FORMULATION AND EVALUATION OF EFAVIRENZ ORAL IN-SITU GEL

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ABSTRACT:
The objective of this work was to develop an oral mucosal drug delivery system to facilitate the local and systemic delivery of Efavirenz for the treatment of human immunodeficiency virus (HIV) infection. An *in situ* gelling system was used to increase the residence time and thus the bioavailability of Efavirenz in oral mucosa. *in situ* gel formulations were prepared by cold method using polymers like poloxamer 407, poloxamer 188, and HPMC K 100 M. Tween 80 and ethanol (1: 1 ratio) were used as the drug dissolving solvent. These formulations were evaluated for pH, gel strength, drug content, *in-vitro* diffusion. All the formulations exhibited fairly uniform drug content (83.21–94.56 %). Drug release study of all the formulations showed controlled release properties.

Keywords: Efavirenz, in-situ gel, Poloxamer, HPMC K100 M.

1. Introduction:

Over the past 30 years' greater attention has been focused on development of controlled and sustained drug delivery systems. The 'in situ gel' system has emerged as one of the best novel drug delivery systems, the in situ gelling system helps for the sustained and controlled release of the drugs, improved patient compliance and comfort by its special characteristic feature of 'Sol to Gel transition. In situ gelling system is a formulation that is in solution form before entering in to the body, but it will change to gel form under various physiological conditions.

In situ gel formation occurs due to one or combination of different stimuli like pH change, temperature modulation and solvent exchange. Smart polymeric systems represent promising means of delivering the drugs; these polymers undergo sol-gel transition, once administered. From the early 1970's natural and synthetic polymers began to be investigated for controlled release formulations. The advantages of using biodegradable polymers in clinical applications are apparent. Various natural and synthetic polymers are used for formulation development of in situ forming drug delivery systems. (1,2)

In situ gel formulations offers an interesting alternative for achieving systemic drug effects of parenteral routes, which can be inconvenient or oral route, which can result in unacceptably low bioavailability and passes the hepatic first-pass metabolism, in particular of proteins and peptides. This novel drug delivery system promotes the importantly ease and convenience of administration, deliverance of accurate dose as well as to prolong residence time of drug in contact with mucosa, that problems generally encountered in semi solid dosage forms. In situ gel formation occurs due to one or combination of different stimuli like pH change, temperature Modulation and solvent exchange (3).

In situ gels offer an important "stealth" characteristic in vivo, owing to their hydrophobicity which increases the in -vivo circulation time of the delivery device by evading the host immune response and decreasing phagocyte activities. In situ gels can be engineered to facilitate drug targeting, especially through mucus membranes, for non-invasive drug administration. (4)

The aim of this study was to develop an in situ gel formulation containing Efavirenz for local and systemic delivery from oral mucosal route. This was needed to increase absorption of the drug leading to an improvement in its bioavailability, to reduce its dosing frequency, and to achieve sustained release effect.

2. Material and Methods:

2.1 Materials:

Efavirenz proclaimed from Gift sample SD-Fine Chemicals, Mumbai, India, polaxamer 188 and polaxamer 407 from SD-Fine Chemicals, Mumbai, India. HPMC K100 M from SD-Fine Chemicals, Mumbai, India, Methyl paraben from Virat lab (HYD), Ultrapure water SD-Fine Chemicals, Mumbai, India.

2.2 Solubility Studies

The solubility of Efavirenz was studied in water and in buffer solutions of different pH. These were HCl buffer (pH 1.2), acetate buffer (pH 4.5), phosphate buffer (pH 6.8 and 5.5),

and borate buffer (pH 9.8). For evaluating the solubility in a particular solvent, an excessive amount of the drug was dissolved in 5 ml solvent and the solution was stirred using magnetic stirrer for 24 hrs at room temperature (25 ℃). After 24 hrs the sample was removed from stirrer and allowed to settle down. The supernatant solution was separated and filtered, and appropriate dilution was made with the respective solvents. Absorbance of diluted solution was measured at 287 nm and the concentration of soluble drug was calculated (5).
Similarly, the solubility of Efavirenz was studied in surfactants like tween 80, tween 20. Solubility was also studied in oleic acid, castor oil, and the mixture of tween 80 and ethanol (1: 1 ratio). In this case solubility study was conducted for 48 hrs at 37 ± 5 ℃.

2.3 Standard Graph in 6.8 pH phosphate Buffer

Ultra Violet Scan was taken using UV double beam Spectrophotometer (LABINDIA UV 2000) for 100 ug/ml solution between the wavelengths 200-400nm against 6.8 pH phosphate Buffer as the blank which gave the highest peak at 287 nm and the same was taken as λ max of Efavirenz.

2.4 Preparation of *in situ* gel formulation

In situ gel was prepared by the cold method. A weighed amount of poloxamer 407 and 188 was slowly added to 15ml water in a beaker with continuous stirring using a magnetic stirrer at a speed of 500 rpm for 2 hrs. This solution was kept overnight in refrigerator. HPMC K-100 (0.5%w/v) and the preservatives (methyl paraben 0.01%, w/v) were added to poloxamer dispersion with continuous stirring. The preservative solution was prepared by solubilizing it in hot water. It was mixed with above dispersion after cooling. The weighed amount of drug was dissolved in the mixture of tween 80 and ethanol (1:1). The drug solution was then mixed in the above described poloxamer dispersion (6).

Table 3: Formulation table of Efavirenz in situ gel composition (%w/v)

Ingredient	F1	F2	F3	F4	F5	F6	F7	F8	F9
Efavirenz	15	15	15	15	15	15	15	15	15
Tween 80	3	3	3	3	3	3	3	3	3
Ethanol	3	3	3	3	3	3	3	3	3
Poloxamer 407	4	3	4	4	2	2	2	3	3
Poloxamer 188	2	2	2	2	2	2	2	2	2
HPMC K100M	0.10	0.35	0.35	0.60	0.10	0.35	0.60	0.60	0.10
Methyl paraben	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Distilled water	30	30	30	30	30	30	30	30	30

2.5 Evaluation parameters:

2.5.1 Clarity: The formulations were visually checked for the clarity

2.5.2 pH: pH of each formulation was determined by using Digital pH meter and all values were recorded immediately after preparation (7).

2.5.3 Measurement of the gel strength: A sample of 50 of the gel was placed in a 50 ml graduated measuring cylinder. A weight of 14.33 g was placed on the gel surface. The gel strength indication for the ophthalmic gel at physiological temperature, was determined by the time taken by the cylinder to penetrate 5 cm into the gel (8).

2.5.4 Drug Content: The drug content was determined by taking 1 ml of the formulation and diluting it to 100 ml with distilled water. Aliquot of 5 ml was withdrawn and replaced with 5 ml distilled water. Efavirenz concentration was determined at 287 nm by using UV-Visible spectrophotometer (9).

2.5.5 *In-vitro* Drug Release Study: *In vitro* release study of the formulated oral in-situ gel was carried out by using diffusion cell through egg membrane biological membrane. Diffusion cell with inner diameter 24mm was used for the study. Formulation was placed in donor compartment and Freshly prepared 100 ml artificial or fluid (sodium chloride 0.670g, sodium bicarbonate 0.200g, calcium chloride Dihydrate 0.008g, potassium chloride 0.248g, distilled water Q.s 100ml.) was placed in receptor compartment. Egg membrane was mounted in between donor and receptor compartment. The position of the donor compartment was adjusted so that egg membrane just touches the diffusion medium. The whole assembly was placed on the thermostatically controlled magnetic stirrer. The temperature of the medium was maintained at 37°C + 0.5°C. 1mL of sample was withdrawn from receiver compartment after 30 min, 1, 2, 3, 4, 5, 6, 7 & 8 hrs and same volume of fresh medium was replaced. The withdrawn samples were diluted to 10ml in a volumetric flask with distilled water and analyzed by UV spectrophotometer at 287 nm (10).

2.5.6 Fourier - Transform Infrared Spectroscopy (FTIR)

FT-IR was carried out to determine if there is any potential interaction between the dug and the carrier used, which is indicated by the disappearance of important functional group of the drug.

2.5.7 X-Ray Diffraction (XRD)

The geometry of an X-Ray Diffractometer is such that the sample rotates in the path of the collimated X-Ray beam at an angle while the X-ray detector is mounted on an arm to collect the diffracted X-Rays and rotates at an angle of 2Ɵ. The instrument used to maintain the angle and rotate the sample is termed a goniometer (11).

2.5.8 Differential Scanning Calorimetry (DSC):

Thermal properties of drug, and Oral insitu gel formulations were investigated using a Perkin-Elmer DSC-7 differential scanning calorimeter/TAC-7 thermal analysis controller with an intracooler-2 cooling system (Perkin-Elmer Instruments, USA). About 3 to 5 mg of product was placed in perforated aluminum sealed 50-µl pans, and the heat runs for each sample was set from 40°C to 200°C at 5°C/min, under an inert environment using nitrogen. The apparatus was calibrated using pure metals like indium with known melting points and heat of fusion (12).

3 Results & Discussion

3.1 Pre-Formulation

3.1.1 Organoleptic Evaluation

Table 5: Organoleptic properties of Efavirenz

Properties	Results
Description	Amorphous
Taste	Tasteless
Odour	Odourless
Colour	White

3.1.2 Solubility Studies

The solubility of efavirenz was found to be low in distilled water 0.0176 mg/ml and was high in borate buffer (pH 9.8), due to hydrogen bond formation between efavirenz and borate buffer. Although, the solubility was found to be high in borate buffer, pH 9.0, the drug didn't show a pH dependent solubility in the GI tract pH range 1.2- 7.4. The solubility studies were also performed in different surfactants/cosurfactants/oils/. Tween 80 and ethanol (1:1) had higher solubility than other vehicles. Although Efavirenz had higher solubility in tween 80 in comparison to its mixture with ethanol, its higher viscosity restricted not using this compound for the formulation development.

3.1.3 Standard Calibration Curve of Efavirenz in 6.8 pH Phosphate Buffer

A standard graph was drawn using the average values of six trials by plotting a graph between absorbance versus concentration of Efavirenz shown in table 6 and figure 1, which shows there is no pH effect on drug. Efavirenz showed good linearity between 5- 30 mcg/ml with coefficient of 0.9994.

Table 6: Standard Calibration Curve of Efavirenz in 6.8 pH Phosphate Buffer

S.No.	Concentration(µg/ml)	Absorbance
1	0	0.000
2	5	0.086
3	10	0.177
4	15	0.256
5	20	0.351
6	25	0.431
7	30	0.510

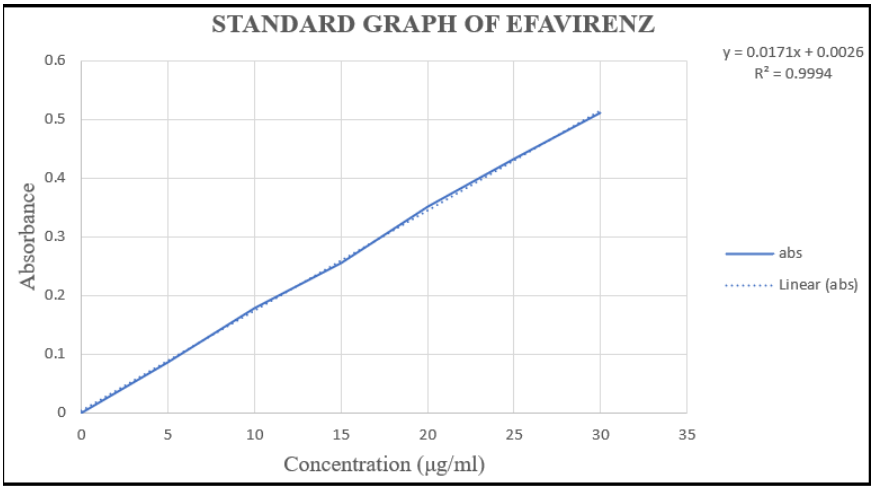

Figure 1: Standard Calibration Curve of Efavirenz in 6.8 pH Phosphate Buffer

3.2 Evaluation of in-situ gel

3.2.1 pH:

The pH of all the formulations was found to be in the range of 7.01-7.08, respectively. The pH of the formulations was neutral. This indicated the non-irritancy of the formulation in oral cavity.

Table 7: pH values of formulations F1-F9

S.NO	Formulation code	pH
1	F1	7.05
2	F2	7.02
3	F3	7.01
4	F4	7.03
5	F5	7.08
6	F6	7.05
7	F7	7.06
8	F8	7.03
9	F9	7.02

3.2.2 Measurement of the gel strength:

The results obtained for strength test of all the formulations are mentioned in Table 8. It has been observed that gel strength increased with the increase in the concentration of mucoadhesive polymer in the formulation.

Table 8: Gel Strength values of formulations F1-F9

S.NO	Formulation code	Gel strength (gm/cm)
1	F1	16.31
2	F2	14.28
3	F3	15.22
4	F4	16.43
5	F5	16.24
6	F6	14.21
7	F7	13.21
8	F8	14.23
9	F9	12.35

3.2.3 Drug Content:

All the formulations reflected uniform drug content ensuring adequacy in the method of preparation of the in situ gel. Drug content was found to be within the range of 87.42-94.56%

Table 9: Drug Content values of formulations F1-F9

S.NO	Formulation code	Drug content
1	F1	88.90
2	F2	91.23
3	F3	92.61
4	F4	87.42
5	F5	94.56
6	F6	83.21
7	F7	92.88
8	F8	89.73
9	F9	90.11

3.2.4 *In-vitro* Drug Release Study

In vitro release study of the formulated oral in-situ gel was carried out by using diffusion cell through egg membrane biological membrane. The drug release rate was high lasting for up to 8 hrs. Initial burst release was higher in in situ gel formulations. Among the in situ gel formulations, F5 formulation containing HPMC K100 M showed increased drug release rate.

Table 10: In-vitro diffusion studies

Time (hr)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	11.01	13.82	13.99	11.23	14.24	13.87	15.92	13.86	11.93
2	24.85	27.77	20.73	23.65	25.21	24.68	20.18	25.98	24.54
3	33.16	31.72	33.54	31.91	35.32	33.51	31.83	33.85	34.83
4	46.92	42.83	42.58	39.03	44.87	44.69	40.11	42.57	44.81
5	52.18	56.61	57.67	51.84	56.88	53.81	52.83	53.81	54.68
6	61.72	65.14	67.21	69.51	69.91	61.97	60.88	69.81	64.84
7	76.18	81.72	78.32	78.81	82.73	79.09	77.83	80.54	80.72
8	87.98	91.38	85.71	90.38	94.15	90.22	89.32	88.11	91.71

3.1.4 FTIR STUDIES:

FT-IR was carried out to determine if there is any potential interaction between the dug and the carrier used, which is indicated by the disappearance of important functional group of the drug. FTIR data interpretation data of pure drug, Poloxamer 407, HPMC K 100 M & optimized formulation F5 was shown from the Table 7 - 10. This indicated that there is no potential chemical interaction between the drug and the carrier used and the molecular structure of Efavirenz was retained.

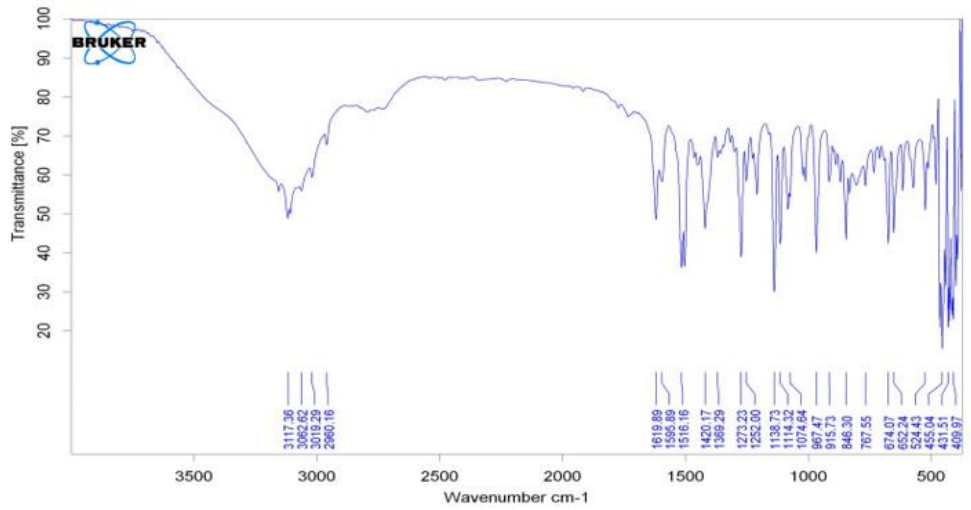

Figure 2: FTIR of Pure drug

Table 7: Characteristics peaks and frequency of pure drug

S.NO	Characteristics Peaks	Frequency Range(cm^{-1})	Frequency(cm^{-1})
1	O-H Stretching	3000-2500	2431.19
2	OH Bending	2500-2000	1761.82
3	C-H stretching	1500-1000	723.64

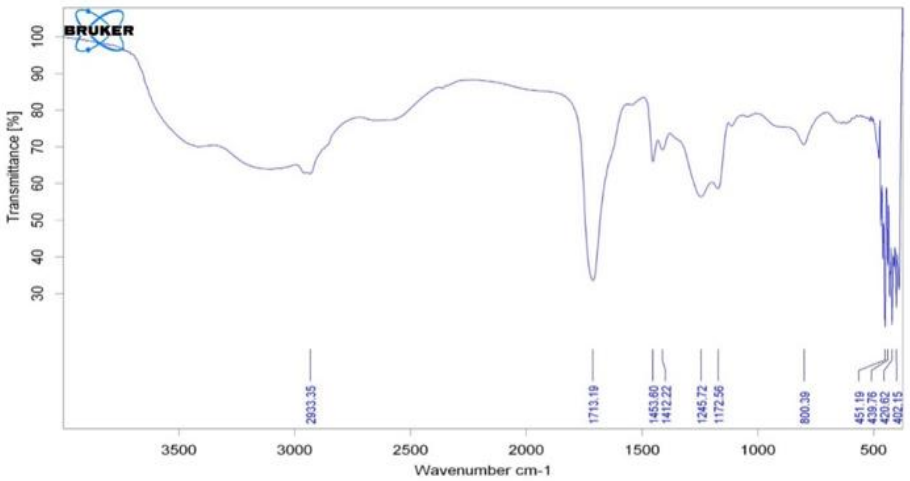

Figure 3: FTIR of Poloxamer 407

Table 8: Characteristics peaks and frequency of Poloxamer 407

S.NO	Characteristics Peaks	Frequency Range(cm^{-1})	Frequency(cm^{-1})
1	OH stretching	3500-3000	3420.38
2	OH Bending	2000-1500	1561.33
3	C-H stretching	1500-1000	823.21
4	C=O stretching	1000	576.42

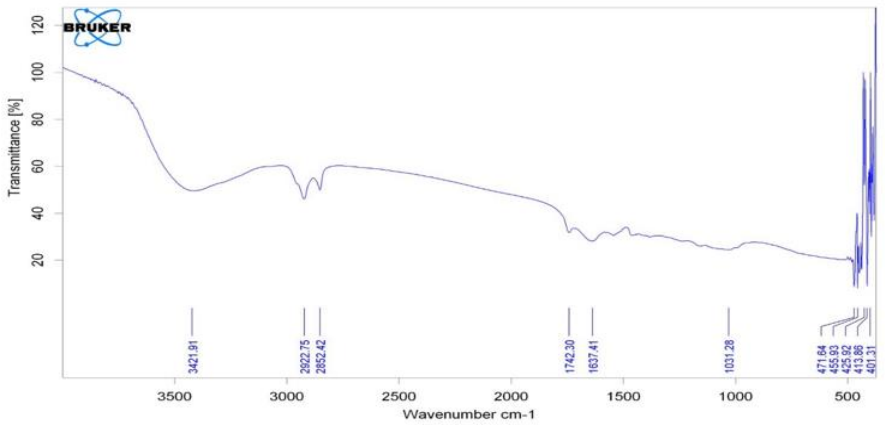

Figure 4: FTIR of HPMC K100M

Table 9: Characteristics peaks and frequency of HPMC K100M

S.NO	Characteristic Peaks	Frequency Range(cm^{-1})	Frequency(cm^{-1})
1	OH Stretching	3500-3000	3624.23
2	OH Bending	2000-1500	2861.14
3	C-H Stretching	1500-1000	1013.62
4	C=O Stretching	1000	763.13

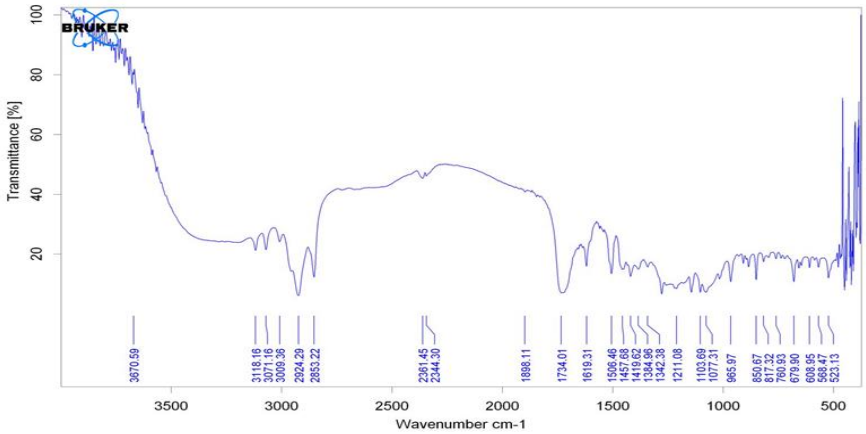

Figure 5: FTIR of optimized formulation

Table 10: Characteristic peaks and frequency of Optimized formulation F5

S.NO	Characteristic Peaks	Frequency Range(cm^{-1})	Frequency(cm^{-1})
1	OH-Stretching	3500-3000	3230.44
2	OH Bending	3000-2500	1536.58
3	C-H Stretching	1500-1000	1033.24

3.1.5 DSC ANALYSIS

DSC indicated there is no crystallization and as well as no other chemical reactions between drug and optimized formulation. The results for DSC of all the drug, optimized formulation are mentioned in table no 9&10

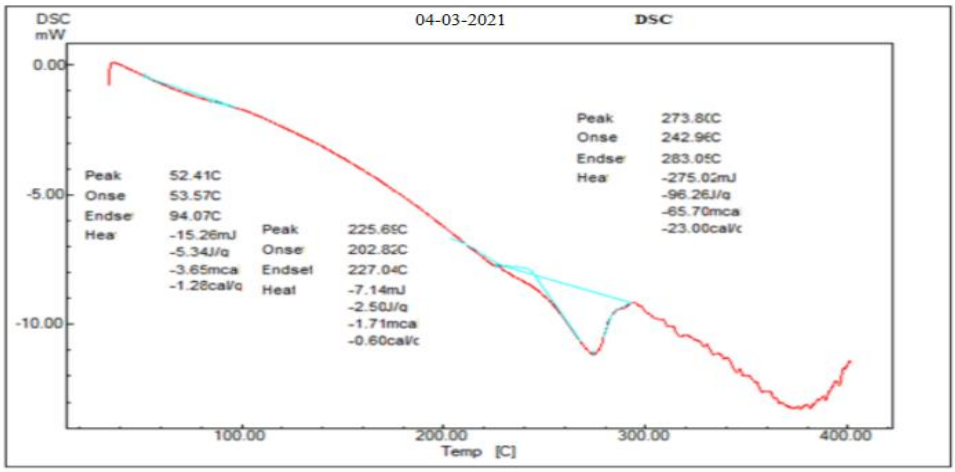

Figure 6: DSC Analysis of Pure drug

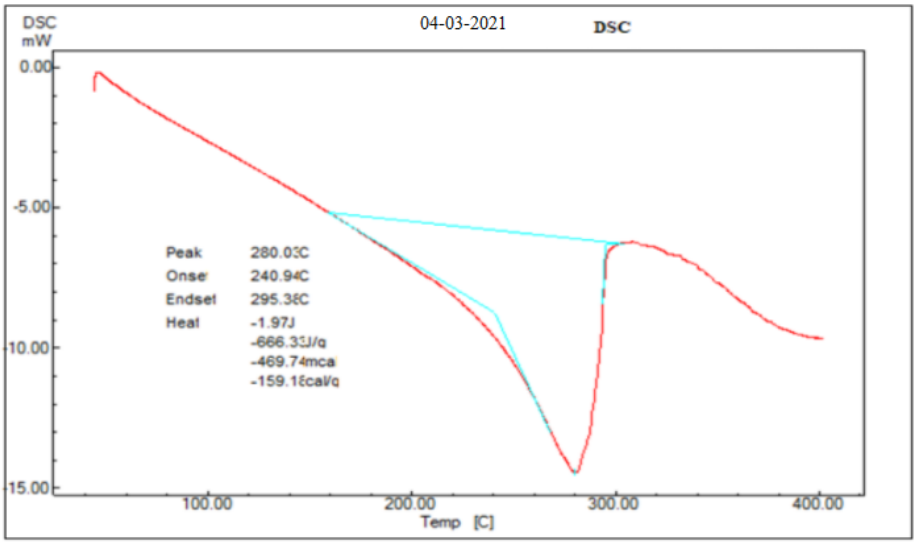

Figure 7: DSC Analysis of Optimized formulation F5

5.3.3 XRD ANALYSIS

XRD indicated there is no unknown crystalline material in between drug and oral insitu optimized gel.

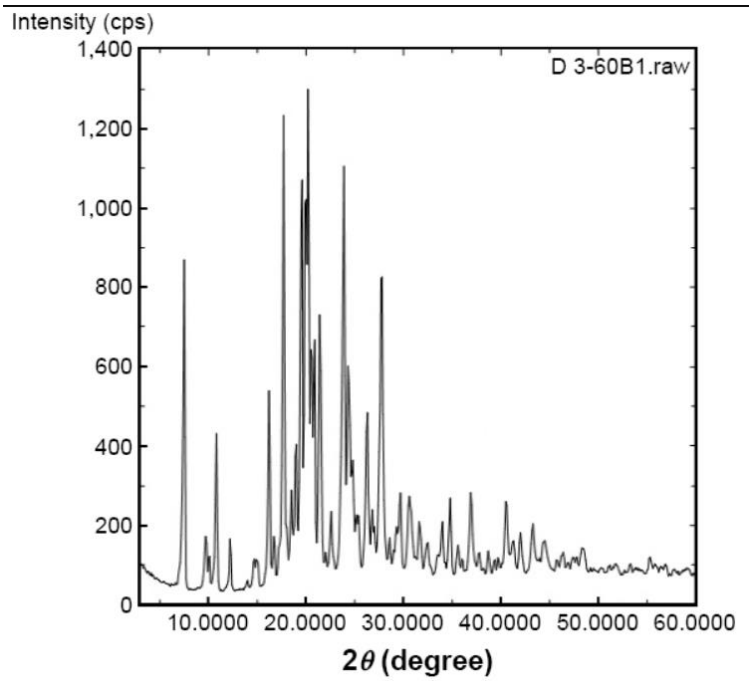

Figure 8: XRD Analysis of pure drug

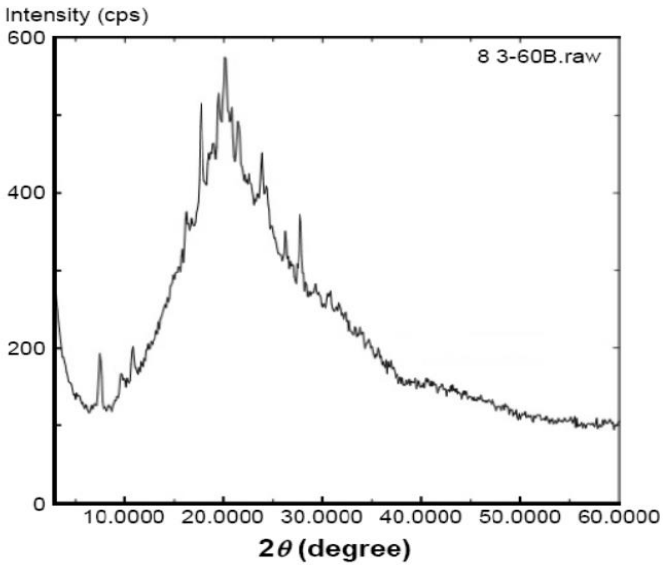

Figure 9: XRD Analysis of Optimized formulation F5

References

1. Nisha Patel, Gajanan Shinde and Rajesh KS. Ophthalmic in situ gel, A genesis journal Pharmagene., 2014; 2(4): 29-33.
2. Nerkar Tushar, Gujarathi Nayan A, Rane Bhushan R, Bakliwal Sunil R. Pawar S.P. In situ gel: Novel Approch in sustained and controlled drug delivery system. International Journal of Pharmaceutical sciences., 2013; 4(4): 1-18.
3. Saraswat R.1, Bhan C. S., Gaur A. A Review on Polymers Used In In-Situ Gel Drug Delivery Systems, May-Jun 2011; 1(2)
4. Zhidong L, Jaiwei L, Shufang N. Study of an Pharma alginate- HPMC based in situ gelling ophthalmic delivery system for gatifloxacin. Int J., 2006; 315: 12-7.
5. Wen-Di Ma, Hui Xu, Chao Wang,Shu-Fang Nie, Wei-San Pan Pluronic F127-g-poly(acrylic acid) copolymers as in situ gelling vehicle for ophthalmic drug delivery system, int. j. of pharmaceutics, 2008: (350):247-256.
6. Sirish Vodithala, sadhna Khatry, Nalini Shastri, M.Sadanandam, Formulation and evaluation of ion activated ocular gels of ketorolac tromethamine International Journel of current Pharmaceutical Reasearch, 2010;2(3).
7. Jothi M, Harikum SL and Geeta Aggarwal, In-situ ophthalmic gels for the treatment of eye diseases, International Journal of Pharmaceutical Sciences and Research, 2012; 3: 1891-1904.
8. Rajas NJ, Kavitha K, Gounder T, Mani T, In-Situ ophthalmic gels a developing trend, Int J Pharm Sci Rev and Res, 2011; 7: 8-14
9. Shastri DH, Patel LD, Novel alternative to ocular drug delivery system: Hydrogel, Ind J Pharma Res, 2010; 2: 1-13.

10. Guo DD, Xu Cx , Quan JS, Song CK, Jin H, Kim DD, Choi YJ, Cho MH, Cho CS, synergistic anti-tumour activity of paclitaxel incorporated conjugated linoleic acid coupled poloxmer thermosensitive hydrogel in vitro and in vivo, Biomat, 2009; 30: 4777-4785.
11. Jothi M, Harikumar SL and Geeta Aggarwal, In-situ ophthalmic gels for the treatment of eye diseases, International Journal of Pharmaceutical Sciences and Research, 2012; 3:20:59
12. Cao S, Ren X, Zhang Q, Chen E, Xu F, Chen J, et al. In situ gel based on gellan gum as new carrier for nasal administration of mometasone furoate. Int J Pharm 2009; 365:109-15.