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Optimization of *in-situ* gelling system for nasal administration of celecoxib

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ABSTRACT

Nasal drug delivery has become more prominent compared to other conventional routes as it improves both systemic and local availability and effects of drugs. The in-situ gels are colloidal sol which undergoes sol to gel transition through internal trigger like temperature or pH, when administered in-vivo at the target sites like ocular, nasal, vaginal, etc. Administration of Celexocib as in-situ gelling system for nasal route could enhance the bioavailability and therapeutic activity of the drug. In this paper, optimization of the in-situ gelling system of Celecoxib using polymers like pluronic F127, carbopol 934P and the effect of incorporating inclusion complex of drug with β-cyclodextrin into the insitu gels were studied. The rheology of the sol and gel systems was found show pseudoplastic behaviour, and with incorporation of drug inclusion complex, the gel viscosity was found to decline. The dissolution studies showed 100% drug release for the gels containing inclusion complex of drug, as compared to in-situ gels with plain drug providing sustained release maximum 12% drug at the end of 6 hours. Characterization studies such as FTIR, XRD, DSC and SEM confirmed the formation of inclusion complex of drug, and also no significant interaction between drug and polymers when incorporated into the in-situ gelling system. Celecoxib thermoresponsive nasal in-situ gelling system with 15% pluronic F127 was optimized to provide sustained release effect. The addition of cyclodextrin complexed drug in the formulation prove enhancement in the dissolution of the hydrophobic drug in aqueous environment, whereby in-vivo bioavailability can be improved.

Key words: *In-situ* gels, Celecoxib, pluronic F127, carbopol 934P, β-cyclodextrin.

INTRODUCTION

In-situ gelling systems have become more prominent among the various novel drug delivery systems, in the recent times. These are polymeric formulations which remain in sol form before administration into the body and then undergo transition to form gel *in-situ*. Use of biodegradable, water soluble polymers makes them more acceptable and excellent drug delivery systems. *In-situ* gels can be prepared by various methods using single or multi polymeric combination, based on the drug release required and the site of application. There are many mechanisms such as solvent exchange, UV-irradiation, ionic cross linkage, temperature modification, pH change, etc., by which *in-situ* gels can be formed [1]. Specific characters such as prolonged and sustained release of the drug, stability, biocompatibility, reproducible and accurate quantities of administration makes the *in-situ* gel dosage more reliable for commercial use. [2]

Celecoxib is a non-steroidal anti-inflammatory drug, which is a selective cyclo oxygenase-2 (COX-2) inhibitor. It is very poorly water soluble and show bioavailability upto 40% through the oral route. It is used for the relief of pain, fever, swelling and tenderness. It is also used for familial FAP, acute pain and menstrual cramps. The maximum recommended daily dose is 400 mg for all indications. The half life of Celecoxib is about 11-12 hours and it has pka of 11.1. The side effects include headache, abdominal pain, dyspepsia, diarrhea, nausea, flatulence and insomnia. Hence, it is rationale to administer Celecoxib in a sustained release dosage form especially at target sites, which can enhance the bioavailability and also reduce repeated administrations.

EXPERIMENTAL SECTION

Materials

Celecoxib was obtained as gift sample from Shasun Pharmaceuticals Limited, Pondicherry. Pluronic F127, Carbopol 934P and β-cyclodextrin were obtained from Sigma Aldrich, Mumbai. All the chemicals and solvents used in the study were of analytical grade.

Methods

Preparation of *in-situ* gel

Thermoresponsive *in-situ* gels of Celecoxib were prepared using 15%, 18% and 20% (w/w) of Pluronic F-127. The polymer was slowly added to cold distilled water with continuous stirring and the mixture was kept overnight at 4^{o}C to ensure complete homogenity. Accurately weighed quantity of drug was dissolved in ethanol and this solution was added to the polymer dispersion. 20µl of benzalkonium chloride was added as preservative and then the formulations were stored as sol in the cold temperature for further evaluation studies. As the temperature was increased aggregated micelle lead to hard sphere crystallization and gelling occurred. [3]

The pH responsive *in-situ* gels were prepared using 0.3 %, 0.5% and 0.6% (w/w) of carbopol 934P, wherein the polymer was slowly added to distilled water with continuous stirring and sonicating to ensure complete solubilisation. Required amount of drug was accurately weighed and dissolved in 2% sodium lauryl sulphate (SLS) and this mixture was added to the polymer solution. 20µl of benzalkonium chloride was added as a preservative and then the prepared formulations were evaluated for physico chemical properties and analytical characterizations. [4] The formulation design is shown in table 1.

Preparation of Celecoxib/βCD inclusion complex

The inclusion complex of Celecoxib with β-Cyclodextrin was prepared by kneading method, in three different ratios as 1:1, 1:2 and 1:3. The mixture of drug and carrier was triturated in a mortar with a small volume of water-methanol (1:2 v/v) solution. The thick slurry that formed was kneaded for 1 h and then sieved through sieve no.44 and finally air dried for 24 h. The dried mass was pulverized and again sieved through sieve no. 60. [5]

Preparation of *in-situ* gel with inclusion complexed drug

The prepared Celecoxib/βCD inclusion complex was added to the optimized *in-situ* gelling formulation. The effect of drug with cyclodextrin in the gelling system was studied by the dissolution studies. [6] The formulation design is shown in table 2.

Evaluation of pH and drug content:

The pH of the formulations was determined using the pH meter by dipping the electrode in contact with the surface of the sol systems. The uniformity of drug content in all the formulations was evaluated by dispersing standard volume of the formulation into ethanol to dissolve the drug and further diluting suitably and measuring the absorbance at 248.6 nm spectrophotometrically. [7]

Determination of sol – gel transition temperature, time and pH:

The different formulations prepared with Pluronic F127 were evaluated for gelation time and temperature. 2 ml of formulation were transferred to test tube and placed in the water bath. The temperature of water bath was increased slowly by 1ºC/min, whereby the exact sol to gel transition temperature was noted, when the change in flow ability of the sample was observed. To check the transition time, the formulations were kept in water bath maintained at 37ºC and the time taken for conversion of sol to gel was noted. Gelation was observed visually when the meniscus would no longer move upon tilting and the flow ability of sol was reduced. [8]

Determination of gelation pH

The different formulations prepared with Carbopol 934P were evaluated for gelation pH. The pH of the carbopol sol system was increased gradually by the addition of 0.1 N NaOH, and the pH was noted upon each addition, finally to arrive at the gelation pH where the flow of the sol was reduced and resulted in gelling. [9]

Viscosity measurements

Viscosity determination of the prepared *in-situ* gels (sol and gel) was carried out using Brookfield DV – II + model viscometer. The formulation under study was placed in the sample holder and the suitable spindle selected (No. 63) was lowered perpendicular into the sample and rotated at constant angular velocity. The viscosities were measured by increasing the rpm from 10 to 200, at cold temperature. The gel viscosity was measured for FP-1 to FP-3 and FcyP-1 to FcyP-3 by increasing the temperature to the gelation point of the sample. In case of FC-1 to FC-3, the pH

of the solution was increased from 4.5 to 7 by adding 0.1 N NaOH. Then the viscosities of the gels were measured at different rpm. The viscosity measurement was carried out in triplicate for all the trials. [10]

Phase solubility study

The phase solubility technique permits the evaluation of the affinity between β-cyclodextrin and Celecoxib in aqueous media, based on the method reported by Higuchi and Connors. The apparent stability constant (Kc) of complexes was calculated from the phase solubility diagram using the equation,

$$Kc = \frac{\text{Slope}}{\text{So}\ (1 - \text{Slope})}$$

The slope was obtained from the initial straight line portion of the plot of Celecoxib concentration against β-CD concentration, and S_0 was the equilibrium solubility of Celecoxib in water. [11]

Ethanol and distilled water (1:1) was used to determine the saturation solubility of the drug, wherein standard amount of drug was added individually into the test tubes containing 10ml of β-cyclodextrin solutions of increasing concentrations 0, 2mM, 4mM, 6mM, 8mM and 10mM. The test tubes were sealed with paraffin tape and shaken at 50rpm. After equilibrium of 72h, the solutions were filtered through the Whattman filter paper and then centrifuged at 3000 rpm for 10min, and the supernatant was suitably diluted to determine the concentration of Celecoxib in each solution by UV- Vis spectroscopy at 248.6nm.

Thermal behaviour changes of Celecoxib (TG-DSC)

Thermo gravimetric and differential scanning calorimetric analysis was used to characterize the thermal behaviour of individual polymers, drug, as well as the complexes. TG-DSC thermo grams were obtained from Thermo Gravimetric - Differential Thermal Analyser (TA instrument, Q100, USA) using an automatic thermal analyzer system. [12] Approximately 50 mg of sample was placed and sealed by crimping in a standard aluminium pan and heated from 0-500˚C at a heating rate of 10˚C/min under constant purging of nitrogen at 30 ml/min.

Interaction of drug and polymers (FT-IR)

FTIR (Perkin Elmer System 200, USA) analysis was done to determine the interaction of drug and polymers due to chemical reactions. Infrared spectra of freeze dried gel was recorded and compared with pure drug and polymer spectra. The KBr pellet mode was followed for the study, where the dried samples were mixed with KBr (saturated and dried) and pressed under hydraulic pressure of 150kg/cm^2 to form a thin disc. The IR rays were passed over the disc within the wave number range of 400-4000cm^{-1}. [13]

Crystallinity behavior of the drug-(XRD)

The X-ray diffractometry was carried out to investigate crystallinity of the drug in formulation, which was carried out using an X-ray diffractometer (D8 FOCUS, BRUKER, USA). The pure drug, pure cyclodextrin and cyclodextrin inclusion complex in powder form were scanned for 2θ values from 10^o to 60^o with a step size of 0.01^o/min. The measurement was made with Cu-Kα radiation at 1.5418 Å, voltage 40 kV, current 35 mA, temperature range −170 ˚C to +450 ˚C. [14]

Surface Morphology of the complex-(SEM)

The surface morphology of complexed drug was examined by scanning electron microscopy (JSM 6701F, JEOL, Japan), after they were sputter coated with gold using auto fine coater (JFC 1600, JEOL, Japan), and compared with pure drug and polymer. A small amount of powder was spread on an aluminum stub in the sample chamber and the photographs were taken at acceleration voltages of 20 kV electron beam. [15]

In-vitro **drug release studies**

The *in-vitro* drug release study was carried out in USP dissolution apparatus using dialysis membrane, soaked in phosphate buffer (PB) pH 6.4. The dissolution chamber was filled with 100 ml of phosphate buffer pH 6.4 maintained at 37°C and the sample was taken in a dialysis bag which was mounted in the basket. The position of the basket was adjusted so that the formulation just touches the dissolution medium. The content of the basket was stirred at 50 rpm, from which an aliquot of 10 ml of sample was withdrawn at suitable time intervals and replaced with the same volume of fresh medium. These samples were analyzed spectrophotometrically at 248.6 nm. [16]

Release kinetic studies

The *in-vitro* release data were analyzed with various kinetic models to describe the drug release mechanism. The following plots were made as cumulative % drug release vs. time (zero order kinetic model), log cumulative of % drug remaining to be released vs. time (first order kinetic model), cumulative % drug release vs. square root of time

(Higuchi model); log cumulative % drug release vs. log time (Korsmeyer-Peppas model), time vs cube root drug to be released (Hixson model). [17]

RESULTS AND DISCUSSION

Formulation of *in-situ* Gelling System

The thermo responsive and pH sensitive *in-situ* gelling system of Celexocib was developed by varying the composition of the gelling polymers, Pluronic F127 and Carbopol 940 respectively. Also the effect of incorporation of inclusion complex of drug with cyclodextrin was also studied in the optimized formulation. [18]

Characterization of the *in-situ* gels

Determination of pH

pH was determined for all the *in-situ* gel formulations both in sol and gel form. Thermoresponsive pluronic and β-cyclodextrin inclusion complex(FP1, FP2, FP3, FCyP1, FCyP2, and FCyP3) showed pH 6.9 in sol form whereas pH responsive carbopol (FC1, FC2 and FC3) sol forms showed acidic pH range of 4.8-5 and get converted to gel by adding 0.1N NaOH. In gel form it showed alkaline pH range of 6.5-7. [19]

Uniformity of drug content

This is one of an important requirement for any type of dosage form. The drug amount in the formulation should not deviate beyond certain specified limits from the labelled amount. Drug content was estimated for all the *in-situ* gel formulations (FP1, FP2, FP3, FC1, FC2, FC3, FCyP1, FCyP2, and FCyP3) by using UV-Spectrophotometer at 248.6nm. The drug content of all these formulations with 100 µg/ml concentration showed nearly a 100% drug content showing our formulations, satisfactorily can be used for drug release profile. [20]

Sol – Gel transition pH/temperature and time

The sol-gel transition time and temperature was noted for all the thermoresponsive formulations. Pluronic and β-cyclodextrin inclusion complex (FP1, FP2, FP3, FCyP1, FCyP2, and FCyP3) formulations get converted to gel within 20 secs and at 36^{o}C. These temperatures seemed to be suitable for *in-situ* gelling system for administration into the nasal cavity which minimizes the loss of administered drug caused by mucociliary clearance from the site of application. The gelation pH was observed to be pH 6.5-6.8 for the pH responsive system. Upon further increase in pH upto 11.0 caused gel to sol transition effect. [21] The results of the Celecoxib *in-situ* gel characterization is shown in table 3.

Phase solubility study

According to Higuchi and Connors classification, the phase solubility study for βCD inclusion complex is of A_L type was studied to check the formation of soluble complex. The phase-solubility diagram for the complexes of Celecoxib with β-cyclodextrin is shown in Figure 1. Mixtures of ethanol and water were used to increase the solubility of hydrophobic drug Celecoxib. The aqueous solubility of Celecoxib was increased linearly as a function of the concentration of βCD with a slope of <1 showing that the increase in the solubility was due to the formation of 1:1M complex. [24] The apparent solubility viscosity (K_c) obtained from the slope of the linear phase solubility diagram was found to be 300.9M^{-1}. This value of stability constant (K_c) indicated that complexes formed were quite stable. [22]

Viscosity of sol and gel

The viscosity of the thermo responsive pluronic sol systems measured using Brookefield viscometer at cold temperature was found to show shear thinning property (Pseudoplastic behavior), where an increase in the shear rate as angular velocity (rpm) showed a decrease in the viscosity of the formulations (Figure 2).[23] The viscosity of 15%, 18% and 20% pluronic sol (cold temperature) was found to be the in the narrow range of 18 – 28 cp, whereas the gel viscosity (above room temperature) showed wide variation in their range measured as 204 – 1046 cp, 385 – 2028 cp and 448 – 6179 cp respectively, when the shear rate was varied between 200 to 10 rpm. This property of viscoelastic fluids possessing low viscosity under high shear rate and vice versa are often preferred in pharmaceutical preparations, which enable easy administration of sol preparations in the site of application followed by conversion into gel *in-vivo*.

Table 1 Composition of thermoresponsive and pH responsive *in-situ* gels of Celecoxib

S. No.	Ingredients	FP-1	FP-2	FP-3	FC-1	FC-2	FC-3
1	Pluronic F127	15%	18%	20%	-	-	-
2	Carbopol 934 p	-	-	-	0.3%	0.5%	0.6%
3	Benzalkonium chloride	20µl	20µl	20µl	20µl	20µl	20µl
4	Celecoxib	250mg	250mg	250mg	250mg	250mg	250mg
5	Distilled water	q.s. to 100 ml	q.s. to 100 ml	q.s. to 100 ml	q.s. to 100 ml	q.s. to 100 ml	q.s. to 100 ml

Table 2 Composition of *in-situ* gels of Celecoxib-β cyclodextrin inclusion complex

S. No.	Ingredients	FCyP-1	FCyP-2	FCyP-3
1	Pluronic F127	15%	15%	15%
2	Benzalkonium chloride	20µl	20µl	20µl
3	Celecoxib	75mg	75mg	75mg
4	Cyclodextrin	75mg	150mg	225mg
5	Distilled water	q.s. to 30 ml	q.s. to 30 ml	q.s. to 30 ml

Table 3 Physiochemical characterization of Celecoxib *in-situ* gels

Formulation	Sol pH	Gelling time (secs)	Gelation temp (°C)	Gel pH	Drug content (%)
FP1	6.9	20	36	-	94±1.5
FP2	6.9	20	37	-	100±0.3
FP3	7.0	20	37	-	97±0.5
FC1	4.8	-	-	6.5	103±0.1
FC2	4.9	-	-	6.7	106±0.4
FC3	5.1	-	-	6.8	108±0.1
FCyP1	6.9	20	37	-	89±1.1
FCyP2	6.9	20	37	-	94±1.2
FCyP3	6.9	20	37	-	96±0.8

Table 4 *In-vitro* drug release kinetics of Celecoxib *in-situ* gelling system

	R^2 Value					
Formulations	Zero-order	First-order	Higuchi	Korsmeyer – peppas	Hixson	n value
FP-1	0.8897	0.9067	0.9886	0.9953	0.9013	0.588
FP-2	0.9175	0.9268	0.9833	0.9976	0.9238	0.632
FP-3	0.8181	0.8271	0.9829	0.9829	0.8241	0.505
FC-1	0.8506	0.8544	0.9023	0.9133	0.8531	0.630
FC-2	0.8859	0.8876	0.8872	0.9161	0.8871	0.725
FC-3	0.8497	0.8579	0.9981	0.9994	0.8552	0.536
FCyP-1	0.9589	0.9692	0.9130	0.9757	0.9661	0.811
FCyP-2	0.9507	0.9923	0.9629	0.9953	0.9930	0.709
FCyP-3	0.6177	0.9800	0.9595	0.9928	0.9929	0.480
Capsule 1:1	0.9287	0.9428	0.8804	0.9425	0.9395	0.822
Capsule 1:2	0.3817	0.6455	0.8972	0.9960	0.5691	0.247
Capsule 1:3	0.2550	0.6347	0.8448	0.9990	0.5303	0.192

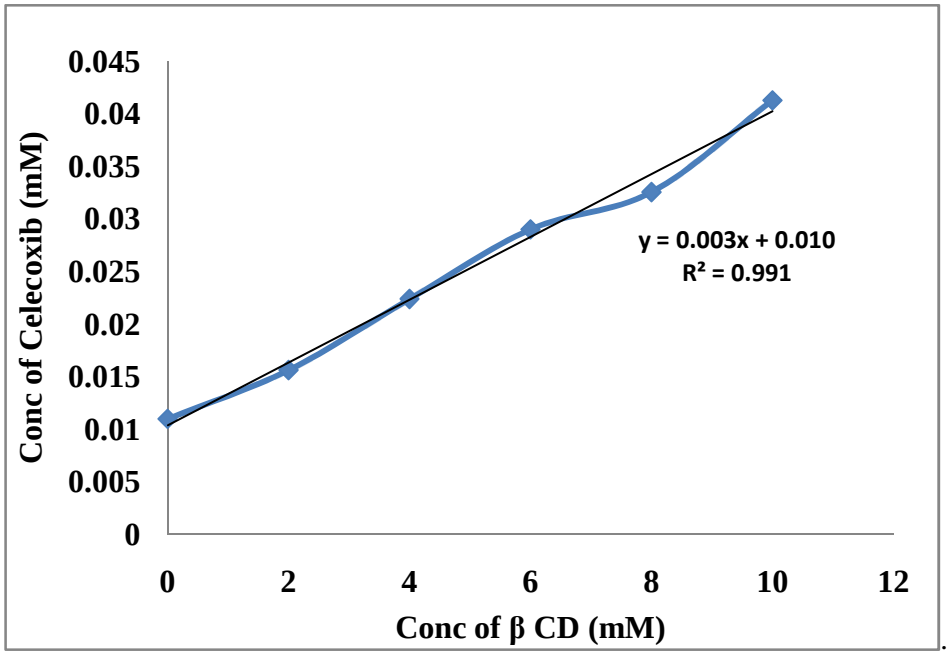

Figure 1 Phase solubility study of Celecoxib complexed with βCD.

The similar behaviour was observed in the pH sensitive sol system also (Figure 3), where the sol viscosity (at pH 4.8, 4.9, 5.1) of the formulations with 0.3%, 0.5% and 0.6% of carbopol was found to be in the range of 13.5 cp to 96 cp. And the viscosity of these gels (at pH 6.5, 6.7, 6.8) was observed to be 47 – 75 cp, 102 – 240 cp and 266 – 923 cp, respectively at 200 to 10 rpm. The sol – gel viscosity of both systems showed that thermo responsive

pluronic systems had more significant viscosity changes during the transition, compared to the pH sensitive (carbopol) system. Also at higher pH (above pH 11), the carbopol gels converted into sol systems where the stiff gel consistency could not be viewed.

When the pluronic 15% formulation was incorporated with the drug-cyclodextrin inclusion complex prepared as 1:1, 1:2 and 1:3 ratio, the sol viscosity of the samples was found to be 17 – 40 cp, whereas the gel viscosity ranged between 145 – 1112 cp. At 200 rpm, the viscosity of these gel systems was found to be as low as indicted with the values of 272 cp, 230 cp, 145 cp with respect to the formulations containing 1:1, 1:2 and 1:3 drug-cyclodextrin complex respectively (Figure 4), which may be due to the enhanced solubilization of the drug in higher ratio of complexation that can alter the viscosity slightly.

When CD complex of drug was incorporated in the samples, the viscosity of the gels decreased when higher rotation speed was applied. The decrease in viscosity indicated the possibility of interaction between the complexes and polymer chains, which could affect formation of micelle aggregation of pluronic. The CD inclusion complexes were known to interact with water-soluble polymers through hydrogen bonds, thus forming ternary complexes containing the drug molecule, CD and the polymer chain. [9, 10] This interaction can reduce the interaction between polymer chains and therefore, at increased rotation speed, the polymer chains could be disentangled and well aligned in the direction of the flow. Further experiments, such as viscoelastic measurements, are needed in order to investigate the mechanisms of interaction between the polymer chains and the complexes in gel samples.

Analytical characterization

Thermal behaviour changes of Celecoxib-(TGA-DSC)

The DSC thermogram of the pure sample of Celecoxib showed an endothermic peak at 162.39 ºC which corresponds to the melting point of the drug, [24] followed by its decomposition point at the temperature of 320.65 ºC, where a corresponding sharp reduction in the percentage weight of the sample was also observed in the thermo gravimetric curve. The pure sample of pluronic F127 surfactant showed the similar pattern of curve with endothermic melting point at 58.05 ºC which undergoes decomposition at 391.49 ºC with the sharp decline in the TG curve at respective temperature.

The lyophilized samples of the 15% pluronic F127 system containing the drug and the drug-cyclodextrin inclusion complex exhibited only the characteristic melting point of polymer at 57.01 ºC and 57.02 ºC with their decomposition point slightly shifted to 389.98 ºC and 388.97 ºC respectively. The melting point of the drug was not observed in these samples, because of the higher polymer concentration in the formulations, wherein the amount of drug present was insignificant for its detection. The other important reason for the masking of the endothermic peak of drug was due to encapsulation of the small amount of drug within the higher ratio of polymeric system, which was also confirmed by the TG curve showing weight loss only after 388 ºC, compared to the pure drug showing a sudden weight loss before 320ºC itself. (Figure 5)

Interaction of drug and polymers (FT-IR)

The FT-IR spectrum (Figure 6) of pure drug Celecoxib showed the characteristic peaks at the specific wave numbers which identified the functional groups of the molecule. The peak obtained at 3099.19 cm^{-1} 1594.78 cm^{-1} and 1498.14 cm^{-1} correspond to the C=C-H asymmetric stretching, C-C=C symmetric stretching and C-C=C asymmetric stretching of the aromatic ring respectively. The functional peaks of N-H stretching, C-F stretching and S=O stretching was observed at the respective wave numbers of 3339.72 cm^{-1}, 1402.88 cm^{-1} – 1103.42 cm^{-1} and 1347 cm^{-1} – 1164.80 cm^{-1} which indicated the identity of the drug.

The lyophilized sample of *in-situ* gel formulation prepared with 15% pluronic F127 containing the drug showed characteristic predominant peaks of the polymer with mild shift in the peaks of drug, compared to the pure sample, which indicate minimum interaction of the drug and polymer. The peaks of the polymer was observed at 3733.36 cm^{-1} for O-H stretching of alcohol, 1242.42 cm^{-1} and 1111.26 cm^{-1} for C-O stretching of alcohol, 1360 cm^{-1} for C-O-C alkyl stretching, 2970.57 cm^{-1} and 2886.17 cm^{-1} for H-C-H asymmetric and symmetric stretching, 1467.41 cm^{-1} for H-C-H bending. The peaks of the drug for specific functional groups such as S=O stretching, N-H stretching and C-F stretching was slightly shifted to 1147 cm^{-1}, 3501.13 cm^{-1} and 1372 cm^{-1} – 1061 cm^{-1} respectively.

The freeze-dried sample of the *in-situ* gel formulation prepared by incorporating the drug-cyclodextrin inclusion complex into the pluronic gel, had shown the characteristic peaks of pluronic, cyclodextrin and the drug with mild shifts in their wave numbers due to the mechanism of formed inclusion complex by inter molecular hydrogen bonding. The presence of active drug could be confirmed by the characteristic peak observed at 3432.48 cm^{-1} for N-H stretching, 1343 cm^{-1} for S=O stretching and 1456 cm^{-1} for C-C=C asymmetric stretching of the aromatic ring. The peaks observed for pluronic and cyclodextrin polymer in the inclusion gel sample were at 2970.82 cm^{-1} and

2889.29 cm^{-1} for H-C-H asymmetric stretching of alkane chain, 1468.07 cm^{-1} for H-C-H bending, 1110.98 cm^{-1} for C-O stretching of alcoholic group and 1061.47 cm^{-1} for the C-O stretch of the anhydride linkage. The cyclodextrin wave numbers obtained for the inclusion gel sample was compared to the pure cyclodextrin spectra showing characteristic peaks for O-H and C-O stretching of alcohol at 3390.81 cm^{-1} and 1259.78 cm^{-1} respectively, C-H stretching of alkane chain at 2923.44 cm^{-1}, C-O-C stretching at 1158.27 cm^{-1}, C-O stretching of anhydrides at 1080 cm^{-1} and 1028 cm^{-1}. From the presence of characteristic peaks of the drug in the formulations with mild shift in the wave numbers, the formation of inclusion complex and the entrapment of drug without significant changes could be confirmed. [25]

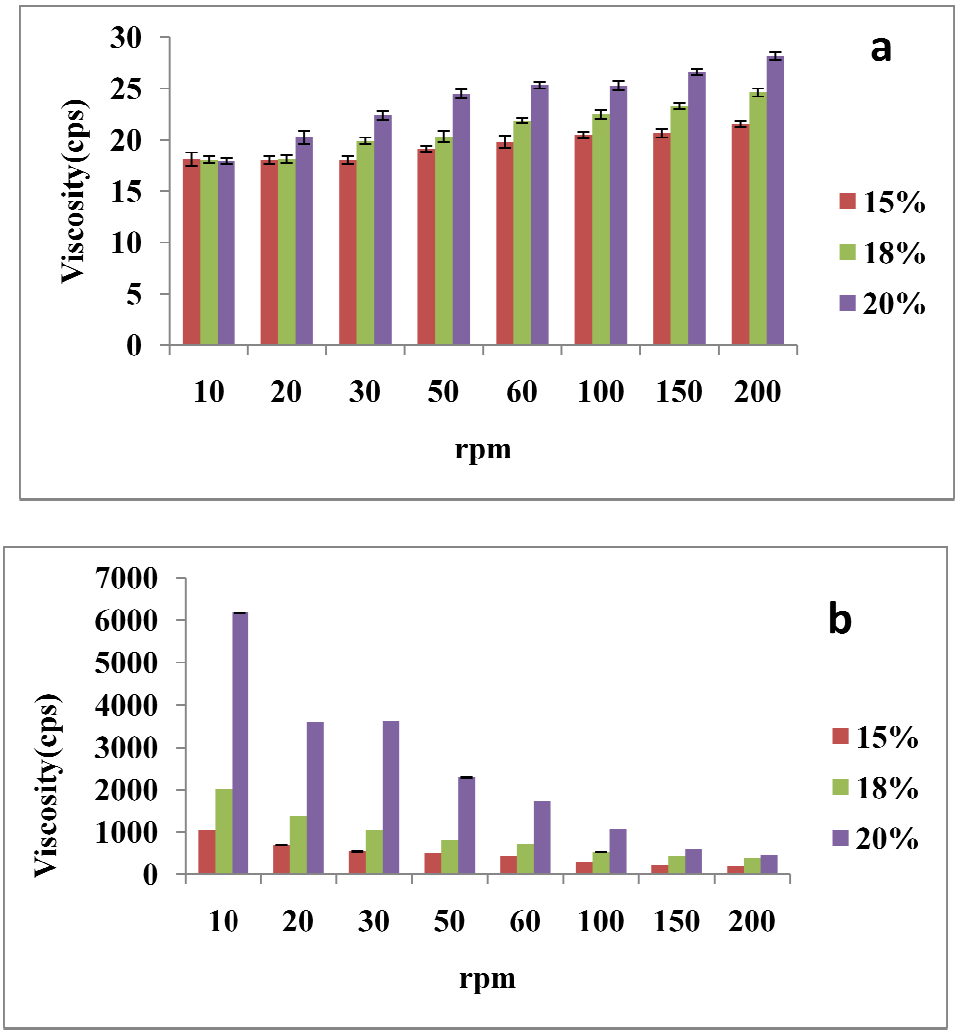

Figure 2 Viscosity of Celecoxib thermosensitive gelling system a) Sol b) Gel

Crystallinity behavior of the drug-(XRD)

The pure drug was found to be highly crystalline in nature showing sharp intense peaks in the XRD spectra at the scattering angle of 2θ, especially the characteristic diffraction peaks observed at 16.14 and 21.52 confirming the three dimensional ordered structure. The pure cyclodextrin was observed with less intense peaks corresponding to its low crystallinity nature, which is one of the reasons for its high solubility.

The XRD spectrum of complex of drug with cyclodextrin (1:2) prepared by kneading method, was devoid of diffraction peaks of the drug and exhibited only less intense peaks of the carrier. This was due to solid state conversion of crystalline form of drug into amorphous nature, which attributed to the modification in the arrangement of the molecules in the crystal lattice, confirming the enhancement of solubility and dissolution of the drug. The encapsulation of the drug into the inclusion molecule especially with higher ratio of the carrier also contributes to the change in XRD peaks. Figure 7 shows the comparison of XRD spectra of pure samples and the *in-situ* gelling formulation. [24]

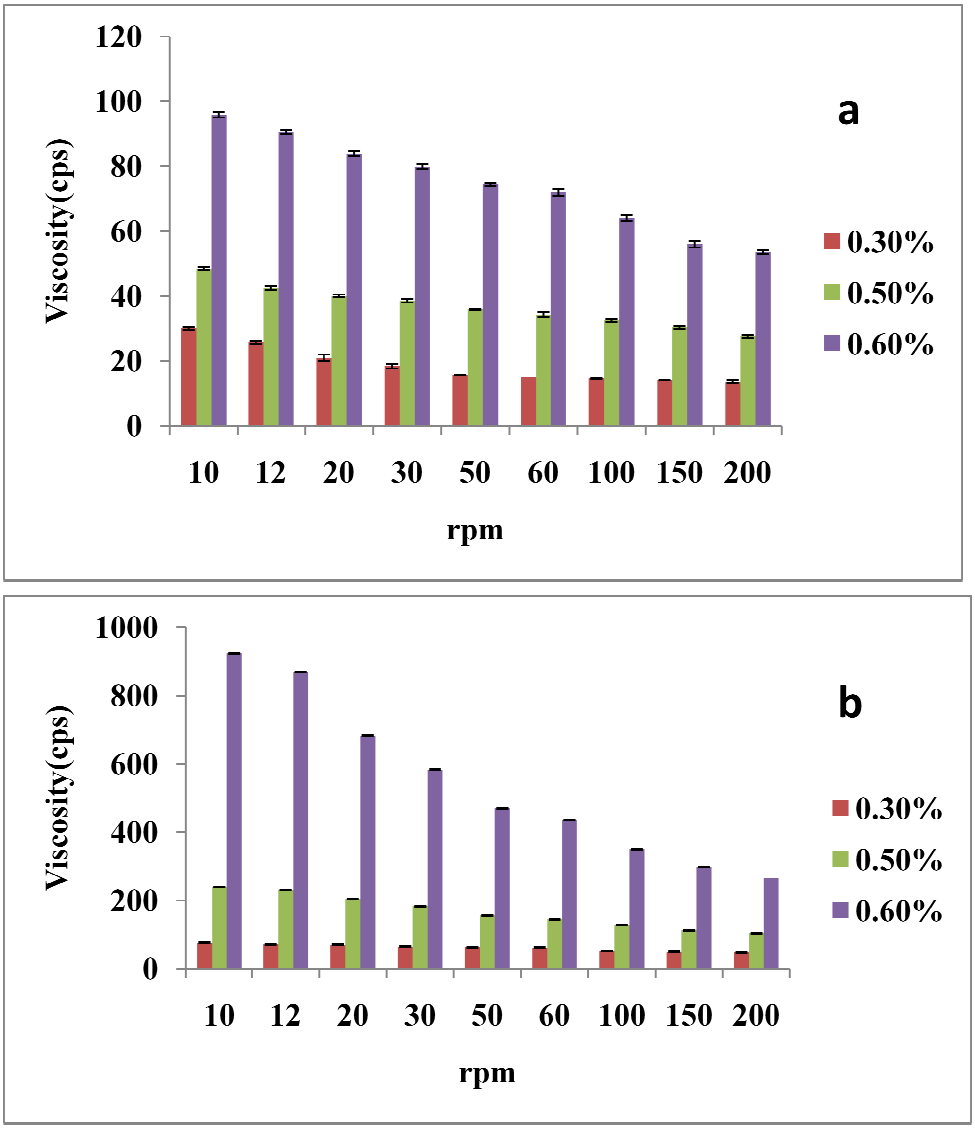

Figure 3 Viscosity of Celecoxib pH sensitive gelling system a) Sol b) Gel

Surface Morphology of the complex-(SEM)

The pure Celecoxib exhibited its characteristic crystalline shaped morphology, and the pure cyclodextrin showed the amorphous nature of the sample. The surface morphology of the complex formed by drug with cyclodextrin by kneading method was compared with the pure drug and cyclodextrin.

The complexed mixture of drug with carrier exhibited the surface morphology showing the breakdown of the crystals of the drug and mixing with the amorphous carrier, wherein the characteristic crystalline shapes of the drug could not be observed (Figure 8). [24]

Drug release studies

***In-vitro* Drug release studies**

The dissolution studies of the *in-situ* gelling systems performed for 6 hours, using modified USP dissolution basket system have showed significant changes in the release profiles of the formulations with respect to variation in the composition of pluronic F127 and carbopol 940 (Figure 9).

Sols containing 15%, 18% and 20% of pluronic F127 converted into gels immediately, in the *in-vitro* dissolution apparatus maintained at 37ºC, which provided sustained release of the drug as gradually increasing from 2-4% in the 1st hour up to 12% in the 6th hour. The percentage drug release was inversely proportional to the concentration of the gelling polymer in the system, because the higher concentration of polymer caused higher number of micelle formation and faster transition of sol to gel, forming more stiff gels from which drug diffusion was slower. The

structure of the gel functioned as an increasingly resistant barrier to drug release as the concentration of polymer increased.

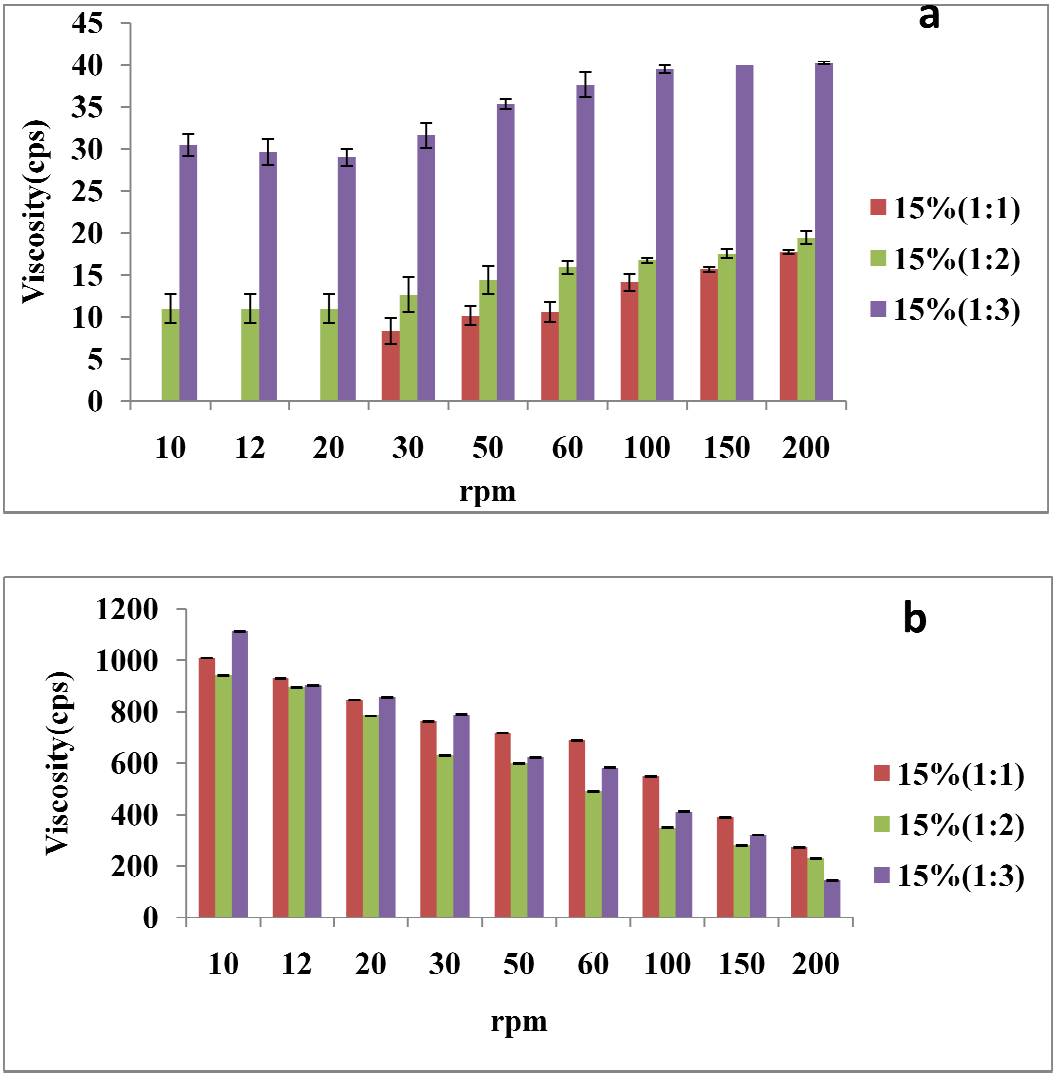

Figure 4 Viscosity of Celecoxib gelling system with 15% Pluronic and drug-cyclodextrin inclusion complex a) Sol b) Gel

In case of carbopol gelling system, the sols prepared with 0.3%, 0.5% and 0.6% of the polymer transformed into gels in the dissolution basket containing phosphate buffer pH 6.8 (mimicking the invivo pH of the nasal fluid), which showed more sustained release of the drug from the system. Since the concentration variation used in the formulation design is narrow, the percentage drug release was around 2% in 1st hour which increased to 4-6% in the 6th hour. The sudden change in the pH of the environment from slightly acidic of the formulation to alkaline nature of the dissolution fluid, the gel formation was observed, which was proportional with the concentration of the gelling polymer.

Among these formulations, the formulation prepared with 15% pluronic was found to be better, where the amount of drug released was significant and more linear profile was observed. So, drug-cyclodextrin inclusion complex at 1:1,1:2 and 1:3 ratio was incorporated into the optimized 15% pluronic gel system.

The pure drug filled in capsules showed the release of 10% at 1st hour after which the release was very poor showing only 15% at the end of 6 hours, due to the poor solubility of the drug in the aqueous media. When the drug and cyclodextrin complex prepared in the ratio of 1:1, 1:2 and 1:3 was filled in capsule and release profile was studied, the percentage of drug release increased as 27%, 48% and 61% respectively at the end of 6 h. As the ratio of cyclodextrin was increased with respect to the drug, the amount of inclusion complexes of drug-cyclodextrin formed was higher, which resulted in enhanced solubility and dissolution of the drug. The encapsulation of the poorly soluble drug in the hydrophobic core of cyclodextrin and its spontaneous dissolution into the media by the

solubilization of hydrophilic surface of the cyclodextrin enhanced the percentage of drug released at each time interval.

When the drug-cyclodextrin complexes were incorporated into the 15% pluronic gelling system, the release profile of the drug changed drastically. The percentage of drug release observed with 1:1, 1:2 and 1:3 drug-cyclodextrin complex included pluronic *in-situ* gel was 16%, 80% and 100% respectively at the end of 6 h. Inspite of the sol-gel transformation, the drug release was found to be higher in these gels containing inclusion complexes, compared to the 15% pluronic gel containing plain drug. This may be due to the lack of complete gelation of the system in presence of cyclodextrin, which could interfere with the micelle aggregation of pluronic as well as its hard sphere crystallization mechanism. The gelling effect of pluronic was overcome by the solubilization effect of cyclodextrin which also added as the synergistic effect with the surfactant property of pluronic in solution, ultimately leading to increase in the amount of drug released. As the complexation ratio was varied, drug release upto 100 % at the end of 6 hours could be observed in 1:3 ratio complex in gel formulation as compared to 61% drug release observed from the same complex filled in capsule. This highest percentage of drug release is more significant, since greater improvement in the bioavailability can be achieved within 6h for this hydrophobic drug, through both the complexation of drug with β–CD and also incorporation in pluronic system.

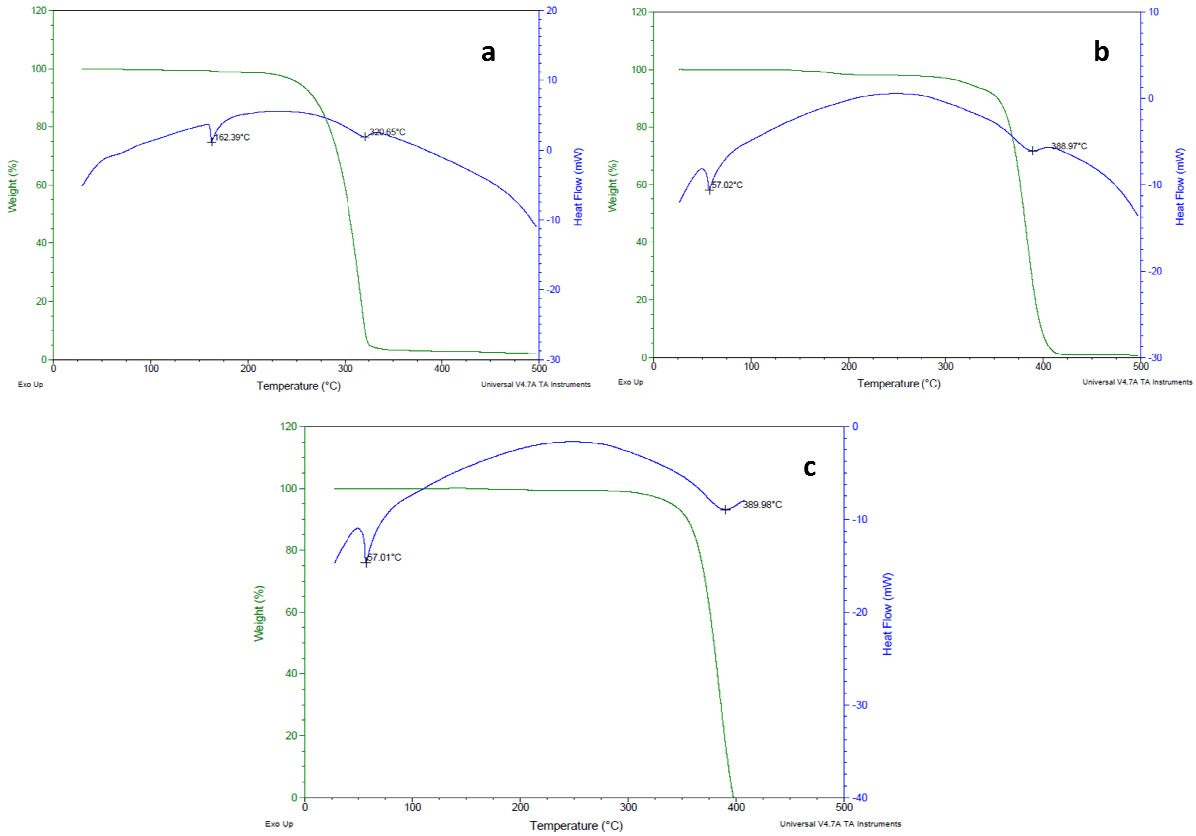

Figure 5 TG-DSC Spectra a) Pure Celecoxib b) Celecoxib *in-situ* gelling system with pluronic F127 c) Celecoxib *in-situ* gelling system containing drug-cyclodextrin inclusion complex in 15% pluronic F127

The mechanism of drug release through a dialysis membrane involved three consecutive processes: i) dissolution of the dispersed drug particles, ii) diffusion of drug across the polymer matrix and iii) permeation through the semipermeable membrane, all these contributing to the overall release of the drug. Complexation of drug enhanced the overall drug diffusion by improving the drug solubility thereby increasing the amount of diffusible species in the sample. Even though the complex could not permeate across the membrane, the drug in the complex was in rapid dynamic equilibrium with the free drug, thus continuously releasing the molecules to the dissolution media. Therefore, cyclodextrin complexation increased the concentration gradient across the membrane, which resulted in an increased drug release.

The presence of the solid phase of drug/cyclodextrin complex in the gel system could provide high drug thermodynamic activity on the membrane surface constantly, thereby replacing the drug molecules lost due to diffusion across the semipermeable membrane by dissolution of the solid phase. Cyclodextrins solubilize lipophilic

drugs in the aqueous vehicle and deliver the drug molecules to the barrier surface where complex dissociation and drug permeation across semipermeable membrane occurred. [26]

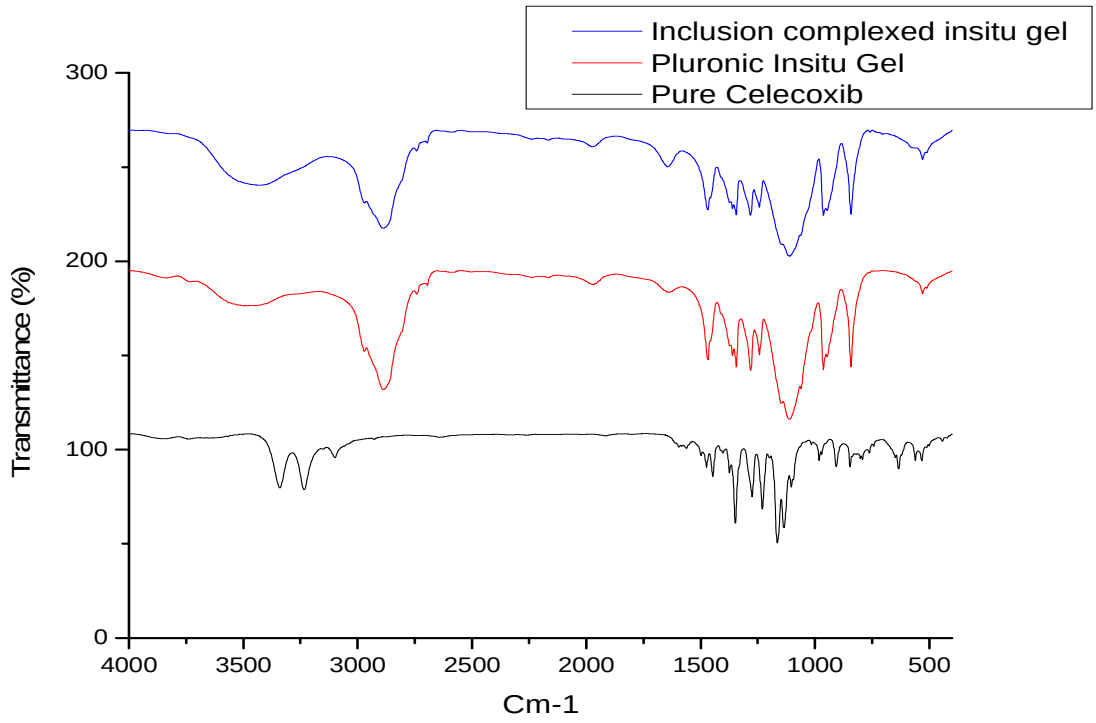

Figure 6 Comparison of FTIR spectra of Celecoxib *in-situ* gelling system with pluronic F127 and Celecoxib in-situ gelling system containing drug-βCD inclusion complex in 15% pluronic F127 with Pure Celecoxib

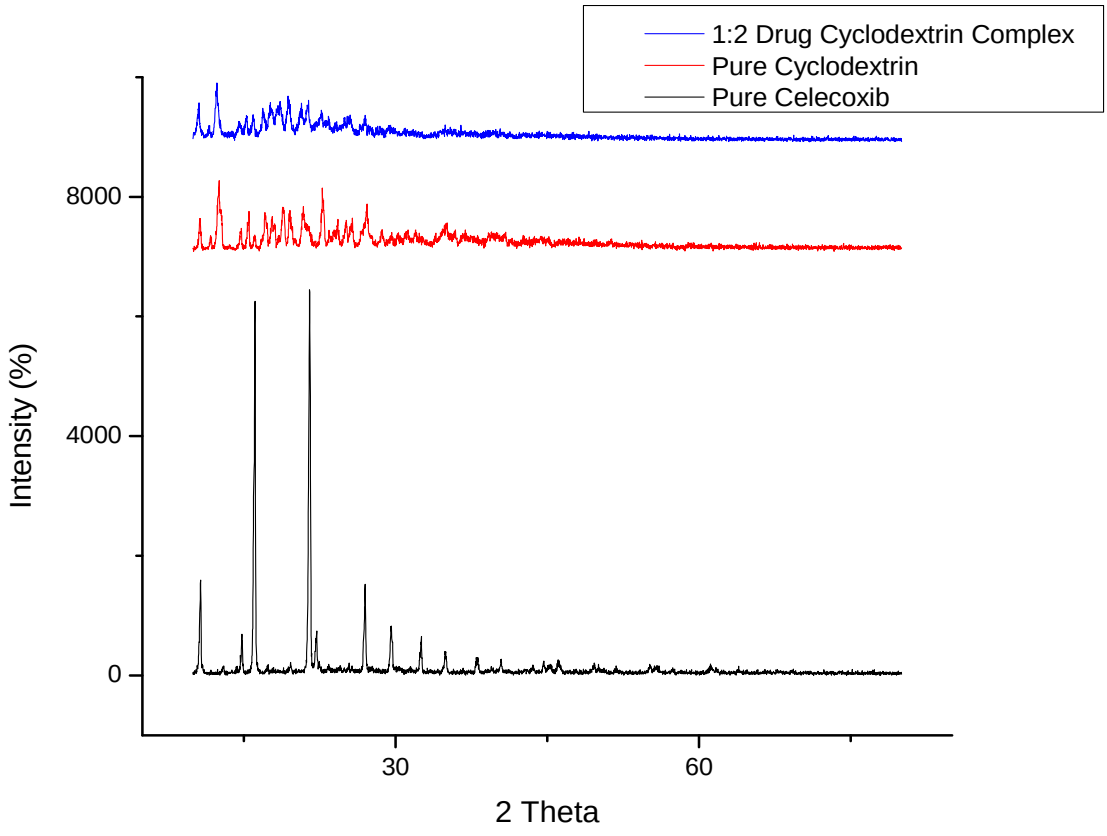

Figure 7 Comparison of XRD spectrum of the pure Celecoxib, pure cyclodextrin and 1:2 inclusion complex

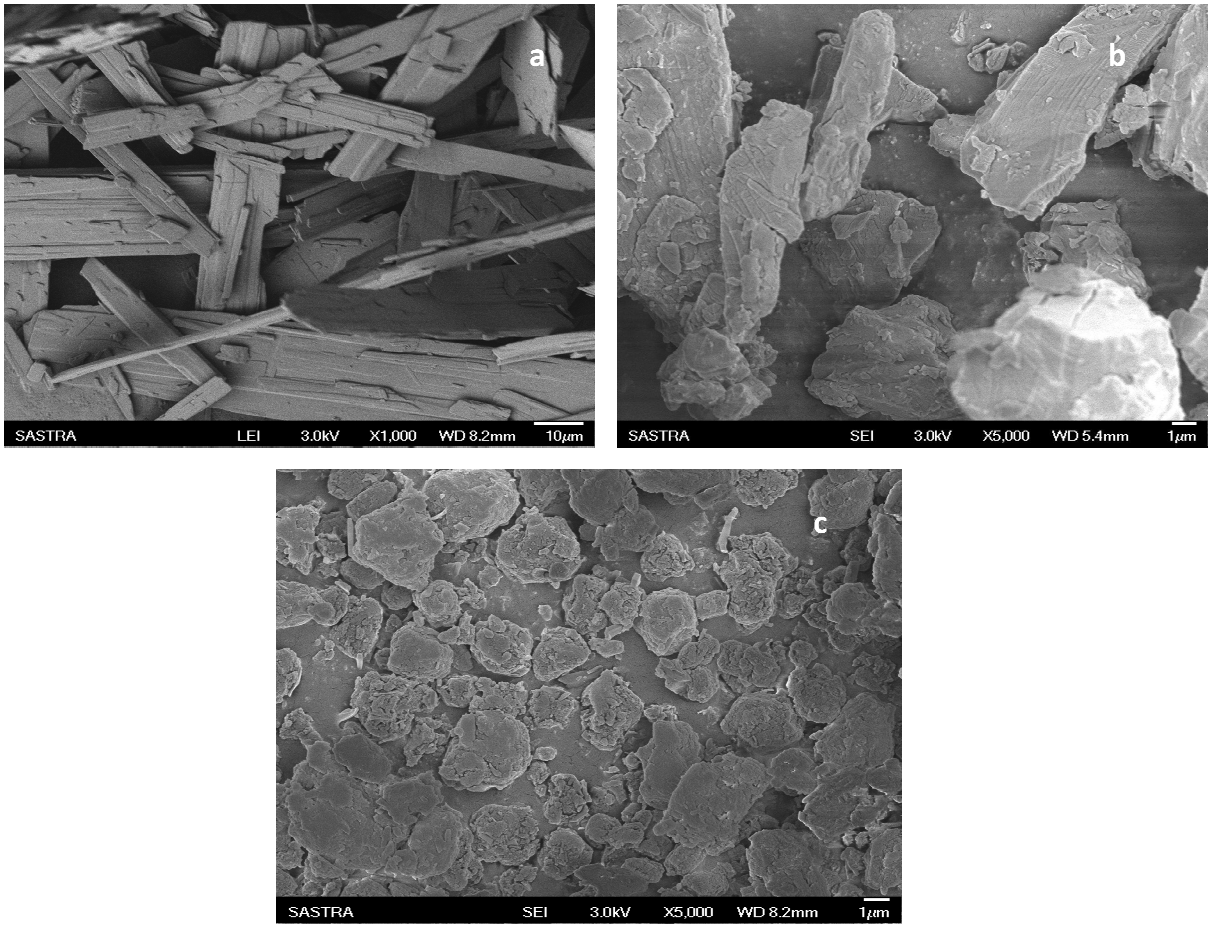

Figure 8 SEM images of a) Pure Celecoxib b) Pure β-cyclodextrin c) Celecoxib-cyclodextrin complex at 1:2 ratio

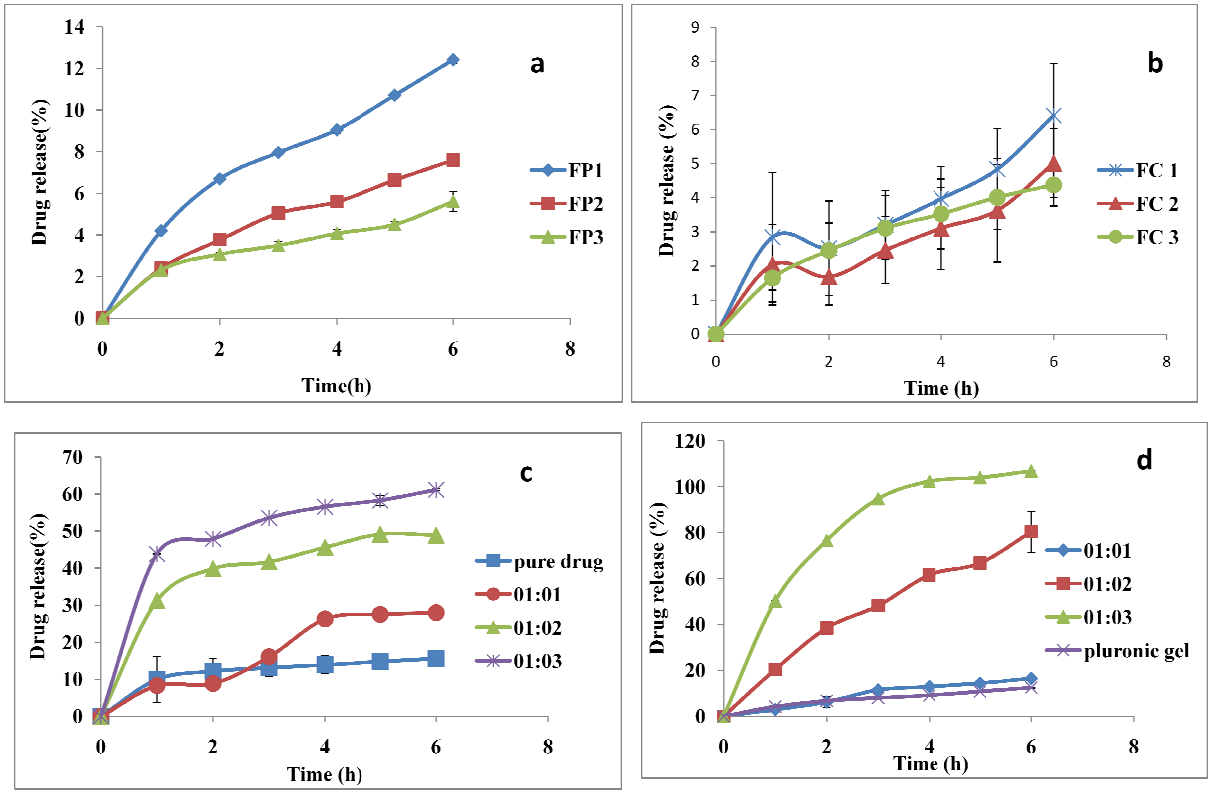

Figure 9 *In-vitro* drug release studies of Celecoxib a) Thermosensitive *in-situ* gelling system b) pH sensitive *in-situ* gelling system c) Cyclodextrin inclusion complex of Celecoxib (capsule) d) *In-situ* gels containing cyclodextrin inclusion complex of Celecoxib (gel)

Release kinetic studies

The *in-vitro* drug release data of the formulated *in-situ* gelling systems were best fitted to Korsemeyer-Peppas model, as shown by the R^2 values in the table 4. Both the thermo responsive and pH responsive system showed similar mechanism pattern for the drug release kinetics. The mechanism of *in-vitro* drug release from these systems can be correlated to the swelling of the polymer, diffusion of the drug and the dissolution of the polymer matrix. The n values obtained with the formulations in the range of 0.480 – 0.822 indicated the mode of release as anomalous non-Fickian diffusion. The similar results were obtained for the cyclodextrin-drug inclusion mixture incorporated thermo responsive gels also. [27]

CONCLUSION

From the results it was concluded that Celecoxib was successfully formulated as a thermoresponsive *in-situ* nasal gelling system using pluronic F127. The optimized formulations containing 15% pluronic provided sustained *in-vitro* drug release over a period of 6 h. Incorporation of drug in the form of complex with βCD, proved maximum drug release of 100% at 6 h, which can improve the overall percentage bioavailability. The optimized formulations can be a competitive alternative to conventional nasal drops. Physicochemical characterization contributed to the understanding of the effect of Celecoxib addition on intermolecular interactions within the gel. *In-vitro* drug release and release kinetics experiments demonstrated the feasibility for the sustained delivery of Celecoxib at therapeutic levels. Mucoadhesion that allows stagnation of the gel in the nose may be an advantage to protect the drug from extensive first-pass effect. In order to increase the effectiveness of the drug a dosage form should be chosen which increases the contact time of the drug in the target site. This dosage form can prolong the residence time of the gel along with its ability to release drugs in sustained manner which will assist in enhancing the bioavailability, increase systemic absorption and reduce the need for frequent administration leading to improved patient compliance. The *in-situ* formulations can also improve the patient acceptability since the formulation is applied in sol form which upon contact with biological environment forms gel causing less irritation and pain.

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