1N HCl buffer of pH 1.2 .

In the table below highest drug release observed was 91.17%, 90.06%, 87.43%, 86.99% for F14 ,F8, F5 ,F10 respectively.

Table No .4: *In vitro* drug release of microspheres

Time (hr)	%DRUG RELEASE															
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F1O	F11	F12	F13	F14	F15	
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
1	4.75	5.06	4.96	6.51	7.70	6.72	3.41	5.79	8.53	6.15	10.13	6.51	6.82	5.12	5.11	
2	6.21	8.75	9.27	11.34	14.97	11.39	10.1	11.13	16.01	10.9	15.27	11.34	13.41	10.31	10.62	
3	10.16	15.44	15.29	16.90	22.23	18.45	15.44	16.53	25.81	19.12	20.48	15.60	20.16	15.65	16.34	
4	15.66	20.79	21.18	24.29	31.74	24.32	21.21	22.61	31.28	26.0	31.22	21.83	26.91	23.77	22.34	
5	23.65	30.91	27.30	31.14	41.51	32.12	29.51	30.04	36.59	33.00	39.23	29.99	32.33	31.76	30.67	
6	31.18	36.44	33.84	38.21	47.66	39.46	36.18	36.80	43.05	40.49	46.52	36.23	37.13	39.24	34.16	
7	37.85	41.86	41.65	44.98	53.15	50.11	42.53	44.61	51.76	48.04	53.15	42.07	43.07	46.74	42.85	
8	46.53	50.30	50.34	51.30	62.63	58.66	50.35	53.26	60.25	58.50	60.09	48.44	50.06	61.81	50.35	
9	51.87	57.70	57.08	58.65	68.24	67.79	58.69	62.64	67.60	68.82	67.51	57.19	55.09	67.47	60.09	
10	64.25	67.71	62.47	66.02	74.22	76.09	67.92	72.60	73.85	73.82	72.97	66.00	62.45	72.82	67.51	
11	69.14	74.21	67.77	70.23	81.04	83.38	75.73	78.11	79.47	80.89	79.47	74.15	67.80	83.36	72.92	
12	74.39	83.09	73.96	77.10	87.43	89.01	86.20	90.06	84.95	86.99	86.44	82.26	73.72	91.17	83.30	

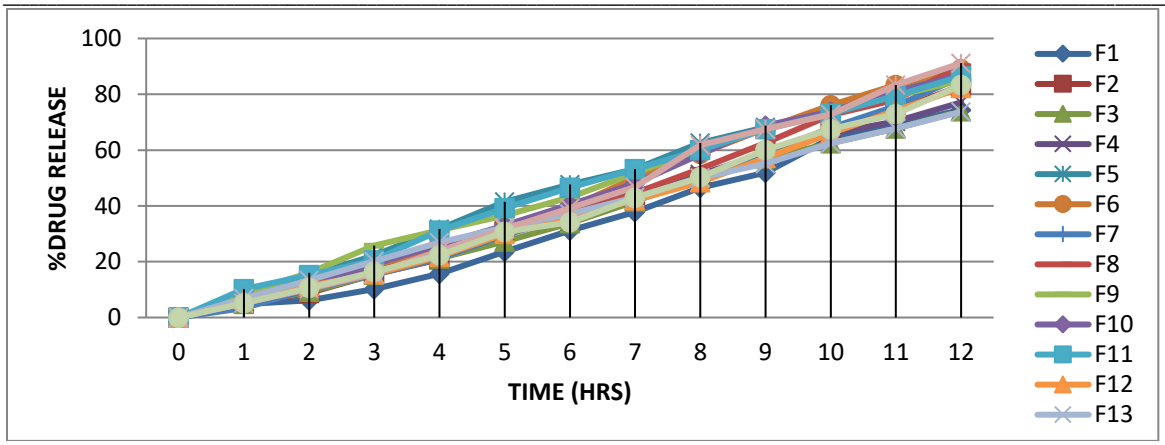


Fig No.6:*In vitro* drug release profile of F1-F15

***In vitro* mucoadhesiveness**

The mucoadhesive properties of the microspheres were evaluated by the in vitro wash-off test as reported by Lehret et al. A 1 cm x 1 cm piece of rat stomach mucosa was tied onto a glass slide (3 inch x 1 inch) using a thread. Microspheres were spread onto the wet, rinsed, tissue specimen, and the prepared slide was hung onto one of the groves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down movements in a beaker containing the simulated gastric fluid (pH 1.2). At the end of every time interval, the number of microspheres still adhering on to the tissue were counted and there adhesive strength was determine using the following formula.

Percent mucoadhesion =

(Weight of adhered microsphere /Weight of applied microspheres) ×100

Table No. 5 : *In Vitro* Mucoadhesiveness

	In Vitro MUCOADHESIVENESS														
TIME (HRS)	% MUCOADHESIVENESS														
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
1	88.97	95.63	95.16	96.53	95.63	98.60	97.80	95.53	92.67	94.36	85.73	98.67	95.73	90.83	88.90
2	85.43	89.50	92.36	93.43	89.50	92.53	94.80	93.43	89.73	92.50	82.57	95.73	92.57	88.60	86.90
4	82.70	85.37	89.76	90.77	85.37	85.33	91.23	89.77	85.90	89.37	78.73	92.90	86.73	85.50	83.50

6	79.73	81.43	87.86	88.67	78.43	82.93	88.13	87.67	81.77	86.34	74.53	90.77	80.53	83.33	81.53
8	76.33	78.30	84.24	86.33	74.30	78.16	85.13	85.33	78.47	83.30	72.07	88.47	77.07	80.47	79.10
10	73.77	76.03	81.26	84.53	73.03	76.80	83.00	81.53	74.63	81.03	70.90	86.63	74.90	78.13	78.96
12	69	72.97	78	82	70	75.33	80.00	83	72	79	68	84	72	77	75

From the above table it is concluded that F12,F8,F7 having highest mucoadhesiveness of 84%,83%,80%respectively.

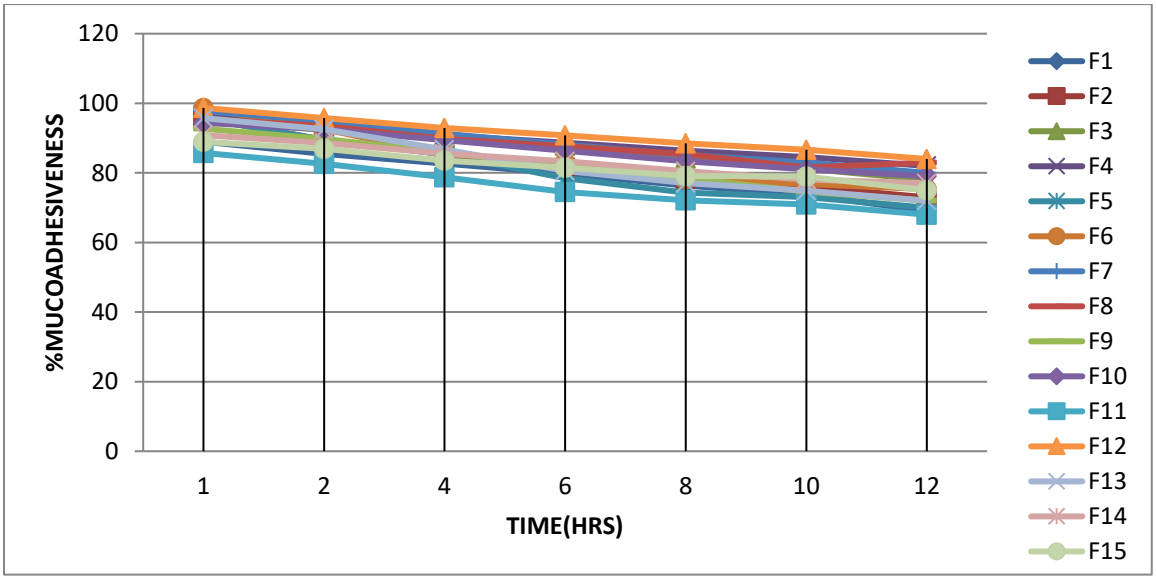


Fig No.7: *In vitro* mucoadhesiveness profile of F1-F15

Scanning Electron Microscopy: Morphology of microsphere batch was examined by scanning electron microscopy. In University of pune, SEM model JEOL JSM 6360A the top view of the microspheres showed a spherical structure with a slight rough surface morphology

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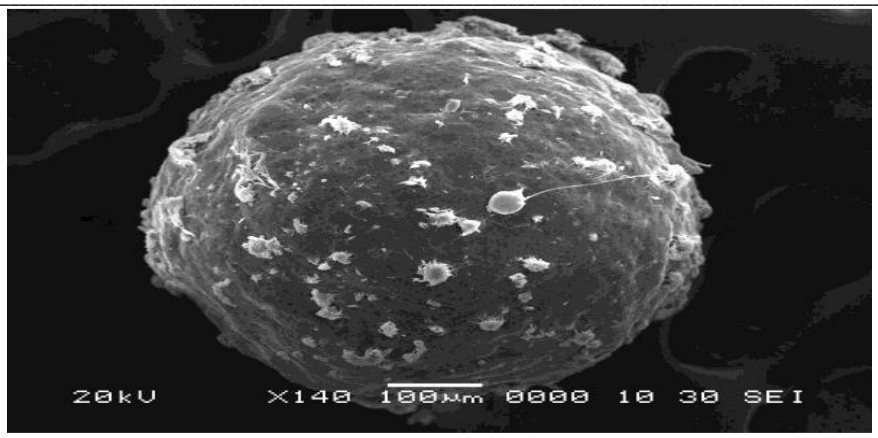

Fig No.8: SEM images of Pioglitazone hydrochloride microspheres

Annova study

Table No.6: Study of responses

Batch code	Variable Level in Coded Form			Particle size (Y1) (µm)	%Drug Release (Y2) (%)	Mucoadhesiveness(Y3) (%)
	X1	X2	X3			
F1	-1	-1	-1	94	74.36	69
F2	1	-1	-1	73	83.09	72
F3	-1	1	-1	105	73.96	78
F4	1	1	-1	83	77	82
F5	-1	-1	1	100	87.08	70
F6	1	-1	1	77	89	75
F7	-1	1	1	100	86.59	80
F8	1	1	1	80	90.69	83
F9	-1.682	0	0	125	84.96	75
F10	1.682	0	0	75	86.96	79
F11	0	-1.682	0	94	86.39	68
F12	0	1.682	0	98	82.48	84
F13	0	0	-1.682	93	73.39	72
F14	0	0	1.682	97	91	77
F15	0	0	0	96	83.36	75

Analysis of data by design expert software [17]

A RSM design was selected and the 3 factors were evaluated at 3 levels, respectively (Table 6). Stirring speed(X1), Conc.of carbapol934(X2) and HPMC K100M (X3) and were selected as independent variables and the dependent variables were particle size, percent drug release and mucoadhesiveness. The data obtained were treated using Stat Ease Design Expert 8.0.7.1 software and analyzed statistically using analysis of variance (ANOVA) .The data were also subjected to 3-D response surface methodology to study the interaction of)Stirring speed(X1), Conc.of carbapol934(X2) and HPMC K100M (X3)

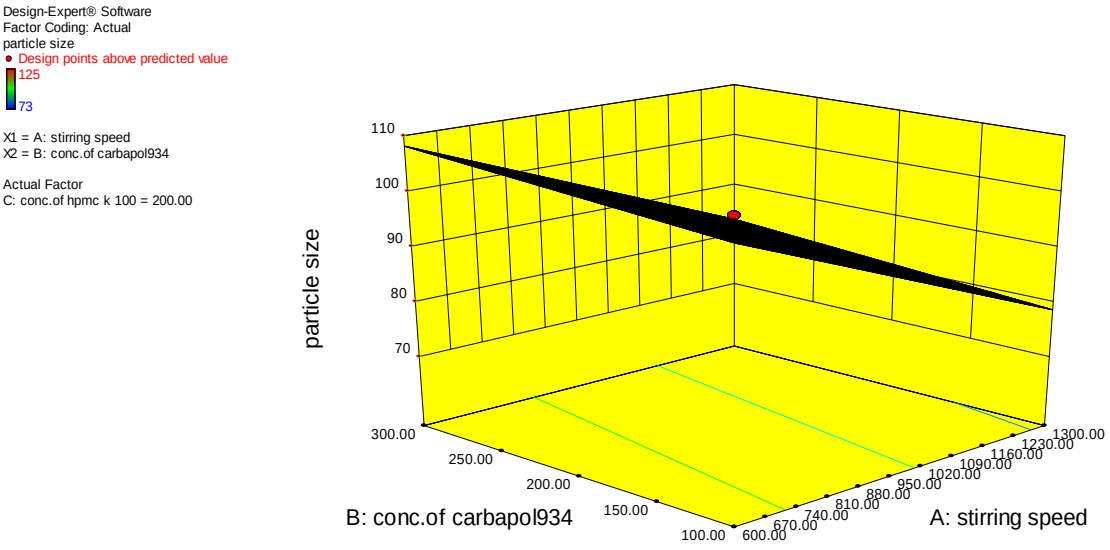

Fig No.9:3D Surface plot of particle size

From the above graph it is concluded that as stirring speed increases particle size decreases and particle size increases as the concentration of carbapol934 increases.

Maximum particle size: 125 µm
Minimum particle size :73µm

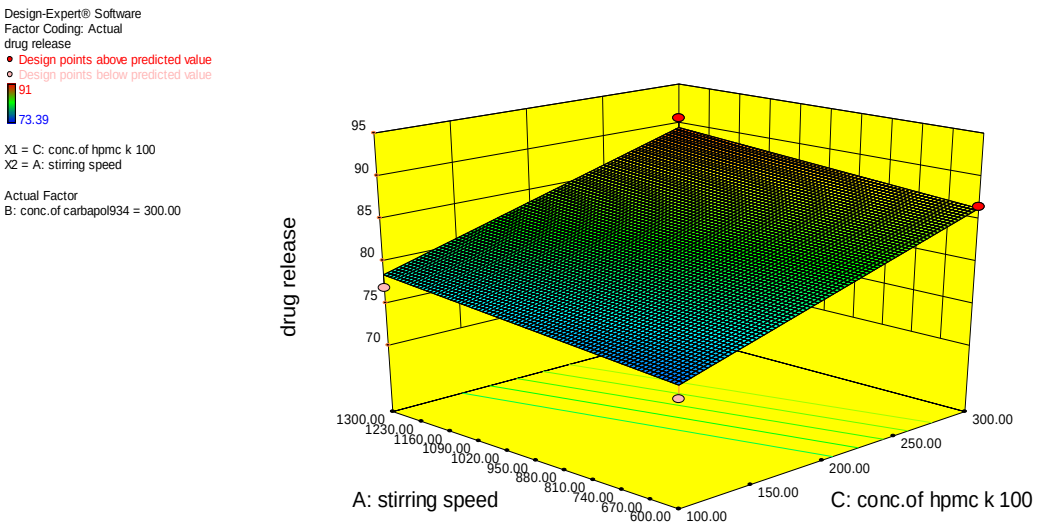

Fig No.10: 3D Surface plot of drug release

As seen in above graph ,as concentration of HPMC K100 and stirring speed increases drug release increases.

Maximum drug release: 91 %
Minimum drug release: 73.39%

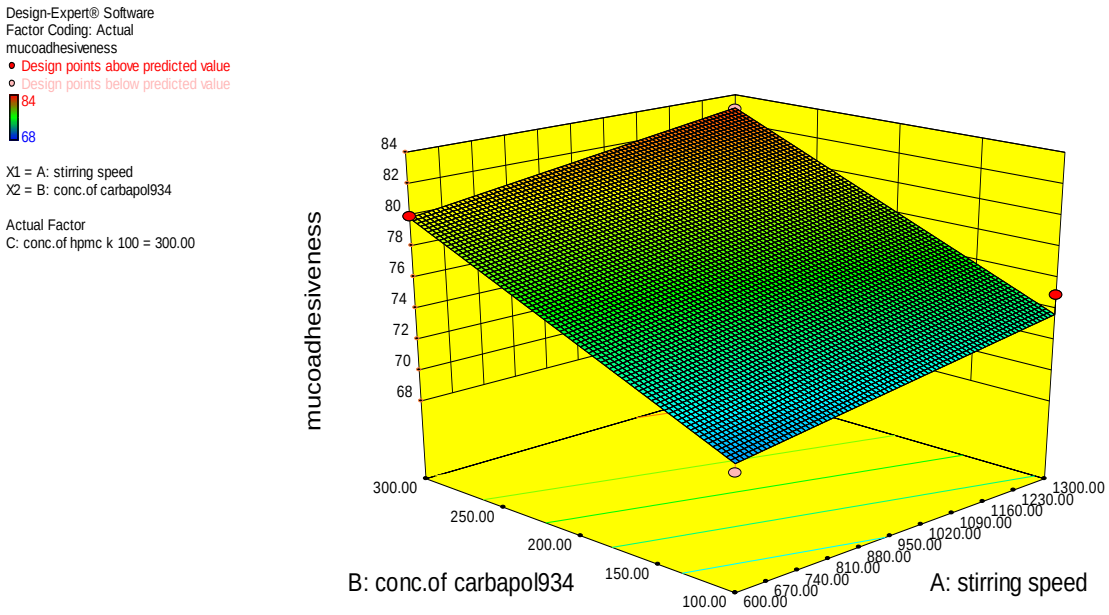

Fig No.11: 3D Surface plot of mucoadhesiveness

In the above graph, mainly concentration of carbapol934 affects mucoadhesiveness, as the concentration of carbapol934 increases mucoadhesiveness increases, stirring speed has less affected.

Maximum mucoadhesiveness: 84%
Minimum mucoadhesiveness: 68%

CONCLUSION

The mucoadhesive microspheres of Pioglitazone hydrochloride were prepared by solvent diffusion evaporation method and optimized using the Response surface method factorial design. The concentration of Carbapol934 and HPMC K100 had significant impact on mucoadhesiveness and drug release. The results of RSM factorial design revealed that the Stirring speed (X1) , Conc. Of Carbapol 934 (X2) & Conc. Of HPMC K 100significantly affected the dependent variables such as,particle size of microspheres, drug release and mucoadhesiveness. Evaluation of formulations, chosen as optimal from grid searches, indicated that the formulation F8 fulfilled maximum requisites because of optimumparticlesize,better muco adhesiveness strength, & sustained release of the drug .

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