Research Article

Curcumin Loaded *in-situ* Nanoemulgel: An Unique Dosage Form for Ophthalmic Drug Delivery

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ABSTRACT

The objective behind this study is to develop *in-situ* ophthalmic nanoemulgel of curcumin using Pluronic F 127 as thermosensitive polymer in order to obtain an unique drug delivery system to retain the drug in ocular region for prolong period of time and increase the patient compliance. Various concentration of Pluronic F 127 influencing different physicochemical parameters, drug release and particle size were investigated. It was observed that gelation temperature of all the formulations were approximately similar to our normal body temperature. *In-vitro* drug release study revealed that curcumin loaded nanoemulgel formulations could able to sustain the drug release for prolong period of time. Droplet size analysis revealed that droplet size of the prepared formulations was within the nano range which concluded that prepared dosage form may be suitable for ocular delivery due to its small size. SEM study stated that the drug particles were homogenously distributed throughout the formulation. FTIR study revealed that there was no drug-polymer interactions and suggested the stable nature of curcumin in the optimized *in-situ* ophthalmic nanoemulgel formulation. DSC and XRD study revealed the amorphous state of curcumin in the optimized formulation. So, it is concluded from the present research that prepared *in-situ* ophthalmic nanoemulgel formulation is an unique drug delivery device to deliver curcumin in the ocular region for prolong period of time to improve the patient compliance as well as reduce the dosage frequency by sustaining and prolonging the systemic absorption of curcumin.

Keywords: Curcumin, *in-situ* nanoemulgel, ophthalmic delivery, drug release, droplet size, interaction studies.

INTRODUCTION

Ophthalmic delivery of different drugs is a major challenge for the formulation scientists due to the poor bioavailability. Poor ocular bioavailability of drugs (<1%) from conventional eye drops is due mainly to the precorneal loss factors that include rapid tear turnover, nonproductive absorption, transient residence time in the cul-de-sac and the relative impermeability of the drugs to corneal epithelial membrane [1]. As a result, the drug is drainage out due to lachrymation and normal tear turnover. The traditional ophthalmic formulations are immediately diluted in the tear and excess fluid containing drug is drained into the nasolachrymal duct. This phenomenon decrease the corneal contact time for the formulations and decrease the ocular bioavailability of the drugs [2]. As a result, in ophthalmic preparations, the drug concentration is high which some time may cause both ocular as well as systemic side effects [3].

In recent years, extensive investigations have been dedicated to the development of newer systems of ocular drug delivery to attain medications with prolonged retention time on the eye surface, minimized dose frequency and improved transcorneal penetration of newly emerging drugs. *In-situ* ophthalmic gel systems have received considerable attention in this respect. These formulations were present in solution form but when exposure to the physiological conditions of eye, undergo sol-to-gel phase transition due to ionic cross-linking or change of pH or temperature. This *in-situ* ophthalmic gel forming system prolonged retention time because of the gel formulation [4]. This approach also has several advantages such as ease administration, reduce dosing frequency, improve patient compliance etc. Nanonisation of active ingredient also have some additional advantages i.e. cross the corneal membrane, target delivery, improve solubility, improve bioavailability of the drug and minimize the chances of side effects.

Curcumin, a golden yellow water-insoluble pigment extracted from turmeric, the rhizome of *Curcuma longa*, belongs to Zingiberaceae family widely used as a coloring agent and spice in many foods. Chemically, it is a bis-a, b-unsaturated b-diketone (known as diferuloylmethane) that exhibits keto-enol tautomerism, having a predominant keto form in acidic and neutral solutions and a stable enol form in alkaline media [5]. It has several pharmacological effects such as antioxidant, anti-inflammatory, antiviral, antibacterial, hepatoprotective etc. Curcumin has extremely low water solubility which limits its bioavailability and clinical efficacy [6]. The maximum amount of ingested curcumin is excreted through faeces in unchanged condition which also limits curcumin to reach to the target sites and shows the pharmacological effects [7].

Pluronic F-127 consists of 70% ethylene oxide and 30% of propylene oxide is a block polymer due to the propylene oxide block is surrounded by two ethylene oxide blocks. It

is also known as poloxamer 407 and least toxic than the commercially available poloxamers [8]. Pluronic F-127 is soluble in water and form a clear, viscous liquid at refrigerator condition. This polymer has an unique reverse thermal gelation property i.e. the polymeric solution is liquid at low temperatures and at higher temperature, the polymer chains form a gel structure [9]. Pluronic F-127 is widely use in controlled drug delivery, ophthalmic drug delivery, parenteral drug delivery etc [10].

So the aim of the present research was to develop *in-situ* ophthalmic nanoemulgel of curcumin using Pluronic F 127 as thermosensitive polymer in order to obtain a unique drug delivery device to retain the drug in ocular region for prolong period of time and increase the patient compliance by reducing the dosage frequency.

MATERIALS AND METHODS

Materials

Curcumin, Ethyl Oleate and Transcutol P were purchased from Loba Chem., India. Pluronic F127 was a gift sample from Albert David Ltd., Kolkata, India. Tween 80 was purchased from Merck Pvt. Ltd., Mumbai, India. Other materials and solvents used were of analytical grade.

Method

Preparation of in-situ ophthalmic nanoemulgel

In-situ ophthalmic nanoemulgel of curcumin was formulated employing the method described by Li & Li [11]. Accurate quantity of PF127 was added slowly in 20 ml of distilled water with continuous stirring. Then the dispersion was stored in a refrigerator until a clear solution was obtained. In a separate container, curcumin was dissolved in the mixture of oil, surfactant and co-surfactant. Then the drug mixture was vortexed (Vortex mixture, CM101, Remi & Techno makes, Mumbai, India) until a clear solution was obtained and stored at room temperature until further use. Then 1 ml of drug solution was added to the polymer solution and homogenized using high speed homogenizer until a homogenous gel was formed. Then the prepared gel was transferred in a suitable container and kept in the refrigerator for further use.

Table 1: Formulation of *in-situ* ophthalmic nanoemulgel.

Formulation	Drug (g)	Oil (ml)	Surfactant (ml)	Co-Surfactant (ml)	Pluronic PF 127 (g)
CP1	1	15	10	5	1
CP2	1	15	10	5	2
CP3	1	15	10	5	3
CP4	1	15	10	5	4
CP5	1	15	10	5	5

CP: Curcumin-Pluronic nanoemulgel.

Characterization of *In-situ* Ophthalmic Nanoemulgel

Physicochemical evaluation

Clarity test

The clarity test of the prepared nanoemulgel formulations was performed by visual observation under white and black background and the clarity of the formulations was noted.

pH

Weighted amount of prepared nanoemulgel was placed in distilled water to prepare 1% solution. Then the pH of resulting solution was determined by using a pH meter (Digital pH meter, Systonic, Hyderabad).

Drug content determination

The drug content of the prepared nanoemulgel formulations was determined by the method of extraction of drug present in the formulations. 1g formulation i.e. *in-situ* ophthalmic nanoemulgel was placed in a 100 ml of simulated tear fluid (STF) (pH 7.4) and was sonicated at 125 W for 30 min (Imeco Sonifier, Imeco Ultrasonics, India). The drug content of the filtrate was determined spectrophotometrically at 424 nm (UV-1800, Shimadzu, Japan). Each determination was made in triplicate.

Gelation temperature

The gelation temperature of the formulations was measured by tube inverting method [12]. The temperature of water bath was gradually increased at a constant rate i.e. 1°C/min. A thin walled test tube containing 2 ml of nanoemulgel formulation (in liquid condition) was placed in the water bath. Then the nanoemulgel was observed for gelation by inverting the test tube at predetermined time intervals. The gelation time was noted when no-flow was observed by inverting the test tube and shown in Table-2.

Rheological studies

Rheological properties of the prepared nanoemulgel formulations were measured in a cone viscometer (Model DV-3, Brookfield, USA). The samples were thermostated

at 34°C by circulating bath connected to the viscometer. The shear rate was increased from 0 to 20 s^{-1} in 15 min. The viscosity was determined from the flow curve obtained at different values of the shear rate. The samples were equilibrated at 34°C prior to each measurement [11]. All measurements were made in triplicate within 24 hours after the nanoemulgels were prepared and shown in Table-2.

In-vitro drug release studies

In-vitro release studies of prepared nanoemulgel formulations were performed by dialysis tubing membrane (Himedia Ltd., India) [4]. The membrane opening was tied to the mouth of a polyvinyl chloride test tube (1 cm diameter) and dipped in a 100 ml beaker containing simulated tear fluid (STF) (pH 7.4, 50 ml). Then the entire system was placed in beaker (250 ml) containing distilled water and maintained at 37±0.5 °C. A small magnetic bead was placed in the beaker and was stirred at 100 rpm on a magnetic stirrer. At predetermined time intervals, 1.0 ml aliquot was withdrawn and after suitable dilutions the absorbance was measured at 424 nm using UV-visible spectrophotometer (UV-1800, Shimadzu, Japan) against a blank. The release studies were conducted in triplicate shown in Figure-1.

Kinetics of drug release

The rate and mechanism of release of curcumin from the prepared nanoemulgel were analyzed by fitting the dissolution data into the zero-order equation,

$$Q = k_0 t$$

where *Q* is the amount of drug released at time t and k_0 is the release rate constant.

First order equation,

$$\ln (100 - Q) = \ln 100 - k_1 t$$

where k_1 is the release rate constant.

The dissolution data was fitted to the Higuchi's equation [13],

$$Q = k_2 t^{1/2}$$

where k_2 is the diffusion rate constant.

The drug release data was further analyzed by Peppas equation [14, 15],

$$\frac{M_t}{M_\infty} = kt^n$$

Where, n is the release exponent; indicative of the mechanism of release, M_t/M_∞ is the fraction of the drug at time t, K is the release rate constant.

The criteria for selecting the most appropriate model were based on the highest values of the coefficient of determination (r^2).

Statistical analysis

In-vitro drug release data of all the nanoemulgel formulations were subjected to one way analysis of variance (one way ANOVA) followed by multiple comparison analysis study to find out whether any significant difference was present among the formulations or not. Statistical analysis of the data was performed using the PRISM software (Graph pad, San Diego, CA). A confidence limit of $P < 0.05$ was fixed for interpretation of the results.

Droplet size analysis

Light scattering technique was used to determine the droplet size distribution of the optimized *in-situ* ophthalmic nanoemulgel. The droplet size distribution was measured by LASER diffraction using photon correlation spectrometer (Zeta Sizer Nano ZS 90, Malvern Instruments, UK). The optimized formulation was diluted 100 times with purified water and placed into the module. The data was shown in Figure-2.

Surface morphology study (SEM)

Surface morphology of prepared *in-situ* ophthalmic nanoemulgel was studied using scanning electron microscope (S 3700 VP FE-SEM, Hitachi High-Technologies, Europe). Prepared *in-situ* nanoemulgel formulation was diluted with triple distilled water and the liquid sample was deposited on a thin aluminum plate (1cm x 1cm). Then the plate was dried at room temperature and directly placed on the stub without staining. Then the photograph of sample was taken using scanning electron microscope chamber at acceleration voltage of 15 kV and chamber pressure of 0.6 mm Hg. The data was shown in Figure-3.

Fourier Transform Infrared Analysis:

Fourier Transform Infrared Analysis (FTIR) of pure drug (curcumin) and drug-loaded optimized formulation were obtained using FTIR analyzer (Prestige-21, Shimadzu FT-IR, Japan). The samples were scanned over the wave number ranges between 4500 to 500 cm^{-1} at the ambient temperature and all the spectra are shown in Figure-4.

Differential Scanning Calorimetric Analysis:

Differential scanning calorimetric (DSC) thermograms of pure drug (curcumin) and drug-loaded optimized formulation were obtained using a Differential Scanning Calorimeter (Diamond DSC, PYRIS, Perkin Elmer, USA). The samples were heated at constant rate of 10 °C/min over a temperature range of 10 °C to 300 °C. The system was purged with nitrogen gas at the rate of 100 mL/min to maintain inert atmosphere. The DSC thermograms were shown in Figure-5.

X-Ray Diffraction (XRD) Studies:

X-Ray Diffraction (XRD) study of pure drug (curcumin) and drug-loaded optimized formulation were assessed for crystallinity by X-Ray Diffractometer (X'Pert Pro,

Panalytical, Nertherlands) using monochromatized Cu K α-1 radiation (λ = 1.54 A) at a voltage of 45 kV and current of 40 mA. Measurements were carried out in the angular scan range from 5° to 40° (2ϑ) at a scan speed of 1°/min. The XRD spectra of all the samples are shown in Figure-6.

RESULTS AND DISCUSSION

In this present research work, *in-situ* ophthalmic nanoemulgel was prepared using thermosensitive polymer Pluronic F 127 for ocular delivery of curcumin and the influence of thermosensitive polymer on the *in-vitro* drug release from the nanoemulgel was studied.

Physicochemical characterization of in-situ ophthalmic nanoemulgel

The pH and drug content of the different Pluronic F 127 nanoemulgel formulations were found to be 7.1 ± 0.4 to 7.3 ± 0.9 and 83.3 ± 3.02 % to 94.5 ± 2.02 % respectively (Table-2). Visual observation suggested that formulation CP1 to CP4 were clear in appearance whereas formulation CP5 was found to be turbid in appearance. This may be due to the fact that in formulation CP5, the maximum amount of polymer was present which increased the viscosity of the formulation and showed some turbidity. The gelation temperature for all the formulations was found within the range of 36.2 ± 1.37 °C to 37.8 ± 2.03 °C (Table-2). It was found that the gelation temperature of all the formulations were approximately similar to our normal body temperature. So it was concluded that when these formulations were come in contact with the body environment they immediately converted from solution to gel state as well as retained in the targeted site for prolong period of time.

The viscosity of all the prepared nanoemulgel formulations containing Pluronic F 127 is shown in Table-2. The viscosity of all the prepared gels was found within the range of 0.736 ± 0.138 cP to 0.983 ± 0.192 cP (Table-2). It was observed that by increasing the concentration of Pluronic F 127 viscosity of formulations were gradually increased. This increase in viscosity resulted in a large increase in resistance to flow of the gels. This may be due to the entanglement of molecule chains. Pluronic F 127 polymer when come in contact with water formed hydrogen bonds between the poly(oxyethylene) unit and water molecules and get dissolved. At lower concentration (i.e. 1% and 2%), Pluronic forms a monomolecular micelle whereas at higher concentration it forms a polymolecular micelle. This several micelles attach together, minimize their interaction with water and increase the viscosity of the system [16, 17].

Table-2: Physicochemical characterization of *in-situ* ophthalmic nanoemulgel.

Formulation	Clarity	pH	Drug Content (%)	Gelation Temperature (°C)	Viscosity (cP.)
CP1	++	7.1 ± 0.4	83.3 ± 3.02	36.2 ± 1.37	0.736 ± 0.138
CP2	++	7.2 ± 0.7	92.3 ± 1.03	36.7 ± 1.04	0.812 ± 0.151
CP3	++	7.2 ± 0.3	94.5 ± 2.02	37.0 ± 1.22	0.887 ± 0.161
CP4	++	7.1 ± 0.7	92.4 ± 3.03	37.2 ± 1.84	0.944 ± 0.079
CP5	+	7.3 ± 0.9	88.1 ± 2.02	37.8 ± 2.03	0.983 ± 0.192

Mean ± SD; n=3; Turbid '+'; Clear '++'.

In-vitro drug release studies

Drug release from the prepared nanoemulgel formulations was depicted in Figure-1. The effect of Pluronic F 127 concentration on the release of curcumin was studied using simulated tear fluid (STF) (pH 7.4). It was observed that as the polymer concentration of the prepared formulations increased; the drug release was proportionately decreased. This was probably due to the increase in number and size of micelles within the gel structure. The viscosity of gel systems was high due to the formation of a three-dimensional network, and the polymer chains probably prevented the movement of molecules. The shorter intermicellar distance leads to greater numbers of cross-links between neighboring micelles, which result in higher viscosity and lower rate of drug release [18].

It was found that curcumin loaded nanoemulgel formulations (CP1 to CP5) were able to sustain the drug released from 8 to 12 hours. Formulation CP1 and CP2 were able to sustain the drug release up to 8 hours and 10 hours respectively whereas formulations CP3 was able to sustain the drug release for 12 hours. This sustained drug release from the Pluronic formulations generally increase drug residence time at application sites through gelling, resulting in improved bioavailability and efficacy.

On increasing the concentration of Pluronic in formulation CP4 and CP5, the release of the drug was too slow and only 78.75 ± 2.35 % and 67.65 ± 2.32 % drug was released after 12 hours. From the *in-vitro* drug release study it was cleared that among all the *in-situ* ophthalmic nanoemulgel formulations, CP3 shows the best drug release profile (more than 90 % drug was release in 12 hours).

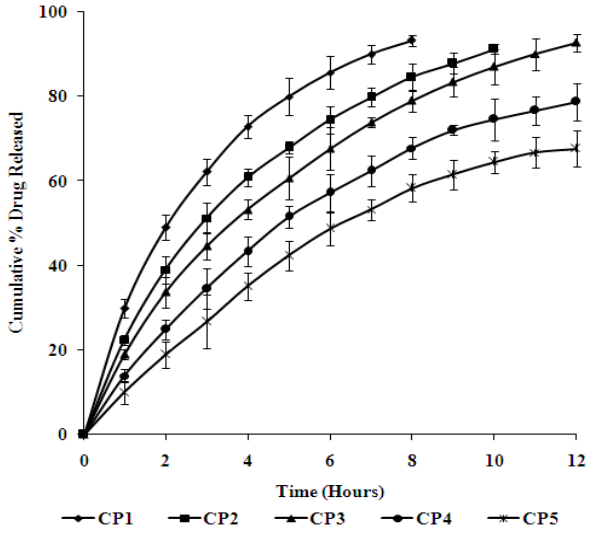

Figure 1: *In-vitro* drug release studies of *in-situ* ophthalmic nanoemulgel (CP1 to CP5).

Kinetic modeling

In-vitro drug release data of all the *in-situ* ophthalmic nanoemulgel were subjected to various kinetics models such as zero order, first order, Higuchi and Korsmeyer model. According to the coefficient of determination (R^2) and release exponent (n) values, drug release data for all the formulations showed best fit in zero order kinetics followed by non fickian diffusion mechanism. The coefficient of determination (R^2) and release exponent (n) values of all the formulations were shown in Table-3.

Statistical analysis

In-vitro drug release data were subjected to one way analysis of variance. One way analysis of variance (ANOVA) suggested a significant difference (at $P < 0.05$) among all the *in-situ* ophthalmic nanoemulgel formulations with respect to their drug release values. Holm-Sidak multiple comparison analysis also suggested significant difference in *in-vitro* drug release among all the formulations. Formulation CP3 shows the best dissolution profile (more than 90 % drug was released in 12 hours) among all the formulations with respect to *in-vitro* drug release. So, Formulation CP3 has been selected as an optimized formulation for further studies.

Table-3: Kinetic modeling of drug release from *in-situ* ophthalmic nanoemulgel*.

Formulations	Drug release kinetics, Coefficient of determination (r^2)				Release coefficient (n)
	Zero order	First order	Higuchi Model	Korsmeyer Model	
CP1	0.978	0.825	0.990	0.997	0.671
CP2	0.977	0.818	0.992	0.996	0.724
CP3	0.970	0.789	0.992	0.994	0.731
CP4	0.955	0.782	0.984	0.998	0.798
CP5	0.948	0.746	0.975	0.997	0.856

*Analyzed by the regression coefficient method.

Droplet size analysis

Droplet size distribution of the Nanoemulsion was determined by LASER diffraction using photon correlation spectrometer (Zeta Sizer Nano ZS 90, Malvern Instruments, UK) at 25°C. Droplet size distribution and size analysis of the optimized *in-situ* ophthalmic nanoemulgel formulation i.e. CP3 was showed in Table-4, Figure-2. The average droplet size of the optimized formulation was found to be 258.7 nm (Figure-2) which is the normal average droplet size range for nanoemulgel. So, from these studies it was concluded that the nano range droplet size of the optimized CP3 formulation may be suitable for ocular delivery due to its small size.

Table 4: Average droplet size of optimized *in-situ* ophthalmic nanoemulgel.

Formulation code	Droplet size (nm)
CP3	258.7

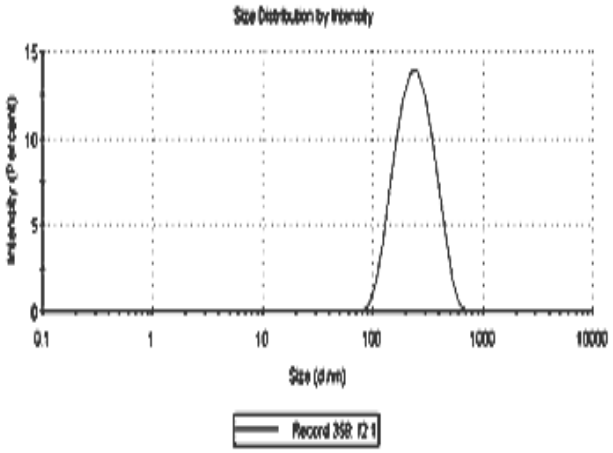

Figure-2: Droplet size analysis of optimized *in-situ* ophthalmic nanoemulgel (CP3).

Surface morphology study (SEM)

SEM study revealed that the droplets of optimized *in-situ* ophthalmic nanoemulgel (CP3) were within the nano range (Figure-3). This study also revealed that due to fairly good solubility of curcumin in the polymeric matrix, the drug particles were homogenously distributed throughout the formulation.

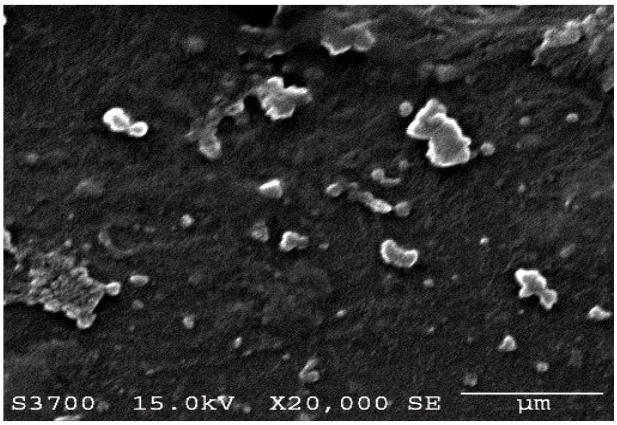

Figure 3: Scanning electron photomicrographs of optimized *in-situ* ophthalmic nanoemulgel (CP3; X20000).

FTIR analysis

FTIR study stated that all the major shoulders of pure curcumin (Figure-4A) were intact in the optimized *in-situ* ophthalmic nanoemulgel formulation (CP3) (Figure-4B). So this study revealed that there was no drug-polymer interactions and suggested the stable nature of curcumin in the optimized *in-situ* ophthalmic nanoemulgel formulation.

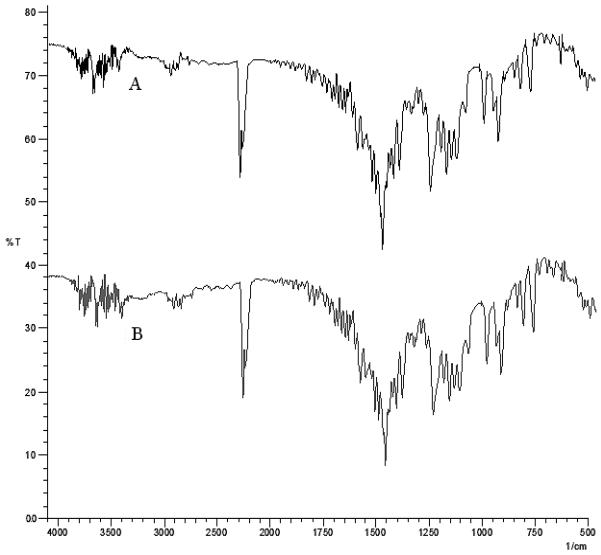

Figure 4: FTIR spectra of curcumin (A) and optimized *in-situ* ophthalmic nanoemulgel formulation (CP3) (B).

Thermal property studies (DSC)

The thermal property of the pure drug and optimized formulation was studied using differential scanning calorimetric analysis (DSC) (Figure-5). The DSC spectra revealed that pure curcumin showed a sharp melting endotherm at 186.28 °C corresponding to its melting point (Figure-5A) whereas when it was incorporated in the *in-situ* ophthalmic nanoemulgel formulation, the melting endotherm was significantly decreased to 114.85 °C (Figure-5B). It was also observed from this study that in the optimized formulation, the sharpness of the endothermic peak corresponding to the melting point of curcumin was reduced which revealed the amorphous state of curcumin in the optimized formulation. This observation was further confirmed by x-ray diffraction study.

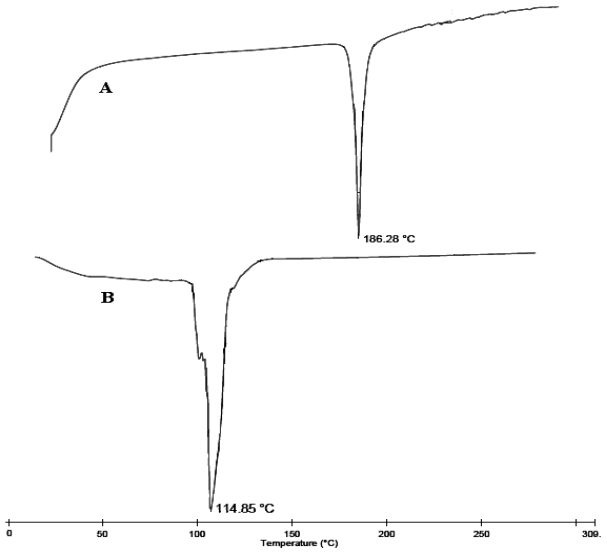

Figure 5: DSC thermogram of curcumin (A) and optimized *in-situ* ophthalmic nanoemulgel formulation (B).

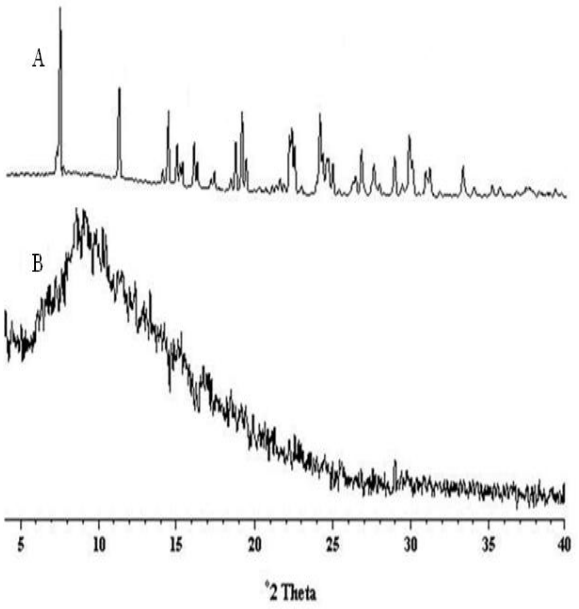

Figure-6: XRD spectra of curcumin (A) and optimized *in-situ* ophthalmic nanoemulgel formulation (CP3) (B).

X-ray diffraction (XRD) studies

X-ray diffraction pattern of pure curcumin and curcumin loaded optimized *in-situ* ophthalmic nanoemulgel formulation are shown in Figure-6. The XRD spectra of pure curcumin exhibited different sharp diffraction peaks

at 2θ values (Figure 6A). In case of curcumin loaded optimized nanoemulgel formulation, no significant difference with respect to the diffraction peaks were observed as compared to curcumin (Figure 6B). So it is concluded that in the prepared optimized *in-situ* nanoemulgel formulation, curcumin is present as an amorphous form.

CONCLUSION

It was concluded from the above research that prepared *in-situ* ophthalmic nanoemulgel formulation is an unique drug delivery device to deliver curcumin in the ocular region for prolong period of time to improve the patient compliance as well as reduce the dosage frequency by sustaining and prolonging the systemic absorption of curcumin.

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