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Research Article

Formulation and Evaluation of Microspheres of Fenofibrate.

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ABSTRACT

The main objective of present research was to formulate & evaluate microspheres of Fenofibrate with sodium alginate & carbopol polymers. Fenofibrate microspheres were prepared by an ionotropic gelation method by using sodium alginate as a crosslinking agent. The developed Fenofibrate microspheres were characterized for micrometric properties, morphology, drug entrapment efficiency, *In-vitro* drug release, drug and polymer interaction studies such as Fourier transform infrared spectroscopy (FTIR) & Differential scanning calorimetric (DSC), X-ray differaction studies & stability study. The Fenofibrate microsphere having mean particle size ranged from 5-50 µm & entrapment efficiency ranged from 92% to 98%. The percentage yield of microspheres ranged from 98-100 %. FTIR spectra of Fenofibrate shows that there is no interaction between drug & polymer ratio in the ideal formulation of F_6. The stability study was carried out for F_6 Formulation at 40 ± 2℃/ 75 ± 5%. The result obtained in this work demonstrate the use of carbopol & sodium alginate polymers for preparation of Fenofibrate microspheres.

KEYWORDS

Ionic gelation method, Sodium alginate, Carbopol, Fenofibrate.

1. INTRODUCTION

A microsphere has been a topic of interest in development of new drug delivery system to prolong the resistance time at a site of application & bioavailability. Microspheres played on important role in development of controlled or sustained drug delivery system. Microspheres multiparticulate drug delivery system which increases sustain action and provides better patient compliance.[1,2,3]

Microspheres have advantages like improved the bioavailability, prevention of incompatibility, provide constant & prolonged therapeutic effects. If the drug to be delivered is subjected to extensive hepatic first pass effect, preventive measures should be advised to either by pass or minimize the extent of hepatic metabolic effects.[4,5]

Fenofibrate used to treat high levels of cholesterol & triglycerides in blood. It is also known as antilipemic & fibric acid so it works by breaking down fats & helping the body to eliminate the triglycerides.[6,7,8]

Fenofibrate is a lipid-regulating agent & is chemically fibric acid derivative. Fenofibrate is lipophilic drug having B.C.S. Class II. Fenofibrate is a lipophilic drug with a low aqueous solubility. A prodrug comprising fenofibric acid linked to an isopropyl ester. Fenofibrate experts its therapeutic effects through the activation of peroxisome proliferation activated receptor (PPARα). This increases lipolysis & elimination of triglycerides rich particles in the plasma by activating lipoprotein lipase & reducing production of apoprotein CIII, The resulting fall in triglycerides produces an alteration in the size & composition of LDL from small, dense particles, to large buoyant particles. These larger particles have a greater affinity for cholesterol receptor & catabolized rapidly. Hence the objective of this work was to formulate the microsphere of Fenofibrate to improve residence of dosage form in GIT, reducing dosing frequency & to enhance the bioavailability.[9]

2. MATERIALS & METHODS

2.1. Materials

Fenofibrate was obtained from Sun pharma Lab Private Ltd., Mumbai, India. Sodium alginate, carbopol, disodium hydrogen phosphate, potassium dihydrogen phosphate, calcium chloride & methanol were procured from chemical store at Rajgad Dnyanpeeth's College of pharmacy, Bhor, Pune, (India). All chemicals used in the study were of analytical grade & used without further purification.

2.2. Method

2.2.1. Preparation of microsphere

The microspheres were prepared by ionotropic external gelation technique. It was a most important method used in preparation of microspheres. Sodium alginate (1-6% w/v) or carbopol (3% w/v) solution was dissolved in deionized water employing mild heat at (50℃) with a magnetic stirring. So stirring it continuously then viscous dispersion is formed. To this dispersion, Fenofibrate (100 mg) was added & sonicated it for 28-30 minutes. The resulting dispersion was then added in 20 gauge hypodermic needle fitted with 10 ml syringe to solution of calcium chloride (5-10% w/v) stirred at 100 rpm. Stirring was continued for one hour to complete to curing reaction & to produce a spherical microspheres. Formed microsphere were

filtered & washed repeatedly with water to remove the excess of calcium chloride which deposited on surface of microspheres . Then microspheres were then dried at 50℃ under vacuum.[10]

Table 1. Formulation composition of microspheres [11]

Formulation code	Amount of Fenofibrate (mg)	Amount of polymer (Sodium alginate + carbopol) (g)	Crosslinking agent ($CaCl_2$) (%)	Distilled water (ml)
F_1	100	0.7	5%	100
F_2	100	0.8	5%	100
F_3	100	0.9	5%	100
F_4	100	0.7	7.5%	100
F_5	100	0.8	7.5%	100
F_6	100	0.9	7.5%	100
F_7	100	0.7	10%	100
F_8	100	0.8	10%	100
F_9	100	0.9	10%	100

2.2.2. Experimental design

The formula optimization was done by 3^2 factorial design using design expert (version 11; Stat-Ease Inc., USA) for mathematical modeling & analysis of responses. The optimal level of variables was determined by 3^2 factorial design including center point. The significant factors selected were concentration of polymers such as sodium alginate & carbopol examining 9 runs.

Variables for experimental designs

Mostly there are two variables for an experimental designs which are as follows,

1. Independent variables

X_1= Concentration of polymer

X_2= Crosslinking agent

2. Dependent variables

Y_1= Particle size

Y_2= Entrapment efficiency

Y_3= t % release

2.3. Evaluation of microspheres

2.3.1. Preformulation studies

Preformulation studies were characterized physical & chemical properties of drug molecule in order to safe, effective & stable dosage form.

2.3.2. Physical appearance

Physical appearances of all the formulations were evaluated for color, solubility, homogenicity & consistency. The melting point of drug was determined by using melting point apparatus.

2.3.3. Bulk characterization [12]

Evaluation of microspheres studied by determining bulk density, tapped density, Carr's index Hausner's ratio, & angle of repose. The properties were determining the following equations,

Bulk density= mass / bulk volume ---Equation 1
Tap density= mass/ tapped volume ---Equation 2
Carr's index= (Tap density – bulk density / Tap density) × 100 ---Equation 3
Hausner's ratio= Tapped density/ Bulk density ---Equation 4
Angle of repose= Tan ɵ= h/r ---Equation 5

2.3.4. Percentage yield[13]

Percentage yield of each formulation was determined according to practical yield or theoretical yield.

Percentage yield= (Practical yield / Theoretical yield) × 100

2.3.5. Entrapment efficiency (%) [14]

100 mg of microspheres were powdered & suspended in phosphate buffer solution (pH 6.8). The solution was kept overnight & filtered through whatmann filter 0.45 µm. Drug content was determined by UV-visible spectrophotometer at 290 nm. The percentage entrapment was calculated by following formula,

Encapsulation efficiency = (Actual drug content / Theoretical drug content) × 100 ---Formula 1

2.4. UV analysis of Fenofibrate[15]

The concentration of Fenofibrate in the samples determined by the UV spectrophotometer (JASCO V-530). A solution of Fenofibrate in methanol gives maximum absorbance at λmax of 286 nm.

2.5. Compatibility study

Compatibility study was performed using FTIR & DSC study.

2.5.1. Fourier transforms infrared spectroscopy (FTIR) [16]

In FTIR study mostly potassium bromide pellet method are used. Samples were thoroughly blended with potassium bromide crystals. The mixture was compressed to make a disc. Then this disc was placed in spectrophotometer & spectra of pure drug & drug excipients combination were recorded. Then FTIR spectra of samples were compared with FTIR spectra of pure drug & excipients.

2.5.2. Differential scanning calorimetric analysis (DSC) [17]

Thermal behavior of pure A.P.I. & Fenofibrate microspheres were studied using differential scanning calorimeter (Schimadzu DSC 60) at heating rate of 10℃/min. Firstly 5 mg samples were accurately weighed into aluminum pans & then sealed. The measurements were performed at a heating range of 50˚- 400˚C under purge nitrogen atmosphere.

2.6. X-ray diffraction study (XRD) [18,19]

X-ray diffractogram of the Fenofibrate & Fenofibrate loaded microspheres were recorded by a differactogram (Brucker AXS D8) using cu line as a radiation which was operated at the voltage 40 kv & current 40 mA. All the samples were measured at the 2ɵ angle range between 5˚& 60˚.

2.7. Particle size[20]

Optical microscopy method was used determination of particle size & size distribution of Fenofibrate microspheres. The size of prepared microspheres were measured by the optical microscopy using a calibrated stage micrometer. The particle size was calculated using the following equation,

$Xg = 10 \times ([ni \times \log X_i] / N)$ ---Equation 6

Where Xg is a geometric mean diameter, ni is number of particle in range, Xi is the midpoint of range & N is total number of particles.

2.8. In-vitro release study[21,22]

In-vitro drug release study was carried out using USP paddle type -II apparatus at 37℃±5℃ & rotational speed of dissolution apparatus was maintained at 100 rpm. 5ml of samples were withdrawn at predetermined time interval upto 6 hrs. 900 ml of phosphate buffer solution 6.8 with 1% sodium lauryl sulphate. The absorbance is measured by UV spectrometry at wavelength of 290 nm & drug content was determined.

2.9. Stability studies[23,24,25]

The stability study was carried out for Fenofibrate formulation as per ICH guidelines. Fenofibrate microspheres formulation was sealed in high density polyethylene bottles & stored at 40±2℃/ 75±5 % RH in closed for good. The samples were evaluated for entrapment efficiency for a period of 3 months.

3. RESULTS AND DISCUSSION

3.1. Preformulation study

3.1.1 Description

The sample of Fenofibrate was found to be white in color & odourless solid.

3.1.2 Solubility

The solubility study of Fenofibrate was found in following table

Table 2. Solubility study of Fenofibrate.

Solvent	Solubility
Water	Practically insoluble
Methanol	Slightly soluble
Ethanol	Slightly soluble
Acetone	Soluble
Ether	Soluble
Benzene	Soluble
Chloroform	Soluble

3.1.3. Melting point

The melting point of Fenofibrate was found to be 80-81℃.

3.1.4. Bulk characterization

The bulk density & tapped density were found to be in the range 0.5 to 0.69 & 0.52 to 0.75. The formulation F_6 showed the compressibility index of 10.76.

Table 3. Bulk characterization of evaluation of microspheres.

Formulation	Bulk density	Tapped density	Carr's index	Hausner's ratio
F_1	0.6	0.75	20	1.25
F_2	0.69	0.73	5.47	1.057
F_3	0.5	0.52	3.84	1.04
F_4	0.54	0.68	20.58	1.259
F_5	0.5	0.69	27.53	1.38
F_6	0.58	0.65	10.76	1.120
F_7	0.62	0.71	12.67	1.145
F_8	0.59	0.63	6.34	1.067
F_9	0.61	0.69	11.59	1.131

3.1.5. Percentage yield

The maximum percentage yield was found of F_6batch & was noted to be 100 % among all the batches. The percentage yield of microspheres were shown in table

Table 4. Percentage yields.

Formulation	% yield
F_1	98
F_2	99
F_3	98
F_4	98
F_5	99
F_6	100
F_7	99
F_8	98
F_9	99

3.1.6. Encapsulation efficiency of microspheres

The percentage encapsulation of microspheres increase with increase in concentration of sodium alginate & carbopol was observed which might due to increased lipophilic & hydrophilic ambience that could accommodate more amount of drug. The 1:2 ratio of sodium alginate & carbopol was used in the microsphere preparation, on the basis of the fact that this ratio is most beneficial for the efficient encapsulation of drugs.

Table 5. Encapsulation efficiency.

Formulation	Encapsulation efficacy
F_1	93
F_2	94

F_3	98
F_4	96
F_5	92
F_6	99
F_7	92.9
F_8	93
F_9	94.5

3.2. Calibration curve of Fenofibrate

Table 6. Calibration curve.

Sr. no	Concentration(µg/ml)	Absorbance
1	**5**	**0.2349**
2	**10**	**0.5016**
3	**15**	**0.8596**
4	**20**	**1.0691**
5	**25**	**1.3442**

3.3. Compatibility study

3.3.1. Fourier transforms infrared spectroscopy (FTIR)

FTIR spectral data were used to confirm the chemical stability of Fenofibrate in microspheres formulation. The FTIR spectra of pure Fenofibrate, FTIR spectra of sodium alginate, FTIR spectra of carbopol, FTIR spectra of physical mixture of drug with sodium alginate & carbopol & Fenofibrate microspheres are as shown in Fig. 1, 2, 3, 4 & 5 respectively.

The spectra of Fenofibrate microspheres showed peak at 817.85 cm^{-1} (C-Cl), 1095.60 cm^{-1} (C-O-C), 1172 cm^{-1} (C=O-O-C), 15.97cm^{-1} (C=C), 2530cm^{-1} (OH).

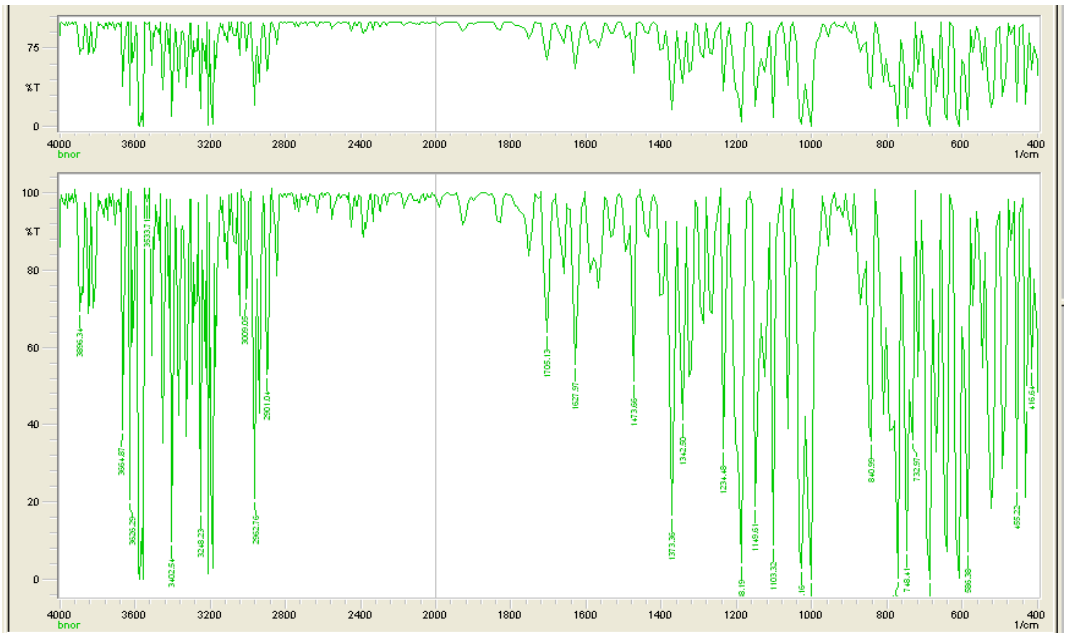

Fig. 1. FTIR spectra of Fenofibrate.

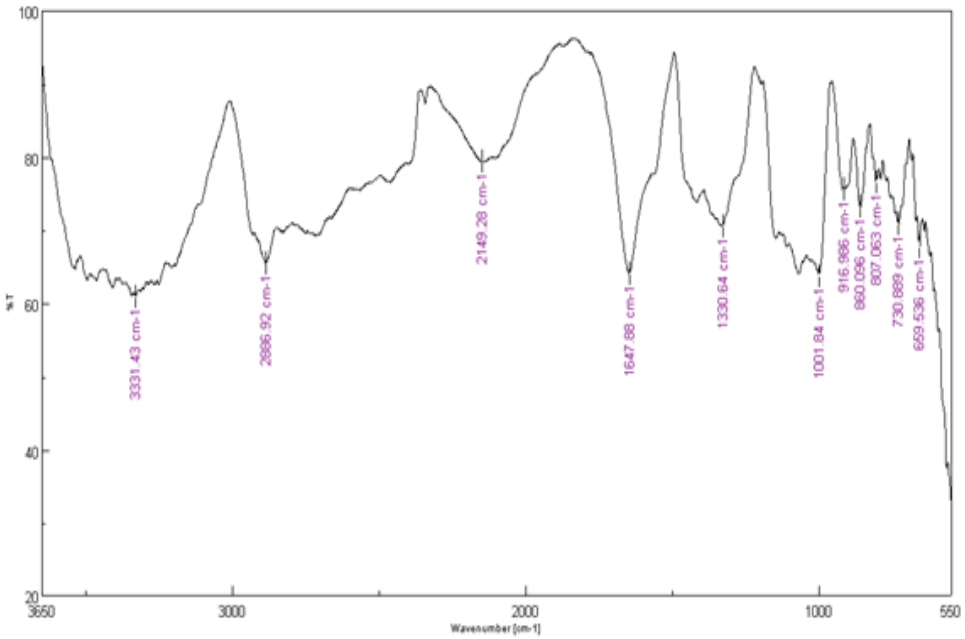

Fig. 2. FTIR spectra of sodium alginate.

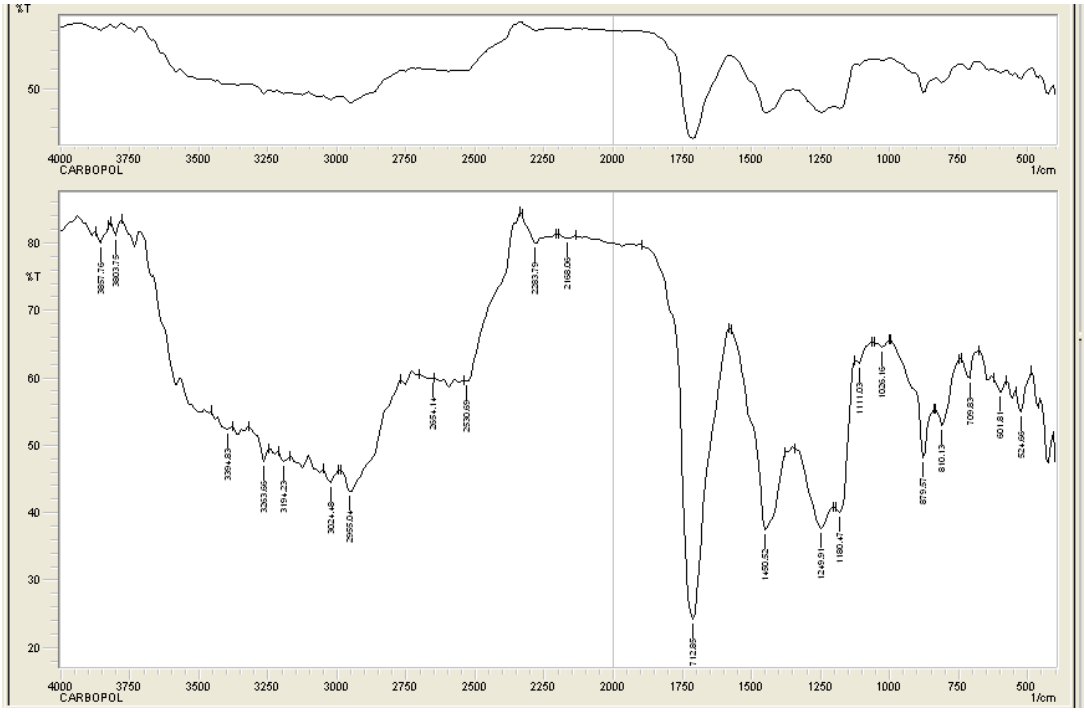

Fig. 3. FTIR spectra of carbopol.

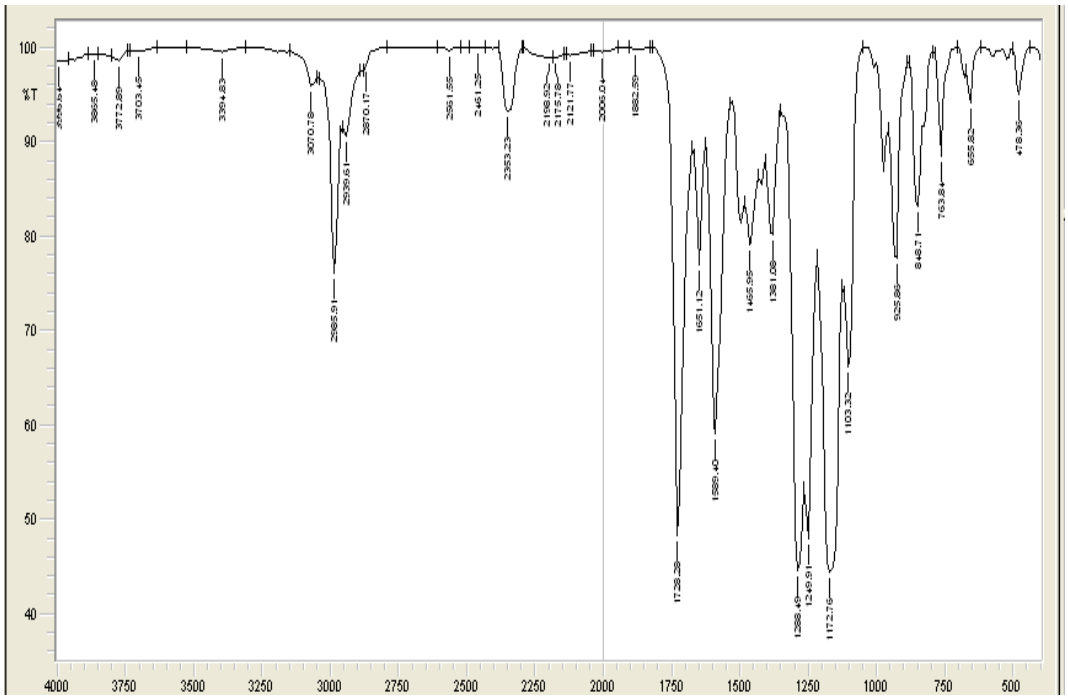

Fig. 4. FTIR spectra of physical mixture of drug, sodium alginate & carbopol.

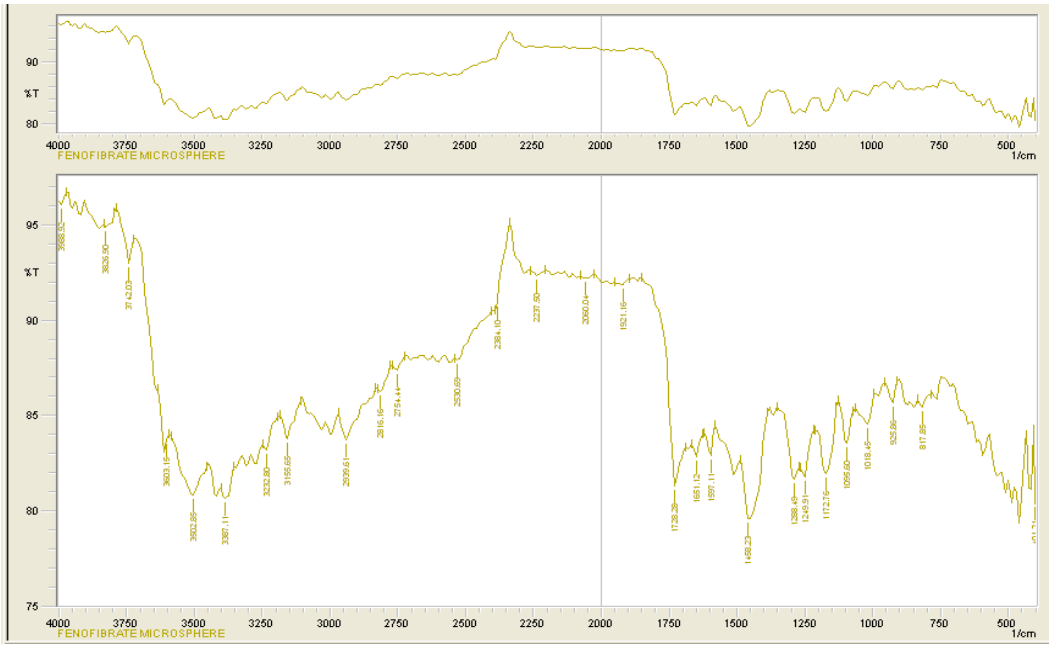

Fig. 5. FTIR spectra of Fenofibrate microspheres.

3.3.2. Differential scanning calorimetric analysis (DSC)

DSC thermogram of drug, Physical mixture & Fenofibrate formulation are as shown in fig 6, 7 & 8 respectively. The thermogram of drug showed a sharp melting peak at 82.39℃. The melting peak of Fenofibrate microsphere formulation showed a sharp melting peak at 78.89℃. The physical parameter like melting point is essential parameter to find the interaction between A.P.I

& excipients. No additional melting point peak was observed in Fenofibrate Thermogram. The formulation findings confirmed the formulation thermal stability & compatibility between drug & excipients since no modification with respect to melting point of drug, sodium alginate & carbopol were observed.

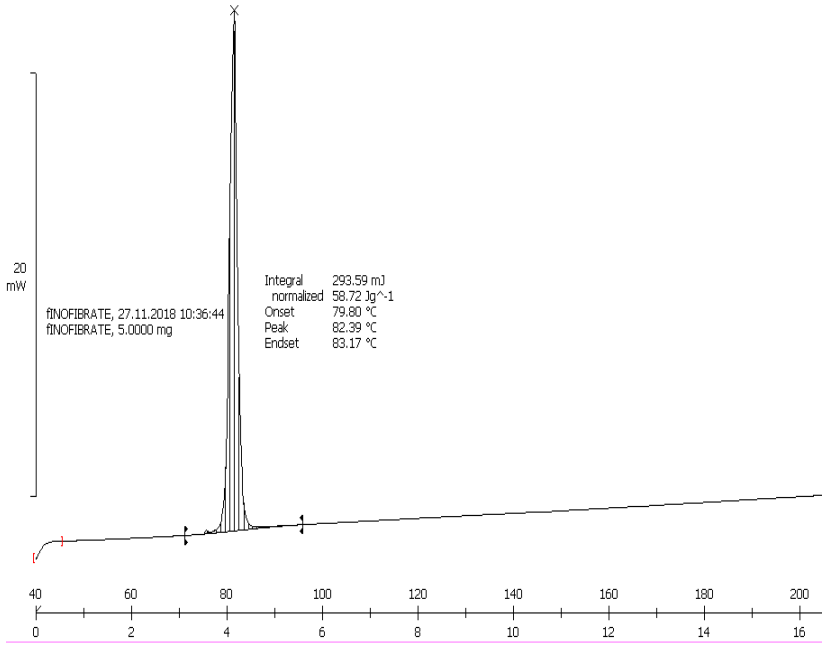

Fig. 6. DSC analysis of Fenofibrate.

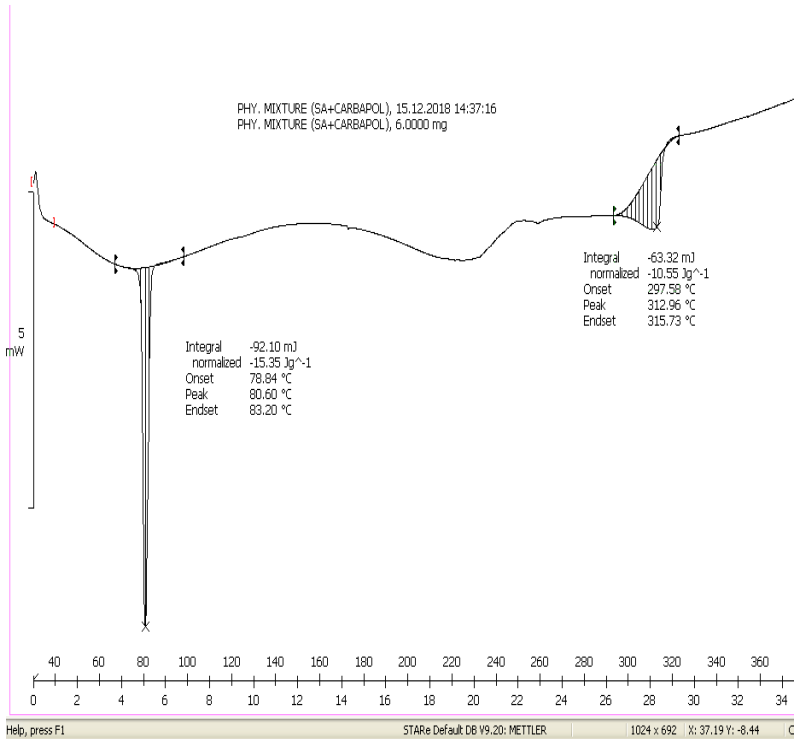

Fig. 7. DSC analysis of physical mixture of drug, sodium alginate & carbopol.

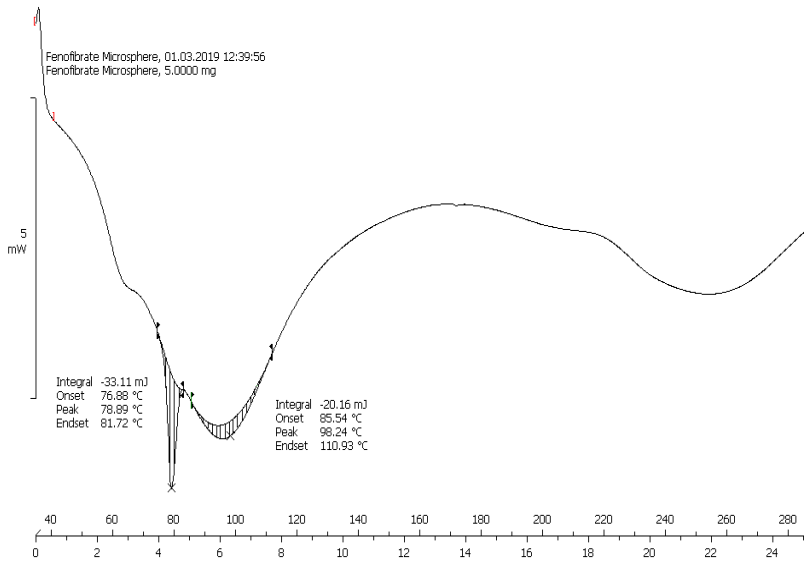

Fig. 8. DSC analysis of Fenofibrate microspheres.

3.4. X-ray diffraction study (XRD)

The x-ray differactogram of Fenofibrate showed sharp peak depicting a typical crystalline pattern. The diminished peak suggests conversion of drug into amorphous form. Also the physical mixture showed less intense peak. Fenofibrate microspheres showed peak, but of low intensity.

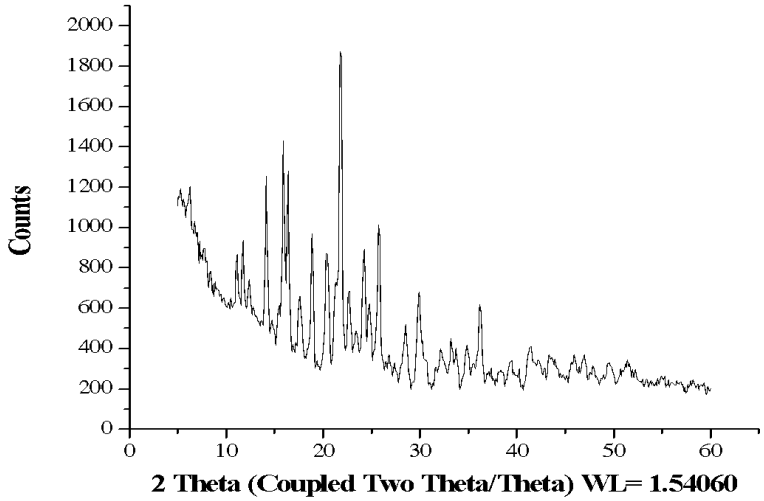

Fig. 9. XRD of Fenofibrate pure drug.

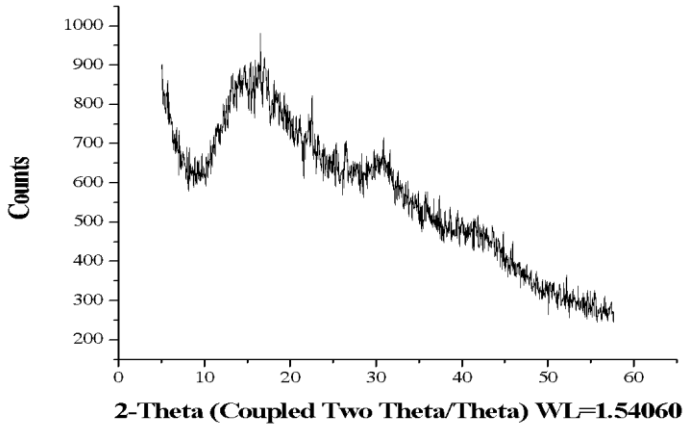

Fig. 10. XRD of Fenofibrate microspheres.

3.5. Scanning electron microscopy

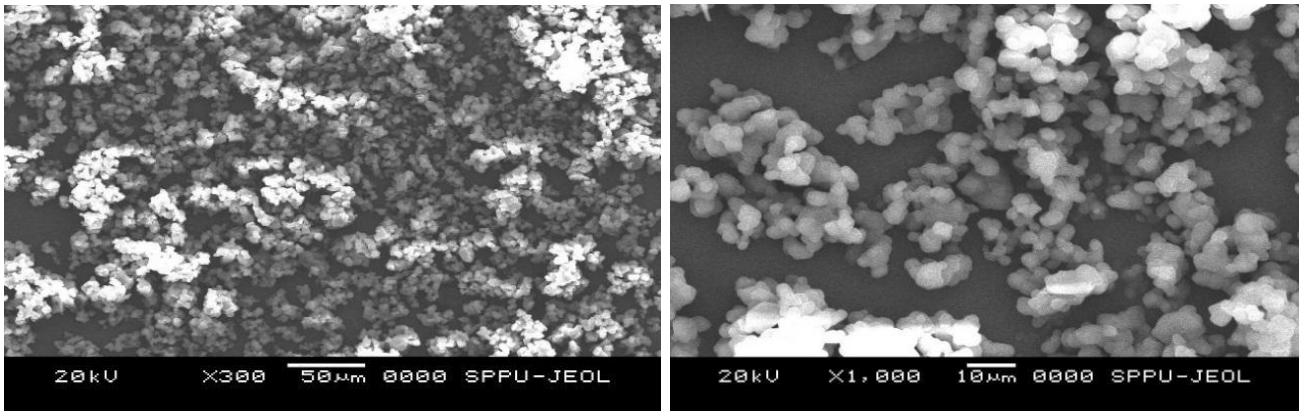

Fig. 11. SEM of Fenofibrate pure drug.

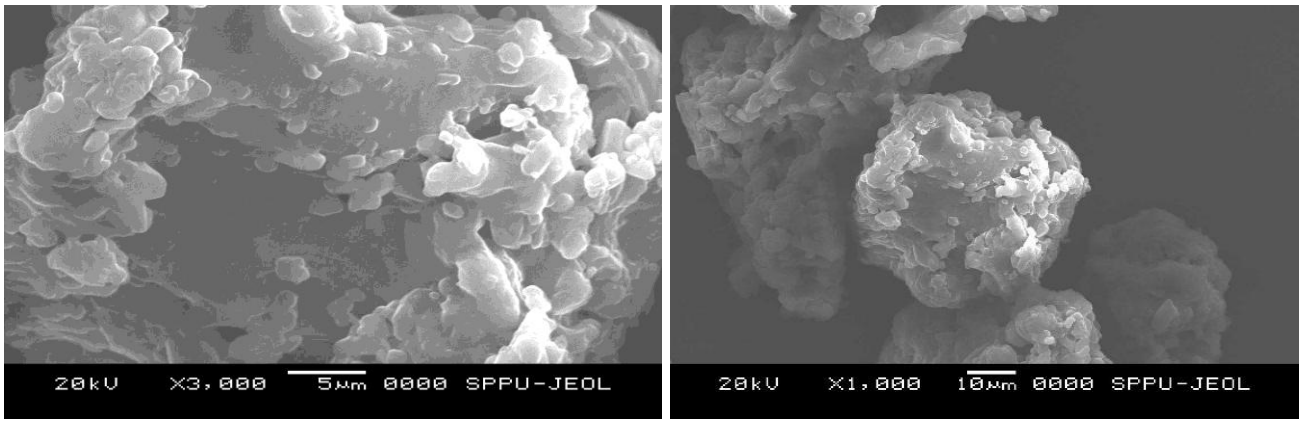

Fig. 12. SEM of Fenofibrate microspheres.

Table 7. *In-vitro* Fenofibrate microspheres release.

Sr. No.	Time (hr)	**0**	**0.5**	**1**	**2**	**3**	**4**	**5**	**6**
1	**F_1**	0	5.77	24.88	35.62	50.09	60.80	70.03	81.01
2	**F_2**	0	8.74	25.06	40.76	55.34	69.65	80.24	89.03
3	**F_3**	0	7.09	19.77	32.29	42.06	61.76	67.72	76.22
4	**F_4**	0	9.54	21.84	31.46	43.77	63.56	71.29	79.06
5	**F_5**	0	4.32	20.43	29.81	51.88	62.09	69.64	77.01
6	**F_6**	0	3.55	12.54	27.01	39.09	55.88	63.21	71.09
7	**F_7**	0	7.89	19.88	37.90	51.64	60.77	70.86	75.77
8	**F_8**	0	4.07	20.31	30.32	49.53	63.45	71.01	79.64
9	**F_9**	0	3.81	12.19	32.11	50.09	61.04	72.45	75.95

3.6. In-vitro Fenofibrate microspheres release

The results of *in-vitro* release are as shown in Figure. The study was performed for pure drug, formulations. Among all the formulation of F6 batch showed good dissolution profile with 89.03% of drug release in 6 hours. Hence it is considered as the best microsphere formulation of Fenofibrate.

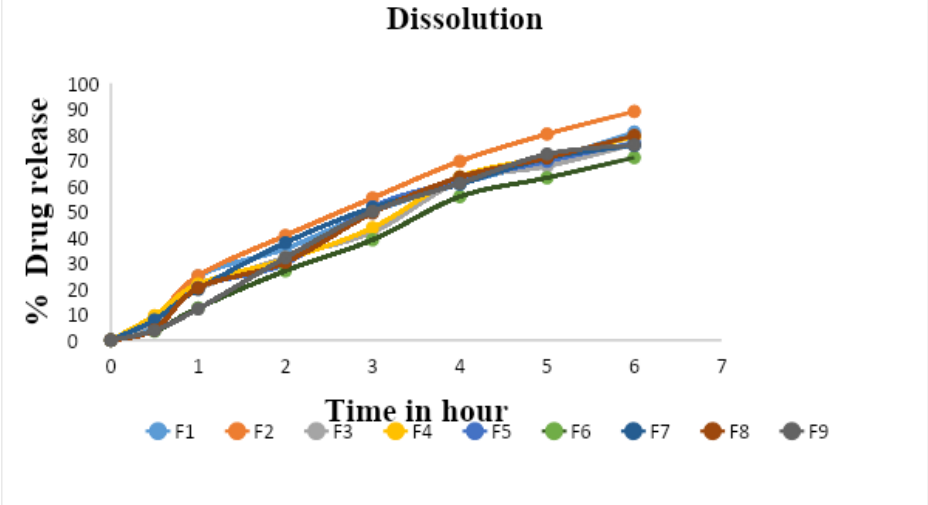

Fig. 13. Dissolution of Fenofibrate microspheres.

3.7. Stability Study

Physical appearance showed no significant variation & change in color. Formulation was stored at temperature of 40 ± 2°C and humidity of 75 ± 5 % for 3 month. After interval at one month samples were withdrawn and analyzed, this formulation was found to be stable under the above conditions.

Table 8. Stability study of Fenofibrate.

Sr. No.	Duration	Drug content (%)	*In-Vitro* dissolution (%)
1	0 day	99	85
2	1 month	97	84
3	2 month	96	82
4	3 month	94	81

4. CONCLUSION

Microspheres formulation of Fenofibrate was prepared by using Ionic gelation method. Sodium alginate & carbopol polymer had significant effect on drug entrapment efficiency & drug release. Particle size was selected by dependant variables. The concentration of sodium alginate & carbopol showed a significant effect with its effect on micromeritic properties. Optimised batch found was F_6 batch because of its sustained release of action, good drug content & product yield capacity.

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