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Formulation and Evaluation of Silk Based Controlled Release Drug Delivery System of an Antidiabetic Drug

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HUMAN

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ABSTRACT

The aim of the present investigation was to formulate and evaluate controlled release tablets of Pioglitazone HCl, using a different proportion of Sericin, HPMC K100M as the release retardant polymer and study the effect of formulation factor such as polymer proportion on the *in-vitro* release of the drug. Pioglitazone HCl belongs to biopharmaceutical classification system class II; hence inclusion complex of Pioglitazone HCl and β-cyclodextrin was prepared to enhance the solubility. Solubility of Pioglitazone HCl was found to be more in 1%w/v solution of ß-cyclodextrin among 0.25%, 0.50% and 0.75%w/v solutions. The sericin was extracted from silk cocoons and this extract of sericin was used for the formulation of tablets. A 3^2 factorial design was applied for study and evaluation of the tablets. The tablets were prepared by wet granulation method using an enteric coating of opadry and evaluated for pre-compression studies. All the values were found within limits of the standard. *In-vitro* release studies were carried out by USP type II paddle apparatus. The interaction of polymer and drug carried out by FTIR studies. The FTIR studies confirmed that there is no interaction between the drug and polymer also the DSC studies confirmed that there is physical interaction between drug and polymer. Data of *in-vitro* release of tablets were fit in different equations and kinetic models. The results showed that Sericin produces a controlled release of the drug. The formulation F7 produced 88.77% drug release at 20 hours.

INTRODUCTION

Silk consists of two types of protein, silk fibroin, and sericin. It contains about 20-30% of sericin and 70-80% of fibroin. Sericin adhesive in nature and helps in the formation of the cocoon by binding fibroin strands together. It is a globular protein with the high content of aspartic acid and serine, has high moisture retaining capacity and can gel under controlled conditions. Sericin is a highly hydrophilic macromolecular protein comprising of 18 amino acids. A molecular weight of protein ranges from 24 to 400 kDa with predominant amino acid groups serine (40%), glycine (16%), glutamic acid, aspartic acid, threonine, and tyrosine. Thus it consists of polar side chain made of hydroxyl, carboxyl, and amino groups that enable easy cross-linking, copolymerization and blending with other polymers to form improved biodegradable materials. Silk sericin is one of the essential components of cocoon filament comprising granular and high molecular proteins with adhesive and gelatin-like characteristics. The silk fibers are composed of a fibrous core protein fibroin (hydrophobic in nature) with sericin surrounding it. Sericin plays a functional role in a coating and adhering the twin filaments and cocoons to protect silk from microbial degradation, animal digestion, and environmental damage. It has other surprising properties, such as resistance to ultraviolet light and oxidation and an ability to absorb and release moisture easily. Their main role is to envelop the fibroin. In presence of sericin, the fibers are hard and tough and become soft and lustrous after its removal. There are different methods of isolation of sericin from silk thread. Solubility, molecular weight, and gelling properties of sericin depend on the methods of isolation.[1-3]

MATERIALS AND METHODS:

1. MATERIALS

Table 1: List of materials with their suppliers

Sr. No.	Name of Materials	Name of Suppliers
1.	Pioglitazone HCl	Yarrow Chem, Mumbai
2.	β-Cyclodextrin	Yarrow Chem, Mumbai
3.	HPMC K100 M	Colorcon, Asia Pvt. Ltd. Goa
4.	Micro crystalline cellulose	Loba Chemie, Mumbai
5.	Iso-propyl-alcohol	Loba Chemie, Mumbai
6.	Starch	Loba Chemie, Mumbai
7.	Magnesium stearate	Loba Chemie, Mumbai
8.	Talc	Loba Chemie, Mumbai
9.	Aerosil	Loba Chemie, Mumbai
10.	Sericin	Swapnroop drugs & pharmaceuticals, Aurangabad
11.	Silk cocoons	Malegaon (BK) Trust

METHODS:

Extraction of sericin from silk cocoons of silkworm *Bombyx mori* [4-5]

Degumming of raw silk fibers: 5 gm of silk was taken 100 ml of distilled water and boil for 1 hour with or without 0.5% sodium carbonate/ sodium hydroxide. The degummed water thus obtained was filtered and taken for precipitation of sericin. Loss of weight of the raw silk fibers was calculated. The percentage yield was calculated using formula, (initial weight – final weight)/ (initial weight) x 100.

Precipitation of sericin:

A. Ammonium sulfate precipitation: 1.5 gm of ammonium sulfate was added to 10 ml of degummed water with continuous stirring. The mixture was left on ice for 30 minutes followed by centrifugation at 8,000 g at 4ºC for 10 min. The pellet formed was washed out 95% ethanol, dried and stored at -20ºC.

Procedure for preparation of tablets[6-16]

1. All ingredients were passed through sieve number 60.

2. The first step for formulation was the preparation of inclusion complexes of drug and β-CD. Pioglitazone HCl and β-CD in 1:2 ratios were used. β-CD impregnating with a little amount of IPA to converted in to paste then the drug is added to the above paste and kneaded for a specified time. The kneaded mixture was then dried and passed through sieve.

3. All the ingredients were mixed in this complex and starch paste was used for formation of wet mass. This wet mass was sieved through sieve number 40.

4. Granules were dried in an oven at a specified period of time. Dried granules were passed through sieve number 20. These granules were mixed in magnesium stearate, talc, and aerosol.

5. Tablets were prepared on a tablet compression machine by wet granulation method. The compression pressure was adjusted to obtain a tablet with hardness in the range of 4-8kg/cm^2.

6. Coating solution was prepared by dissolving Opadry enteric coat powder in IPA with constant stirring.

7. Above prepared tablets were coated in conventional coating pan machine with speed 25 rpm, temp. 55^{0}C spray nozzle 2 min. with a pressure of 40-50 Ib/Sq. weight gain should be up to 5-10%. Prepared tablets were evaluated for release and erosion characteristics.

Table 2: Composition for tablets of Pioglitazone HCl (batches F1-F9)

Batches / Ingredients(mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Pioglitazone HCl: βCD (1:2)	45:90	45:90	45:90	45:90	45:90	45:90	45:90	45:90	45:90
Sericin	0	0	0	60	60	60	90	90	90
HPMC K 100M	0	25	50	0	25	50	0	25	50
MCC	151	126	101	91	66	41	61	36	11
Starch Paste	2	2	2	2	2	2	2	2	2
IPA	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S
Magnesium Stearate	5	5	5	5	5	5	5	5	5
Talc	5	5	5	5	5	5	5	5	5
Aerosil	2	2	2	2	2	2	2	2	2
Total Weight (mg)	300	300	300	300	300	300	300	300	300

RESULTS AND DISCUSSION:

1 Characterization of Drug

1.1 Appearance and Colour

The sample was found to be white, amorphous, odorless powder.

1.2 Melting point

Melting point of the plain Pioglitazone HCl was found to be 192 to 194^0 C which was within the limit as per reported.

1.3FTIR spectrum of pure drug

The IR spectrum of the given sample of Pioglitazone HCl showed similar characteristics peaks to that of a reported spectrum of Pioglitazone HCl.

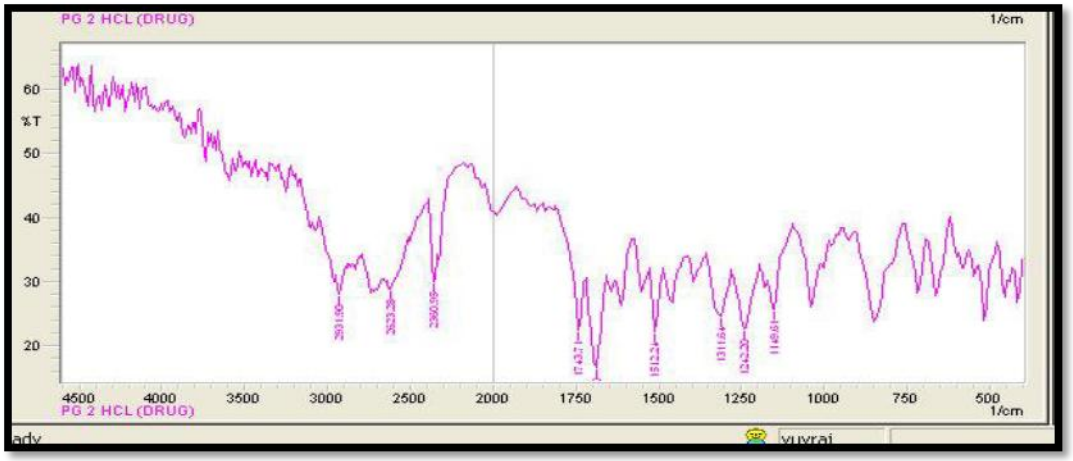

Figure 1: FTIR spectrum of Pioglitazone HCl

Table 3: Data for observed and reported peaks of Pioglitazone HCl

Functional Group	**Observed values cm^{-1}**	**Reported values cm^{-1}**
-C-C- stretching in aromatic	1512.24	1550-1475
-C=C- stretching in alkanes	1689.70	1640-1680
-C-H stretching in alkanes	2931.90	3000-2850
-C=O stretching	1743.71	1760-1690
O-H stretching	2623.28	3300-2500
-C-N stretching in amines	1242.20	1250-1020
-O- stretching	1311.64	1320-1000

The Pioglitazone HCl showed C=C stretching at 1689.70, C-H stretching at 2931.90, O-H stretching at 2623.28, C-C stretching at 1512.24 which corresponds with standards.

1.4 Differential scanning calorimetry (DSC)of Pioglitazone HCl[81]

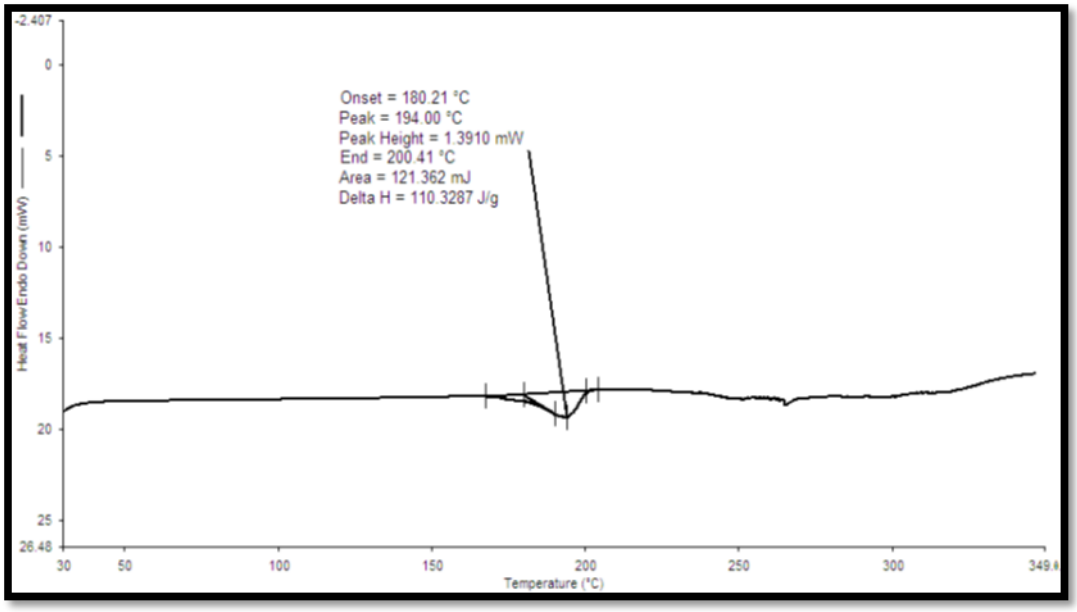

Figure 2: DSC thermogram of Pioglitazone HCl

The thermogram shows the endothermic peak at 194ºC which is near to the actual melting point of the Pioglitazone HCl. From this, it was confirmed that the given drug sample was Pioglitazone HCl.

2. Extraction of sericin from silk cocoons of silkworm *Bombyx mori*

Sericin powder was extracted using the procedure given in section

Evaluation of Sericin

1. **Appearance and Colour-** Sericin was found to be cream to light yellow, smooth powder.

2. **Solubility-** Insoluble in cold water, soluble in hot water.

3. **FTIR Study-** FTIR study of extracted sericin and purchased sericin was carried out and it concluded that the IR spectrum of the given sample of purchased Sericin and extracted Sericin showed similar characteristics peaks to that of a reported spectrum of purchased Sericin.

3. Characterization of Polymer by FTIR

3.1 FTIR spectrum of HPMC K100M

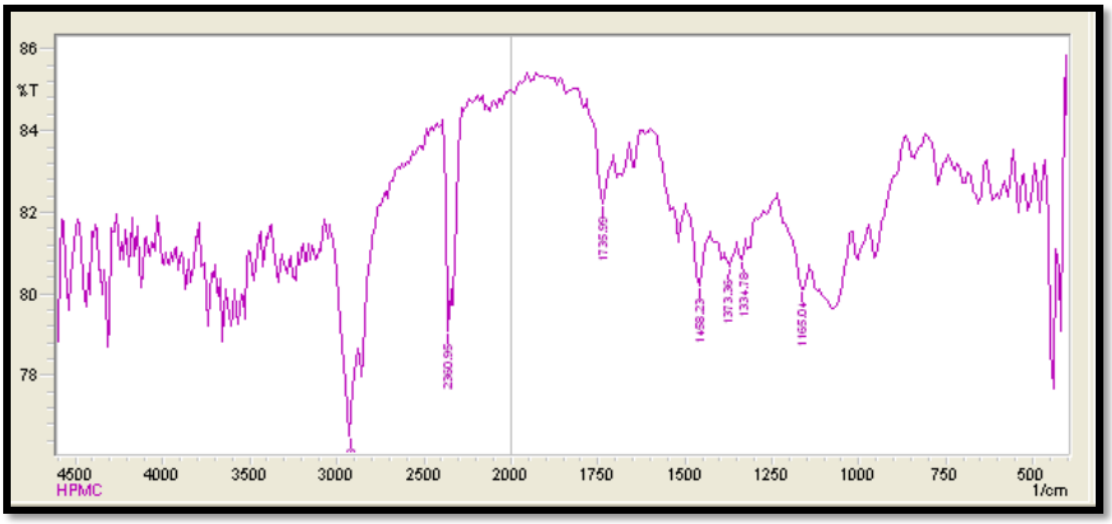

Figure 3: FTIR spectrum of HPMC K100M

Table 4: Data for observed and reported peaks of HPMC K100M

Functional Group	Observed values cm^{-1}	Reported values cm^{-1}
C-H stretching in alkanes	2915.84	3000-2850
-C=O stretching	1743.33	1760-1665
OH-H stretching	3488.60	3500-3200

3.2 FTIR spectrum of Sericin and extracted Sericin: The IR spectrum of the given sample of extracted Sericin showed similar characteristics peaks to that of a reported spectrum of Sericin.

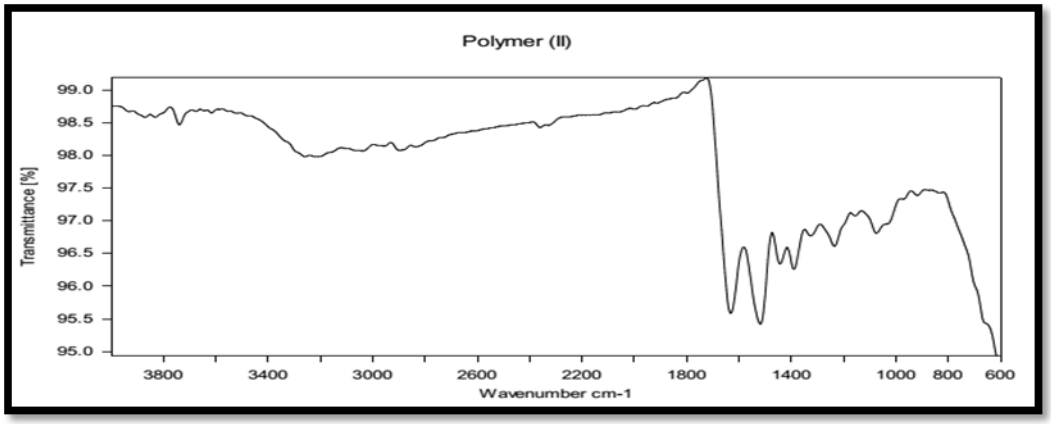

Figure 4: FTIR spectrum of Sericin

Table 5: Data for observed and reported peaks of Sericin

Functional Group	Observed values cm^{-1}	Reported values cm^{-1}
C-O Stretching	1235.39	1300-900
C-H Bending in alkane	1390.46	1500-1300
N-H Bending	1518.38	1700-1500
C=O Stretching	1631.94	1900-1600

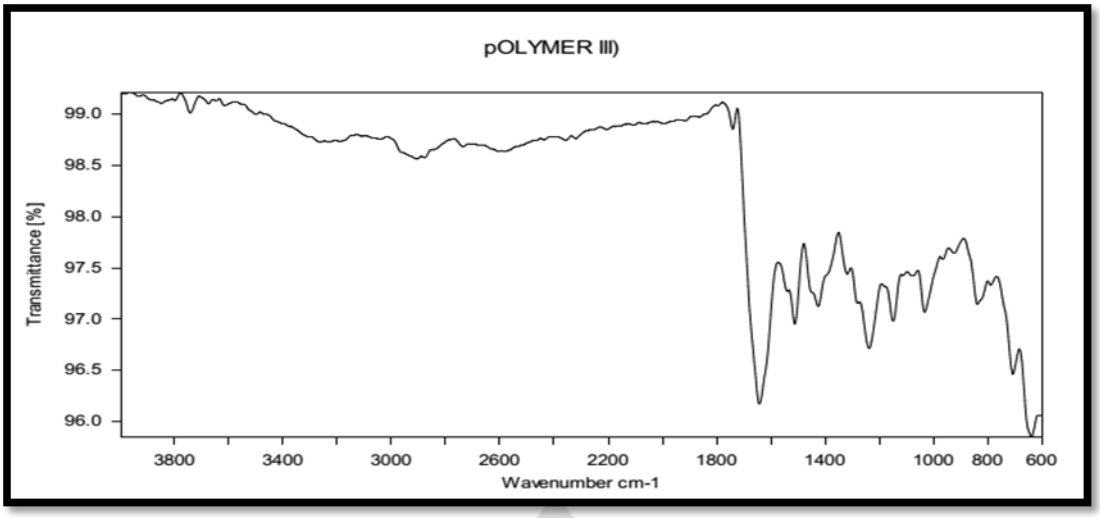

Figure 5: FTIR spectrum of extracted Sericin

Table 6: Data for observed and reported peaks of extracted Sericin

Functional Group	Observed values cm^{-1}	Reported values cm^{-1}
C-O Stretching	1237.28	1300-900
C-H Bending in alkane	1317.91	1500-1300
N-H Bending	1511.09	1700-1500
C=O Stretching	1642.33	1900-1600

3.3 FTIR spectrum of β-CD: The IR spectrum of the given sample showed similar characteristics peaks to that of a reported spectrum of β-CD

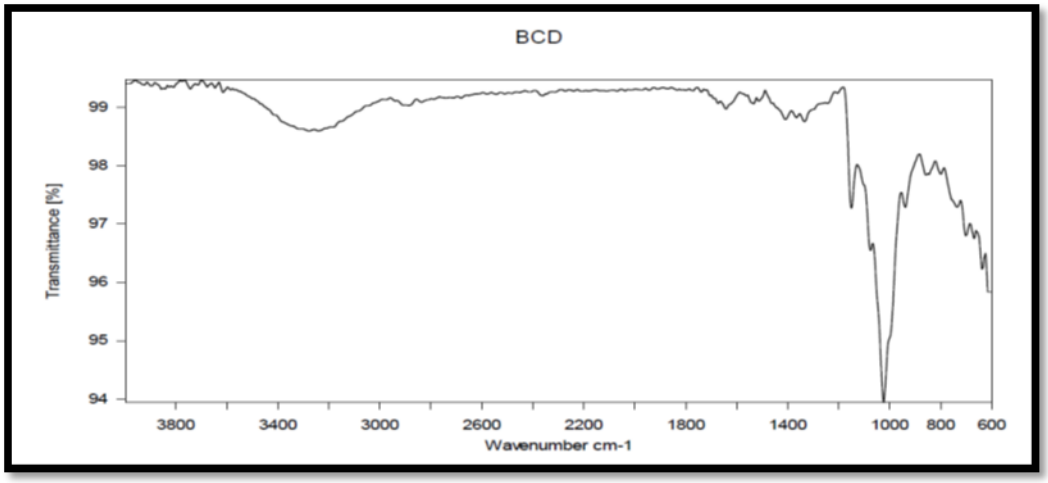

Figure 6: FTIR spectrum of β-CD

Table 7: Data for observed and reported peaks of β-CD

Functional Group	Observed values cm^{-1}	Reported values cm^{-1}
OH stretching in alcohol	3281.20	3550-3200
C-H stretching alkane	2895.76	3000-2850
C-H bending in alkane	1408.32	1500-1300
C-O stretching in ether	1024.01	1320-1000

4. Preparation of calibration curve for Pioglitazone HCl by UV Spectroscopy

4.1 Determination of λ_{max}

The UV spectrum of Pioglitazone HCl in 0.1 N HCl was measured at 200-400 nm and showed absorbance maxima at wavelength 269 nm.

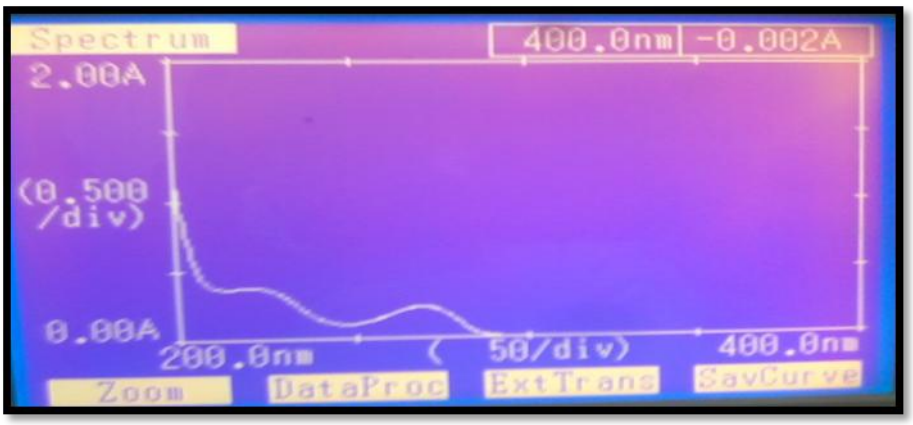

Figure 7: UV spectrum of Pioglitazone HCl in 0.1 N HCl

4.2 Development of calibration curve

Calibration of Pioglitazone HCl was done in 0.1 N HCl and phosphate buffer p^H 7.4 because formulation to be prepared is controlled release dosage form and which is retained in the stomach for 2 hours then comes in the intestine and release drug from stomach to intestine. So 0.1 N HCl have same acidic nature to gastric fluid and phosphate buffer p^H 7.4 have the same p^H as in intestine, due to that both solutions were used for calibration of Pioglitazone HCl.

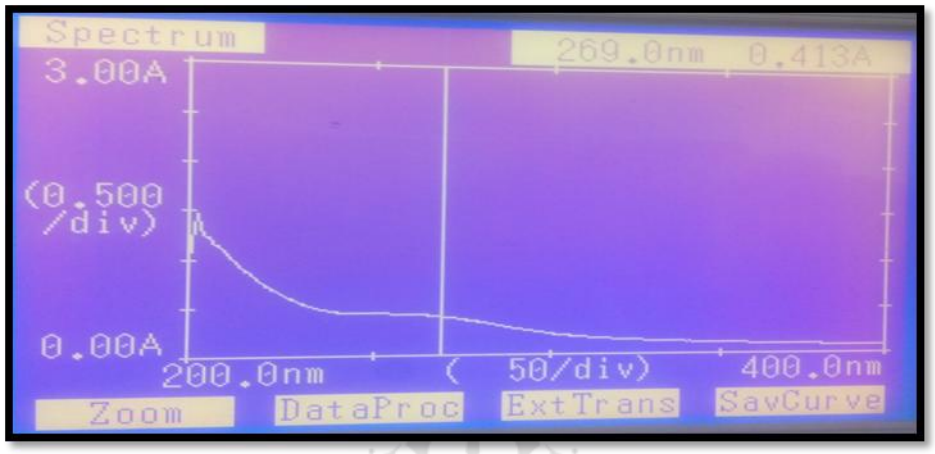

Figure 8: UV spectrum of Pioglitazone HCl in Phosphate buffer pH 7.4

Table 8: Data for calibration curve of various concentration of Pioglitazone HCl

Sr. No.	Concentration (µg/ml)	Absorbance in 0.1 N HCl	Absorbance in Phosphate buffer pH 7.4
1	0	0	0
2	5	0.112	0.033
3	10	0.198	0.097
4	15	0.303	0.152
5	20	0.407	0.194
6	25	0.534	0.236
7	30	0.642	0.289
8	35	0.736	0.357
9	40	0.851	0.398
10	45	0.936	0.413
11	50	1.056	0.487

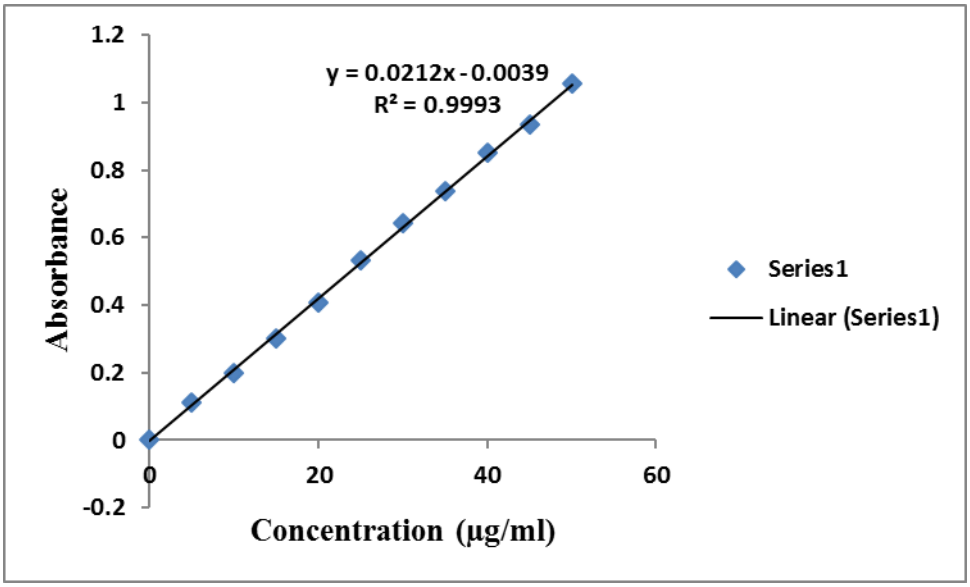

Figure 9: Calibration curve of Pioglitazone HCl in 0.1 N HCl (pH 1.2)

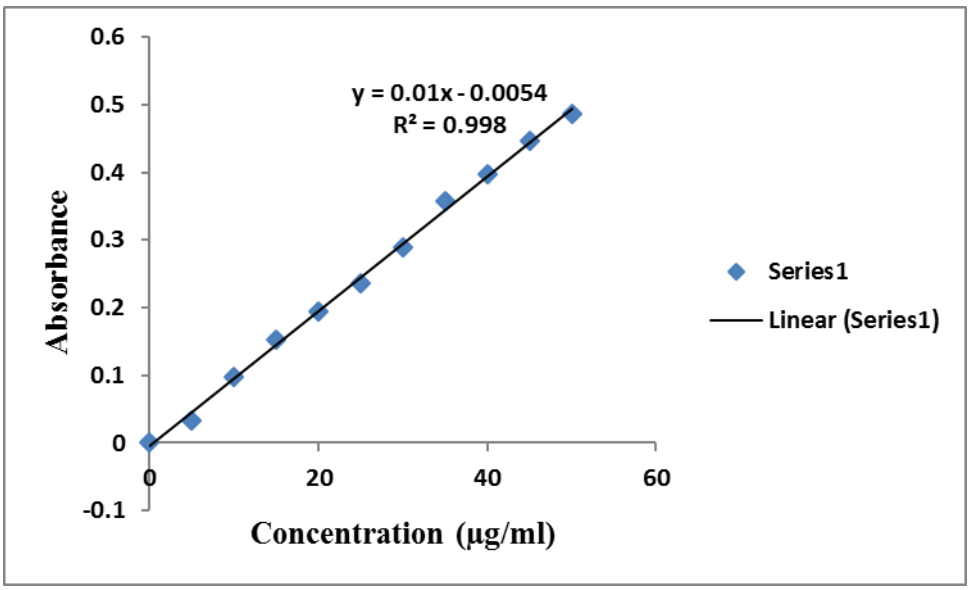

Figure 10: Calibration curve of Pioglitazone HCl in pH 7.4

The regression coefficient for 0.1 N HCl and Phosphate buffer p^H 7.4 was found to be 0.999, 0.998 with slope value 0.021, 0.01 and y-intercept value 0.003, 0.005 respectively. The results indicate that there is a linear relationship between concentration (5-50µg/ml) and absorbance.

5. Solubility Study

Solubility Studies of Pioglitazone HCl in β-Cyclodextrin

Table 9: Solubility studies in β-Cyclodextrin

Sr. No.	Concentration of β-Cyclodextrin (%w/v)	Volume of solvent (ml)	Drug added (mg)	Absorbance at 269 nm	Drug (mg/5ml)
1	0	5	25	0.0023	0.0182
2	0.25	5	25	0.064	0.1725
3	0.50	5	25	0.0397	0.1117
4	0.75	5	25	0.0659	0.1772
5	1	5	25	0.0855	0.2262

Solubility of Pioglitazone HCl is more in 1%w/v solution of ß-cyclodextrin among 0.25%, 0.50% and 0.75%w/v solutions.

6. Preparation of Factorial Batches

A 3^2 factorial design was used for the preparation of controlled release tablet of Pioglitazone HCl. Ingredients given in table 1 was used for formulation development. Pre and post-compression evaluation parameters were studied.

7. Evaluation of factorial batches

7.1 Pre-compression Evaluation

Table 10: Results of powder properties

Sr. No.	Batch No.	Mean BD (gm/ml) ±SD	Mean TP (gm/ml) ±SD	Mean carr's Index %±SD	Mean Angle of Repose^0±SD	Mean Hausner Ratio± SD
1	F1	0.507±0.001	0.587±0.002	13.61±0.45	32.11±1.03	1.15±0.005
2	F2	0.516±0.0005	0.605±0.003	14.40±0.42	31.34±0.67	1.06±0.005
3	F3	0.604±0.003	0.644±0.001	6.2±0.61	27.56±0.96	1.06±0.01
4	F4	0.512±0.001	0.554±0.0005	7.68±0.26	32.48±1.56	1.07±0.005
5	F5	0.507±0.001	0.583±0.0005	13.02±0.28	21.55±0.50	1.14±0.005
6	F6	0.522±0.002	0.561±0.0005	7.01±0.56	26.45±0.83	1.08±0.005
7	F7	0.517±0.011	0.551±0.001	6.10±2.30	24.12±0.57	1.06±0.026
8	F8	0.609±0.006	0.651±0.001	6.49±0.90	33.21±0.64	1.07±0.01
9	F9	0.620±0.001	0.676±0.001	7.91±0.095	29.41±0.35	1.08±0.005

7.2 Post-Compression Parameter

1. Tablets Evaluation

The tablet thickness was found to be in range, the results indicate that there is a uniform size and shape was maintained for all formulations and the die cavities filled accurately as there is very less change in thickness was observed. Hardness and content of uniformity found to be an acceptable limit.

Table 11: Results of an evaluation of tablets

Sr. No.	Batch No.	Weight Variation ± SD (mg)	Thickness ± SD (mm)	Hardness ± SD ($kg/cm^{2)}$	Friability ±SD (%)	Drug content± SD (%)
1	**F1**	316±0.57	4.67±0.01	4.6±0.1	0.74±0.01	99.68±0.184
2	**F2**	316±1.52	4.68±0.015	5.4±0.15	0.72±0.01	99.36±0.315
3	**F3**	315±1.15	4.68±0.01	5.5±0.05	0.67±0.01	99.05±0.363
4	**F4**	316±0.57	4.65±0.005	5.9±0.05	0.57±0.02	98.73±0.315
5	**F5**	317±1.15	4.66±0.005	6.2±0.05	0.42±0.01	99.36±0.483
6	**F6**	316±1.73	4.67±0.011	6.7±0.1	0.57±0.011	98.41±0.660
7	**F7**	317±1	4.68±0.005	7.3±0.1	0.35±0.015	99.68±0.660
8	**F8**	316±1.73	4.67±0.005	7.6±0.05	0.46±0.03	99.05±0.184
9	**F9**	317±1.73	4.66±0.02	7.7±0.05	0.64±0.016	98.73±0.363

2. Weight gain after coating

Table 12: Results of evaluation of tablets

Batch No.	Wo (mg)	Wt (mg)	% Weight gain
F1	299±1.15	316±1	5.68%
F2	300±1	317±1	5.66%
F3	301±2.08	317±1.15	5.31%
F4	300±0.57	316±2	5.33%
F5	301±1.73	317±0.57	5.31%
F6	300±1.52	316±1.52	5.33%
F7	299±0.57	316±2.64	5.68%
F8	300±0.57	317±1.15	5.66%
F9	302±1.52	314±0.57	5.62%

3. *In-vitro* Dissolution Study

Table 13: Cumulative drug release (%) of formulation batches

Time (hrs)	Cumulative Drug Release (%)								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
½	12	7.71	10.26	9.04	6.37	6.57	4.93	5.8	4.26
1	20.77	18.58	15.91	15.81	11.05	11.34	8.48	9.20	7.33
2	29.17	22.21	22.50	19.74	15.35	14.87	13.53	14.49	11.53
3	41.26	36.45	36.25	25.64	20.63	20.63	18.22	18.63	18.62
4	51.11	49.29	41.49	32.67	27.45	27.45	22.65	23.45	25.04
5	65.97	56.54	47.74	42.71	36.28	39.68	26.27	26.87	28.67
6	74.04	61.61	55.96	49.36	41.53	44.33	31.50	31.70	35.10
7	82.52	71.07	60.85	56.01	45.57	48.78	34.73	35.34	37.74
8	86.61	83.05	69.52	62.67	48.62	52.43	38.98	37.58	39.98
9	94.11	89.05	75.99	67.74	54.08	57.09	42.42	40.02	43.23
10	-	93.60	83.48	71.22	56.94	64.55	48.06	43.26	44.47
11	-	97.45	89.77	75.90	61.20	70.02	50.52	47.11	45.52
12	-	-	94.87	79.38	63.87	73.90	54.77	48.96	46.97
13	-	-	98.57	81.07	67.54	77.18	58.63	50.22	49.62
14	-	-	-	83.76	71.61	79.87	63.50	52.67	51.68
15	-	-	-	85.05	77.09	81.75	66.77	57.13	54.34
16	-	-	-	88.54	82.37	83.04	71.94	60.19	57.60
17	-	-	-	91.64	85.66	86.34	74.92	62.46	60.46
18	-	-	-	96.53	90.16	89.23	79.60	64.13	63.53
19	-	-	-	98.62	94.05	94.52	84.48	66.39	65.99
20	-	-	-	-	96.94	97.41	88.77	70.86	68.06

Formulation F1 to F9 all shows different results because of different concentrations of polymer used. As the concentration of Sericin and HPMC K 100M were changed, the drug release affected and gets changed. From the above observations, it was concluded that the optimized F7 batch tablets formulation showed a controlled release of Pioglitazone HCl for 20 hrs and give release up to 88.77 %.

8. Dissolution Kinetic Study

Table 14: Zero- order kinetic model profile for batches F1-F9

Time (min)	Percent Cumulative Drug Release								
	Batch No.								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
30	12	7.71	10.26	9.04	6.37	6.57	4.93	5.8	4.26
60	20.77	18.58	15.91	15.81	11.05	11.34	8.48	9.2	7.33
120	29.17	22.21	22.5	19.74	15.35	14.87	13.53	14.49	11.53
180	41.26	36.45	36.25	25.64	20.63	20.63	18.22	18.63	18.62
240	51.11	49.29	41.49	32.67	27.45	27.45	22.65/	23.45	25.04
300	65.97	56.54	47.74	42.71	36.28	39.68	26.27	26.87	28.67
360	74.04	61.61	55.96	49.36	41.53	44.33	31.5	31.7	35.1
420	82.52	71.07	60.85	56.01	45.57	48.78	34.73	35.34	37.74
480	86.61	83.05	69.52	62.67	48.62	52.43	38.98	37.58	39.98
540	94.11	89.05	75.99	67.74	54.08	57.09	42.42	40.02	43.23
600	-	93.6	83.48	71.22	56.94	64.55	48.06	43.26	44.47
660	-	97.45	89.77	75.9	61.2	70.02	50.52	47.11	45.52
720	-	-	94.87	79.38	63.87	73.9	54.77	48.96	46.97
780	-	-	98.57	81.07	67.54	77.18	58.63	50.22	49.62
840	-	-	-	83.76	71.61	79.87	63.5	52.67	51.68
900	-	-	-	85.05	77.09	81.75	66.77	57.13	54.34
960	-	-	-	88.54	82.37	83.04	71.94	60.19	57.6
1020	-	-	-	-	85.66	86.34	74.92	62.46	60.46
1080	-	-	-	-	90.16	89.23	79.6	64.13	63.53
1140	-	-	-	-	94.05	94.52	84.48	66.39	65.99
1200	-	-	-	-	96.94	97.41	88.77	70.86	68.06
R^2	0.98	0.981	0.987	0.954	0.987	0.966	0.997	0.997	0.953
K	0.171	0.149	0.122	0.087	0.077	0.079	0.07	0.053	0.052

Table 15: Korsmeyer- Peppas kinetic model profile for batch F1-F9

SQRT	Percent Cumulative Drug Release								
	Batch No.								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
5.48	12	7.71	10.26	9.04	6.37	6.57	4.93	5.8	4.26
7.75	20.77	18.58	15.91	15.81	11.05	11.34	8.48	9.2	7.33
10.95	29.17	22.21	22.5	19.74	15.35	14.87	13.53	14.49	11.53
13.41	41.26	36.45	36.25	25.64	20.63	20.63	18.22	18.63	18.62
15.49	51.11	49.29	41.49	32.67	27.45	27.45	22.65	23.45	25.04
17.32	65.97	56.54	47.74	42.71	36.28	39.68	26.27	26.87	28.67
18.97	74.04	61.61	55.96	49.36	41.53	44.33	31.5	31.7	35.1
20.49	82.52	71.07	60.85	56.01	45.57	48.78	34.73	35.34	37.74
21.9	86.61	83.05	69.52	62.67	48.62	52.43	38.98	37.58	39.98
22.8	94.11	89.05	75.99	67.74	54.08	57.09	42.42	40.02	43.23
24.49	-	93.6	83.48	71.22	56.94	64.55	48.06	43.26	44.47
25.69	-	97.45	89.77	75.9	61.2	70.02	50.52	47.11	45.52
26.83	-	-	94.87	79.38	63.87	73.9	54.77	48.96	46.97
27.92	-	-	98.57	81.07	67.54	77.18	58.63	50.22	49.62
28.98	-	-	-	83.76	71.61	79.87	63.5	52.67	51.68
30	-	-	-	85.05	77.09	81.75	66.77	57.13	54.34
30.98	-	-	-	88.54	82.37	83.04	71.94	60.19	57.6
31.93	-	-	-	91.64	85.66	86.34	74.92	62.46	60.46
32.86	-	-	-	96.53	90.16	89.23	79.6	64.13	63.53
33.76	-	-	-	98.62	94.05	94.52	84.48	66.39	65.99
34.64	-	-	-	-	96.94	97.41	88.77	70.86	68.06
R^2	0.97	0.965	0.973	0.98	0.974	0.979	0.955	0.987	0.985
K	4.351	4.196	3.778	3.185	2.951	3.117	2.69	2.018	2.08

Table 16: Data of various kinetic models for formulation

Batch No.	Zero order	Higuchi model	Korsmeyer-Peppas model		Best fit model
	R^2	R^2	R^2	n	
F1	**0.98**	0.97	0.647	0.005	Zero-order
F2	0.981	0.965	**0.987**	0.707	Korsmeyer-Peppas
F3	0.987	0.973	**0.998**	0.68	Korsmeyer-Peppas
F4	0.954	0.98	**0.994**	0.652	Korsmeyer-Peppas
F5	0.987	0.974	**0.988**	0.646	Korsmeyer-Peppas
F6	0.966	0.979	**0.983**	0.653	Korsmeyer-Peppas
F7	**0.997**	0.955	0.983	0.631	Zero-order
F8	**0.997**	0.987	0.994	0.611	Zero-order
F9	0.953	**0.985**	0.977	0.635	Higuchi model

Percentage cumulative drug release and kinetic studies show results are as follows:

1. The formulations prepared by using synthetic and natural polymers shows the different drug release if concentrations of polymers increase it decrease the drug release.

2. The formulation F1 obeyed the zero- order kinetic model, R^2 for this formulation was found to be 0.980.

3. The formulation F2 followed the Korsmeyer-Peppas kinetic model, R^2 for this formulation was found to be 0.987 and n value was found to be 0.707.

4. The formulation F3 obeyed the Korsmeyer-Peppas kinetic model, R^2 for this formulation was found to be 0.998 and n value was found to be 0.68

5. The formulation F4 obeyed the Korsmeyer-Peppas kinetic model, R^2 for this formulation was found to be 0.994 and n value was found to be 0.652.

6. The formulation F5 obeyed the Korsmeyer-Peppas kinetic model, R^2 for this formulation was found to be 0.988 and n value was found to be 0.646.

7. The formulation F6 followed the Korsmeyer-Peppas kinetic model, R^2 for this formulation was found to be 0.983 and n value was found to be 0.653.

8. The formulation F7 obeyed the zero- order kinetic model, R^2 for this formulation was found to be 0.997. This F7 batch gives the good percentage cumulative drug release in 20 hrs. up to 88.77% hence, this F7 batch taken as the optimized batch.

9. The formulation F8 obeyed the zero- order kinetic model, R^2 for this formulation was found to be 0.997.

10. The formulation F9 obeyed the Korsmeyer-Peppas kinetic model, R^2 for this formulation was found to be 0.986.

11. Formulation F1 showed Fickian release and F2-F9 showed Non-Fickian release.

9. Validation parameter study for optimized formulation

9.1 Linearity

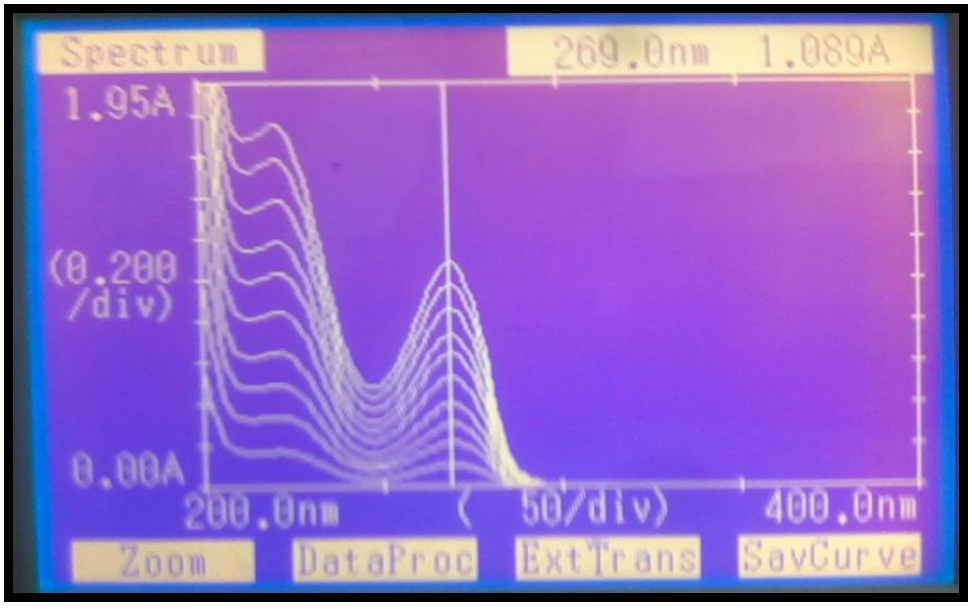

Figure 11: Linearity graph of optimized batch

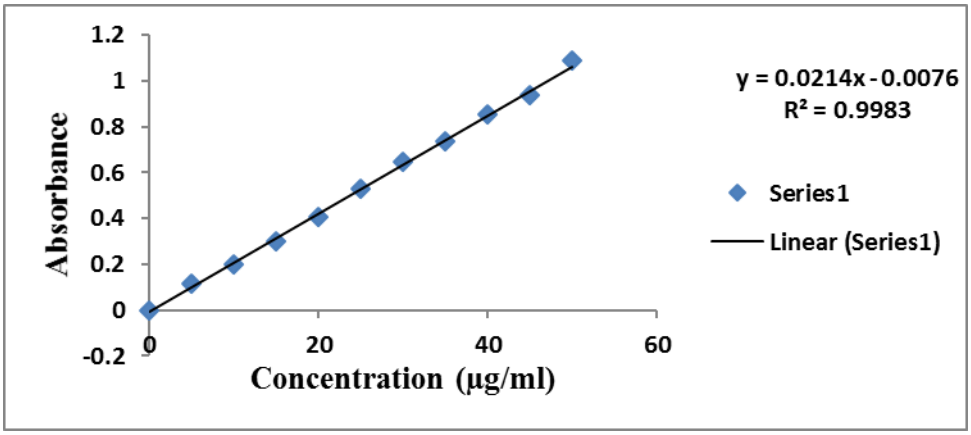

Figure 12: Result of linearity graph for analytical method development

Table 17: Result of Linearity test for analytical method development

Sr. No.	Concentration (µg/ml)	Absorbance in 0.1 N HCl
1	0	0.0
2	5	0.117
3	10	0.201
4	15	0.298
5	20	0.406
6	25	0.531
7	30	0.644
8	35	0.734
9	40	0.853
10	45	0.935
11	50	1.089
Mean	-	0.5808
R^2	-	0.998
Slope	-	0.021

9.2 Precision

A) Intraday Precision

Table 18: Results of Intraday precision test for analytical method development

Time (hrs)	Concentration(µg/ml)	Absorbance at 269 nm
0	10	0.217
1	10	0.213
2	10	0.220
3	10	0.218
4	10	0.229
5	10	0.221
Mean	-	0.219
SD	-	0.0044
RSD	-	0.020
%RSD	-	2.04

B) Inter-day Precision

Table 19: Results of Interday precision test for analytical method development

Time(hrs)	Day		
	Day-I	Day-II	Day-III
0	0.217	0.216	0.221
1	0.219	0.221	0.222
2	0.217	0.222	0.224
3	0.224	0.226	0.228
4	0.221	0.223	0.226
5	0.219	0.223	0.225
Mean	0.219	0.221	0.224
SD	0.00128	0.001	0.0023
RSD	0.0058	0.045	0.010
%RSD	0.58	0.45	1

9.3 Accuracy

Table 20: Results of accuracy for analytical method development

Amount of sample (µg/ml)	Amount of drug added (%)	Total amount present (µg/ml)	Calculated total conc. (µg/ml)	% Recovery ± SD
10	80	18	18.312	99.9±0.002
10	100	20	20.354	101.54±001
10	120	22	22.488	100.06±0.003

9.4 Robustness

Table 21: Results of robustness for analytical method development

Sr. No.	Conc. (µg/ml)	Absorbance at -2 nm (267nm)	Absorbance at 269 nm	Absorbance at +2 nm (271nm)	Mean	SD	% RSD
1	10	0.214	0.217	0.221	0.217	0.0028	1.29
2	20	0.352	0.354	0.355	0.354	0.0037	1.04
3	30	0.598	0.601	0.603	0.600	0.0021	0.35

9.5 Ruggedness

Table 22: Results of ruggedness for analytical method development

Sr. No.	Sample	System	Day	Time	Absorbance	Mean	SD	% RSD
1	Batch no. X (20 µg/ml)	Bio-era	Mon.	11.00 AM	0.355 0.361 0.358	0.358	0.0077	2.16
2	Batch no. Y (20 µg/ml)	Shimadzu	Tue.	2.00 PM	0.354 0.357 0.351	0.354	0.0077	2.16

10. Characterization of optimized formulation batch F7

10.1 Fourier Transforms Infrared Spectroscopy of optimized Formulation F7

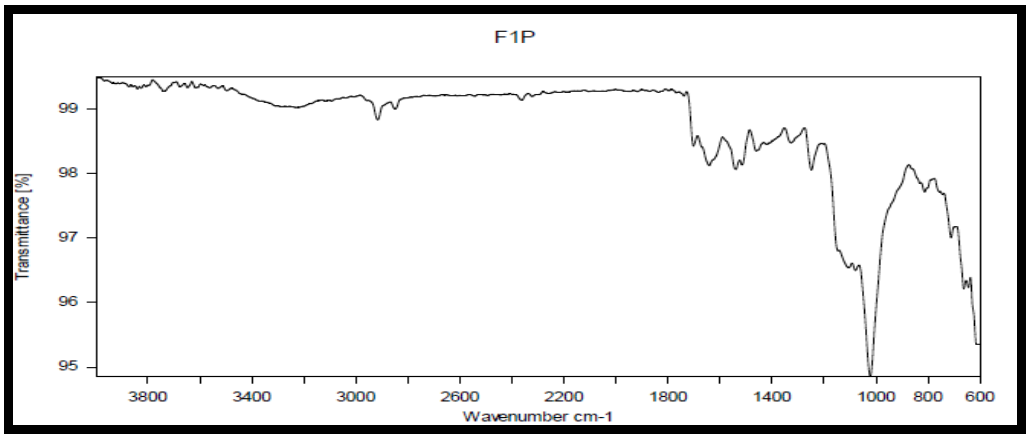

Figure 13: FTIR spectrum of Formulation batch F7

Table 23: Data for observed and reported peaks of Formulation batch F7

Functional Group	Observed values cm^{-1}	Reported values cm^{-1}
-C-C- stretching in aromatic	1538.42	1550-1475
-C=C- stretching in alkenes	1641.23	1640-1680
-C-H stretching in alkanes	2916.10	3000-2850
-C=O stretching	1702.09	1760-1690
O-H stretching	2848.57	3300-2500
-C-N stretching in amines	1248.37	1250-1020
C-O- stretching in others	1022.50	1320-1000
C-H Bending in alkanes	1460.49	1500-1300

These obtained results indicate that there was no evidence for the interaction between PGZ HCl and other polymeric material. These results clearly indicate that the above polymers can be used without any interaction for the preparation of controlled release tablet of Pioglitazone HCl.

10.2 Differential Scanning Calorimetry of optimized batch F7

1. DSC of Pioglitazone HCl

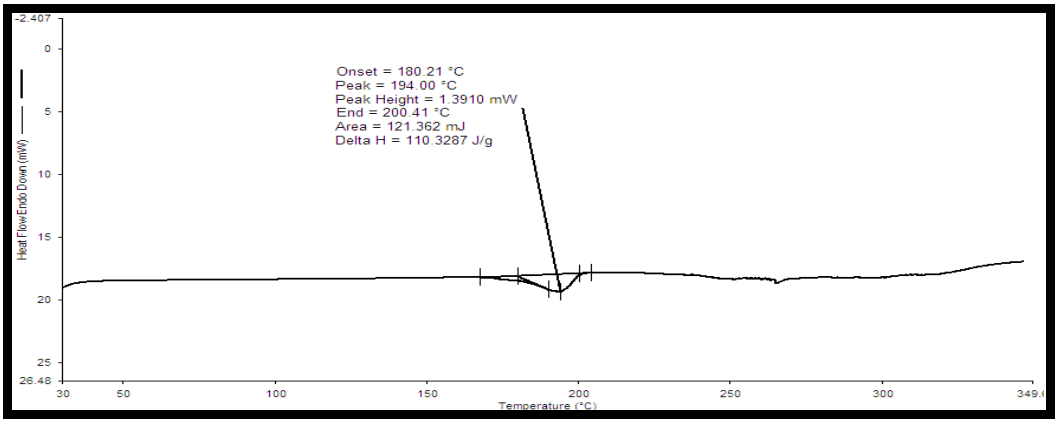

Figure 14: DSC of Pioglitazone HCl

2. DSC of optimized formulation batch F7

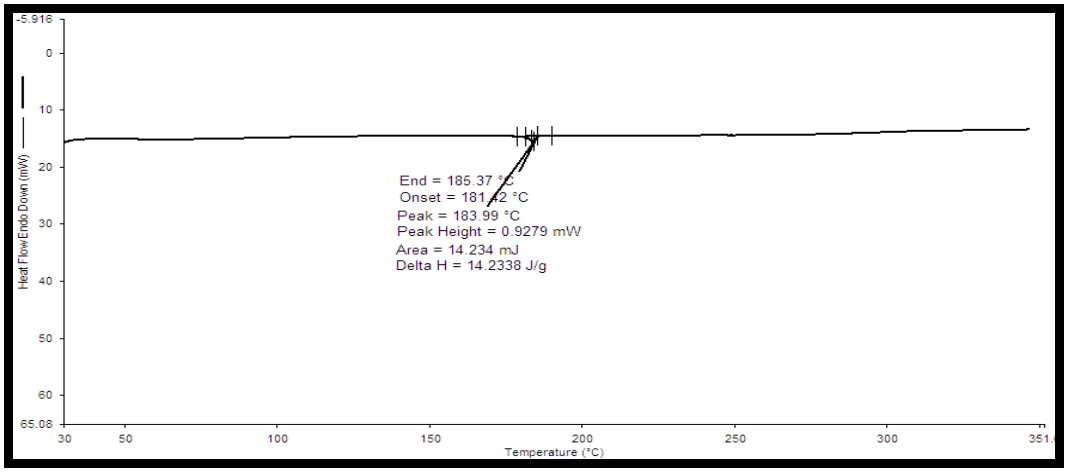

Figure 15: DSC of Formulation batch F7

3. DSC Overlay of pure drug and optimized batch

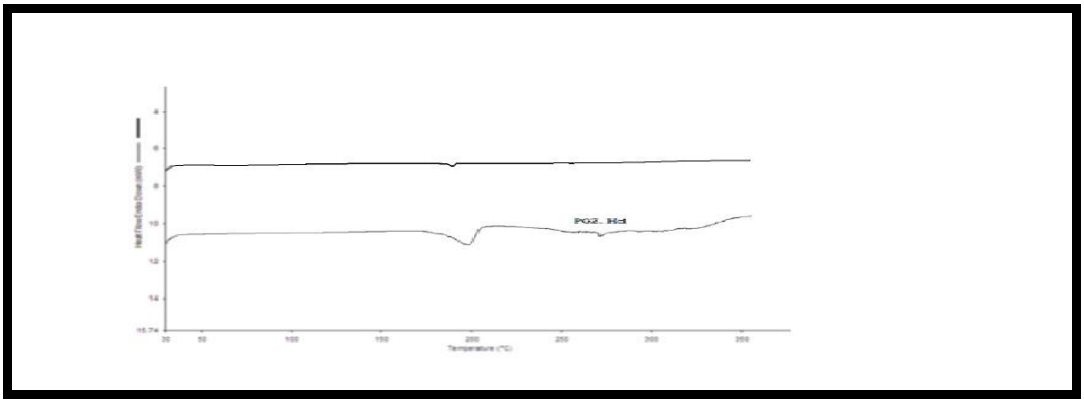

Figure 16: DSC Overlay of pure drug and optimized batch

The DSC thermogram of the drug showed a characteristics endothermic peak at 194^0C and indicating the melting point of the drug in a figure. The optimized formulation batch F7 also showed an endothermic peak at 183.99^0C. The DSC thermogram indicates the shift towards lower temperature so that there is physical interaction between drug and polymers.

Table 24: Interpretation of DSC thermogram of drug and formulation

Drug and Formulation	Onset (ºC)	End (ºC)	Peak (ºC)
Pioglitazone HCl	180.21	200.41	194
Optimized formulation	181.12	185.37	183.99

CONCLUSION

The analytical studies, absorbance maxima confirmed that drug was found to pure and not degraded. The FTIR Study of extracted Sericin and purchased sericin show the same characteristics peak. The solubility study of Pioglitazone HCl was found to be more in 1%w/v solution of ß-cyclodextrin among 0.25%, 0.50% and 0.75%w/v solutions. From the results of the drug content determination, it was assured that there was a uniform distribution of a drug in the tablets. The pre-compression and post-compression studies of formulations show results within an acceptable limit. Release study of Pioglitazone HCl tablets indicates the drug release from the formulations F7 containing Sericin in higher concentration showed a slow release of 88.77% at 20 hr. Based on the R^2 value it was confirmed that it follows zero- order.

So, finally from all results, it was concluded that incorporation of ß-CD with kneading technique showed the increase in dissolution rate and solubility of a drug with a significant release retarding ability for the drug with the high concentration of Sericin, also it was found that Sericin shows the good release retardant activity.

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