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FORMULATION AND EVALUATION OF MODEL CYCLOOXYGENASE INHIBITOR ANTI-INFLAMMATORY DRUG LOADED SOLID LIPID NANOPARTICLES FOR TARGETED DRUG DELIVERY

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ABSTRACT

The objective of the present work was to formulate Solid lipid nanoparticles of Lornoxicam. Solid lipid nanoparticles were prepared by hot homogenization technique and characterized by particle size analysis, Fourier Transform Infrared spectroscopy, Differential Scanning Calorimetry, drug entrapment efficiency, Scanning Electron Microscopy and *in vitro* release studies. At highest speed the result solid lipid nanoparticles were smaller in size and their size increased with increase in lipid concentration. Smaller size solid lipid nanoparticles were obtained with 1 % (1:1 w/v) of lecithin/tween-80. Tripalmitin Solid lipid nanoparticles showed maximum entrapment efficiency, Controlled release and showed maximum release. The *in vitro* release was found to follow Non-Fickian Diffusion mechanism. The surface characters were found to be better with all the lipid carriers. Accelerated stability studies indicated that there were no significant changes when stored between 2-8^{0}C and 75 ± 5% RH. The formulations done with Tripalmitin showed better efficiency with controlled release profile.

KEYWORDS

Solid lipid nanoparticles, Lornoxicam, Hot homogenization Technique, Triglycerides, Stability and *In vitro* release studies.

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INTRODUCTION

Solid lipid nanoparticles by hot homogenization technique is a simple method in nanoparticle préparation with ease, compliance, faster production and also economical. The solid lipid nanoparticles are sub-micron colloidal carriers (50-100 nm) which

are composed of physiological lipid, dispersed in water or in aqueous surfactant solution. SLNs as colloidal drug carrier combine the advantage of polymeric nanoparticles, fat emulsions and liposomes[1, 2]. The object of drug targeting is to achieve a desired pharmacological response at a selected site without undesirable interaction at other sites. At present drug targeting achieved at one of two approaches: the first approach involves chemical modification of the parent compound to a derivative which is activated only at the target site. The second approach utilizes carriers such as liposomes, microspheres, nanoparticles, antibiotics, cellular carriers (erythrocytes and lymphocytes) and macromolecules to direct the drug at its site of action[3]. Lornoxicam is a new non-steroidal anti-inflammatory drug (NSAIDs) of the Lornoxicam class with analgesic, anti-inflammatory and antipyretic properties. It is distinguished from established Lornoxicam by a relatively short half-life (3 to 5 hours), which may be advantageous from a tolerability point. Lornoxicam acts by inhibiting the enzyme (COX). Inhibition of COX-2 is thought to mediate antipyretic, analgesic and anti-inflammatory actions of NSAIDs, while simultaneous inhibition of COX-1 largely but not exclusively accounts for unwanted adverse effects in gastrointestinal tract. Lornoxicam is as effective as the opioid analgesics in relieving postoperative pain following gynecological or orthopedic surgery. It was also effective in relieving symptoms of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, acute sciatica and low back pain[4].

MATERIAL AND METHODS

Material

Lornoxicam was obtained from Chimak Health Care, Himachal Pradesh, India. Glycerol Monosterate was obtained as a gift sample form Loba Chemicals, Mumbai. Tripalmitin was a gift sample from Himedia Pvt Ltd, Mumbai. Stearic Acid was purchased from Genuine Chemicals, Mumbai. Lecithin, Tween 80, Sodium Hydroxide, Potassium Dihydrogen phosphate and Dialysis Membrane are of analytical grade.

Methods

Lornoxicam Solid lipid nanoparticles were prepared by hot homogenization technique using GMS, Tripalmitin and Stearic acid as lipid carriers, Soya lecithin as surfactant and tween 80 as co-surfactant[5]. Drug was dispersed in molten lipid (70^{o}C). This dispersion was added into aqueous medium containing surfactants maintained at same temperature as that of lipid melt. This warm Pre-emulsion so formed is subjected to High pressure homogenization (High pressure Homogenizer IKA Ltd.,) for 3 homogenization cycles at 900 bars and cooled to room temperature to form Solid lipid nanoparticles. The samples were sonicated and analyzed for particle size. The details of formulation designs are tabulated in Table No.1-3.

EVALUATION PARAMETERS

Pre-formulation Studies

Compatibility studies (Fourier Transform Infrared Spectroscopy)

The fourier transform infra-red analysis was conducted for the structure characterization. FTIR spectra of the pure drug, polymers and formulations were recorded by using THERMO NICOLET, Japan FTIR instrument. Approximately 4 mg of samples were mixed with 50mg of spectroscopic grade KBr, samples were scanned in the IR range from 500 to 3500 cm^{-1}, with a resolution of 4 cm^{-1}.

Evaluation parameters of SLNs

Particle Size Analysis

Drug loaded nanoparticles were analyzed by CIS-L50 Particle Size Analyzer. The suspension is taken in a cuvette and diluted with distilled water to give a concentration of 10^{-9} particles with a standard normalizing factor (SNF) value of 1. The mechanism of working of CFS-L50 is TOT (time of transition).

Drug content

Lornoxicam loaded SLNs (1 ml) were diluted to 10 ml of pH 6.8. Final dilution is made with pH 6.8 in its beers range. And total drug content was determined by using UV spectrophotometer at 376 nm by taking pH 6.8 as blank[6].

Entrapment Efficiency

Entrapment efficiency of drug loaded SLNs was determined by centrifugation of samples at 10,000 rpm for 10 min. The amount of free drug was determined in the clear supernatant by UV spectrophotometer at 376 nm using supernatant of non-loaded nanoparticles on basic correction. The entrapment efficiency (EE %) could be achieved by the following equation.

$$EE\ (\%) = \frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}} \times 100$$

Differential Scanning Calorimetry

The compatibility study was analyzed by differential scanning calorimetry (DSC) (Q20,V24.4,Universal**,**USA) DSC was carried out by the action of argon purging with 10ml/min where it is hermetically sealed with aluminium pans. From this sample of 40µl is used. The programme is run at 10^0C/min. The onset peak and end set peaks are recorded for bulk drug and SLN formulations.

Scanning Electron Microscopy

Surface morphology of the drug loaded nanoparticles was determined by using a scanning electron microscope (SEM), Model JSM 6330, JEOL, Japan. The samples are dried thoroughly in vacuum desiccator before mounting on brass specimen studies, using double sided adhesive tape. Gold-palladium alloy of 120^0A was coated on the sample using sputter coating unit (Model E5 100 Polaron U.K.) in Argon at ambient of 8-10 Pascal with plasma voltage about 20 mA. The sputtering was done for nearly 5 minutes to obtain uniform coating on the sample to enable good quality SEM images. The SEM was operated at low accelerating voltage of about 15KV with load current of about 80MA. The condenser lens position was maintained between 4.4- 5.1. The objective lens aperture has a diameter of 240 microns and the working distance WD=39mm.

***In vitro* release study**

In vitro release studies were performed using modified Franz diffusion cell. Cellophane Dialysis membrane having pore size 2.4 nm; molecular weight cut off 12,000–14,000, was used (Membrane was soaked in double-distilled water for 12 hrs. before mounting in a Franz diffusion cell). A volume equivalent to 2 mg of Lornoxicam (Practically calculated) loaded SLNs formulation was placed in the donor compartment and the receptor compartment was filled with 50 ml of 6.8 pH Phosphate buffer. The content of the cell was stirred with the help of magnetic stirrer at 37^0C. Aliquots were withdrawn from receiver compartment through side tube at every hour time interval up to 12 hours. Fresh medium of 6.8 pH Phosphate buffer was replaced each time to maintain constant volume. Samples were analyzed by UV visible spectroscopy at 376 nm.

Accelerated stability studies for the optimized formulation

Accelerated stability studies of the drug loaded SLNs were carried out for 3 formulations from each set as per ICH guidelines, at 40 ± 2^0C /75 ± 5% RH & 2-8^0C /75±5% RH by using Thermolab TH 90S stability chamber for period of 3 month. The samples were observed for particle size analysis, Entrapment efficiency and *in vitro* release studies.

Mathematical modeling for nonlinear curve

To analyze the mechanism for the release and release rate kinetics of the dosage form, the data obtained was fitted in to Zero order, First order, Higuchi matrix and Krosmeyer and Peppa's model. Using Microsoft excel software. Comparing the r^2-values obtained, the best-fit model was selected.

Zero order kinetics

To study the Zero order release rate kinetics the release rate data were fitted to the following equation.

$$Q_t = Q_o + K_o t$$

Where,

Q_t = amount of drug dissolved in time t,

Q_o = initial amount of drug in the solution and

K_o = zero order release constant.

First order kinetics

To study the first order release rate kinetics the release rate data were fitted to the following equation.

$$\text{Log } Q_t = \log Q_o + K_1 t / 2.303$$

Where,

Q_t is the amount of drug released in time t,

Q_o is the initial amount of drug in the solution and

K_1 is the first order release constant.

Higuchi model

To study this model the release rate data are fitted to the following equation

$$Q_t = K_H . t^{1/2}$$

Where,

Q_t = Amount of drug released in time t,

K_H = Higuchi dissolution constant.

Krosmeyer and Peppa's release model

To study this model the release rate data are fitted to the following equation

$$M_t / M_\infty = K.t^n$$

Where,

M_t / M_∞ = fraction of drug release,

K = release constant, t = release time and

n = Diffusion exponent for the drug release that is dependent on the shape of the matrix dosage form.

Hixson-Crowell model

To study the Hixson – Crowell model the release rate data are fitted to the following equation

$$W_o^{1/3} - W_t^{1/3} = Kst$$

Where,

W_o = amount of drug in the dosage form,

W_t = remaining amount of drug in the pharmaceutical dosage form,

Ks = constant incorporating the surface-volume relationship.

RESULTS AND DISCUSSION

Compatibility studies

The Fourier transform infra-red analysis was conducted for the surface structure characterization. FTIR spectrum of the pure drug, polymers and formulations was recorded. The Fourier transform infrared spectroscopy study reveals that there is no interaction between the pure drug, polymers and formulations. Then all the functional groups found in the IR spectrum of pure drug, polymers and formulations. The results are tabulated in Table No.4 and Figure No.1-4.

UV Spectroscopy

The drug solution was scanned for absorption maxima (λ_{max}) spectrophotometrically between 200nm and 400nm. The solution was shown absorption maxima at 376nm.

Particle Size Analysis

The formulations with GMS such as LOMG showed wide distribution in particle size ranging from 780nm to 920nm; similarly formulations with Tripalmitin such as LOMT showed particle size ranging from 740nm to 850nm and formulations with stearic acid such as LOMS showed particle size ranging from 740nm to 800nm. The results are tabulated in Table No.5-7.

Drug content and Entrapment Efficiency

The entrapment efficiencies of the Lornoxicam loaded SLNs was in the order of LOMT>LOMG>LOMS. The higher entrapment efficiency with tripalmitin is attributed to the high hydrophobicity due to the long chain fatty acids attached to the triglyceride resulting in increased accommodation of drugs**.** The results are tabulated in Table No.8-10.

Differential Scanning Calorimetry

It was found that the thermal peaks of drug are identical in formulations with those entire lipid carriers. This indicates that, there is no interaction in the formulations. Results are tabulated in Table No.11.

Scanning Electron Microscopy

The smooth surface of SLNs is because of presence of Lecithin and it could be attributed to its proteinous nature. The surface morphology of the SLNs had not been altered by the type of lipid carrier, concentration of lipid carrier and speed. The figures are shown in Figure No.5.

***In vitro* release study**

In vitro drug release data from the SLNs were carried out for 12hrs and graphically represented as % CDR v/s time profile. The results are shown in Table No.12.

Accelerated stability studies for the optimized formulation

There was significant change of particle size, total

drug content, entrapment efficiency and *In vitro* drug release of formulation which stored at 40 ± 2^0C /75 ± 5% RH than at 2-8^{0}C. At 2-8^{0}C it does not show this changes due to good stability might derive from the slow transition of lipid in SLNs, low temperature and the steric effect of Tween 80.

Mathematical modeling for nonlinear curve

LOMG, LOMS and LOMT were found to follow the zero order models. From the n values obtained it can be said that the diffusion followed Non-Fickian mechanism. Results were shown in Table No.13 and Figure No.6.

DISCUSSION

Solid lipid nanoparticles of Lornoxicam were prepared by hot homogenization technique. The Fourier Transform Infrared Spectroscopy study reveals that there is no interaction between the pure drug and individual, composition of polymers. SEM results showed that their surface of SLNs is spherical and smooth. In-vitro release study showed better release for SLNs with tripalmitin as drug carrier.

Table No.1: Formulation design of Lornoxicam loaded GMS SLNs by hot homogenization technique

S. No	Formulation Codes	Lipid %w/v	Drug (mg)	Conc. of surfactant/ co- surfactant 1:1 (w/v)	Speed (rpm)
1	LORG-I	0.5	8	1.0	6,500
2	LORG-II	0.5	8	1.0	9,500
3	LORG-III	1.0	8	1.0	9,500
4	LORG- IV	1.5	8	1.0	13,500
5	LORG-V	1.0	8	1.0	13,500

Table No.2: Formulation Design of Lornoxicam loaded Stearic acid SLNs by hot homogenization technique

S. No	Formulation Codes	Lipid %w/v	Drug (mg)	Conc. of surfactant/ co- surfactant 1:1 (w/v)	Speed (rpm)
1	LORS-I	0.5	8	1.0	6,500
2	LORS-II	1.0	8	1.0	6,500
3	LORS-III	1.5	8	1.0	9,500
4	LORS- IV	0.5	8	1.0	13,500
5	LORS-V	0.5	8	1.0	9,500

Table No.3: Formulation Design of Lornoxicam loaded Tripalmitin SLNs by hot homogenization Technique

S. No	Formulation Codes	Lipid %w/v	Drug (mg)	Conc. of surfactant/ co- surfactant 1:1 (w/v)	Speed (rpm)
1	LORT-I	0.5	8	1	6,500
2	LORT-II	0.5	8	1	9,500
3	LORT-III	0.5	8	1	13,500
4	LORT-IV	1.0	8	1	9,500
5	LORT-V	1.5	8	1	13,500

Table No.4: Data obtained from Compatibility study of Drug, Lipid and Formulations by FTIR spectroscopy

S.No	Lipid/Drug/ Formulation	Important IR Spectral peaks of different groups, wave length in cm^{-1}							
		C-H Stretch (Aromatic)	C=O Stretch	C-Cl	C-H Stretch (Aliphatic)	C-O-C Stretch	P=O	OH Stretch	S=O
1	Lornoxicam	3062.56	1593.23	783.35	------	-----	-----	-----	1326.99
2	G.M.S	------	1735.29	------	2919.33	1185.24	-----	-----	-----
3	Stearic acid	------	1702.79	------	2919.87	1301.43	-----	-----	-----
4	Tripalmitin	3497.29	1735.15	------	2920.02	------	-----	------	-----
5	LORG	3061.83	1734.78	725.27	2918.80	1183.53	-----	-----	1325.89
6	LORS	3061.93	1702.23	724.97	2919.60	1186.07	-----	-----	1321.68
7	LORT	3061.78	1734.85	725.05	2920.33	1183.14	-----	-----	1326.56

Table No.5: Mean Particle Size of Lornoxicam Loaded SLNs using G.M.S as a lipid carrier

Formulation code	LORG-I	LORG-II	LORG-III	LORG-IV	LORG-V
Mean Particle Size	790nm	780nm	800nm	920nm	780nm

Table No.6: Mean Particle Size of Lornoxicam loaded SLNs using Stearic-Acid as a lipid carrier

Formulation code	LORS-I	LORS-II	LORS-III	LORS-IV	LORS-V
Mean Particle Size	790nm	800nm	800nm	740nm	780nm

Table No.7: Mean Particle Size of Lornoxicam loaded SLNs using Tripalmitin as a lipid carrier

Formulation code	LORT-I	LORT-II	LORT-III	LORT-IV	LORT-V
Mean Particle Size	790nm	780nm	740nm	800nm	850nm

Table No.8: Drug Entrapment Efficiency with GMS as a lipid carrier

Formulation code	LORG-I	LORG-II	LORG-III	LORG-IV	LORG-V
Practically total drug content	88.42%	87.58%	89.40%	86.74%	87.30%
Drug entrapment efficiency	46.12%	45.23%	48.80%	51.14%	47.42%

Table No.9: Drug Entrapment Efficiency with Stearic-Acid as a lipid carrier

Formulation code	LORS-I	LORS-II	LORS-III	LORS- IV	LORS-V
Practically total drug content	89.68%	89.96%	87.44%	90.24%	90.52%
Drug entrapment efficiency	38.22%	42.60%	45.49%	37.20%	37.92%

Table No.10: Drug Entrapment Efficiency with Tripalmitin as a lipid carrier

Formulation code	LORT-I	LORT-II	LORT-III	LORT-IV	LORT-V
Practically total drug content	88.56%	88.04%	87.16%	86.60%	89.68%
Drug entrapment efficiency	50.12%	49.87%	47.52%	48.14%	53.21%

Table No.11: Data obtained from Compatibility study of drug and polymer by DSC

S.No	Drug/Polymer/ Formulation	Peaks			
1	Lornoxicam	-----	-----	221.78^{0}C	-----
2	G.M.S	52.97^{0}C (Known)	-----	-----	-----
3	Tripalmitin	72.63^{0}C (Known)	-----	-----	-----
4	Stearic acid	53.17^{0}C (Known)	-----	-----	-----
5	Lecithin	63.21^{0}C	-----	-----	-----
6	LORG	52.97^{0}C (G.M.S)	59.33^{0}C (Lecithin)	213.46^{0}C (Lornoxicam)	-----
7	LORS	53.17^{0}C (Stearic acid)	55.35^{0}C (Lecithin)	68.75^{0}C (Lecithin)	223.91^{0}C (Lornoxicam)
8	LORT	72.52^{0}C (Tripalmitin)	60.33^{0}C (Lecithin)	-----	220.46^{0}C (Lornoxicam)

Table No.12: *In vitro* drug release study of formulated SLNs

S.No	Time (hrs)	% Cumulative drug release														
		LORG I	LORG II	LORG III	LORG IV	LORG V	LORS I	LORS II	LORS III	LORS IV	LORS V	LORT I	LORS II	LORS III	LORS IV	LORS V
1	1	10.99	12.16	11.43	11.87	7.76	12.31	10.55	11.87	13.48	14.21	9.82	9.08	9.38	7.91	8.50
2	2	19.95	21.15	19.05	18.6	16.8	18.45	19.05	19.35	22.35	22.95	17.1	15.15	17.55	14.7	14.4
3	3	26.85	29.1	24.75	22.8	22.8	25.65	25.2	25.05	29.4	29.55	23.7	20.85	24.15	20.7	17.85
4	4	31.35	35.55	31.05	30.6	29.25	32.55	31.8	31.05	35.85	36.3	31.95	24.6	31.05	26.8	21.15
5	5	38.4	40.95	36.9	32.7	35.7	42.45	37.35	36.75	43.35	43.05	39.15	31.65	37.8	33.75	27.3
6	6	44.4	47.55	41.7	41.25	42.45	47.7	40.8	41.4	49.35	48.75	46.35	36.45	42.6	39.9	32.7
7	7	50.4	54.75	47.25	45.6	48.45	52.5	48.9	47.4	55.65	55.35	54.15	40.05	50.25	45.15	35.1
8	8	55.35	60.6	53.1	49.2	54.45	58.8	54.45	53.1	64.65	61.65	60.45	45.15	56.7	50.85	40.65
9	9	61.5	65.7	57.75	57.0	60.45	64.2	58.65	58.2	68.1	68.7	67.05	50.1	63.75	55.5	46.35
10	10	66.9	73.2	63.15	60.45	65.4	69.75	64.8	63.3	73.95	74.4	73.8	55.35	72.9	61.2	50.55
11	11	72.3	78.6	68.55	66.3	72.15	75.45	70.2	70.35	80.55	80.85	81.75	61.65	78.75	66.45	54.3
12	12	78.75	84.45	72.9	74.1	79.2	81.15	75.9	78.6	86.7	87.3	89.1	65.7	86.7	72.75	58.8

Table No.13: Mathematical modeling for nonlinear curve of LORT-I

S.No	Formulation Code	Zero order	First order	Higuchi	Peppa's		Hixon Crowell	Best fitting model
		R^2	R^2	R^2	R^2	n	R^2	
1	LORT 1	0.9991	0.9207	0.9351	0.9986	0.8783	0.9685	Zero order
2	LORT 2	0.9953	0.9841	0.9496	0.9973	0.7987	0.9932	Zero order
3	LORT 3	0.9981	0.9218	0.9304	0.9981	0.8836	0.9649	Zero order
4	LORT 4	0.9972	0.9812	0.9450	0.9997	0.8821	0.9933	Zero order
5	LORT 5	0.9952	0.9892	0.9449	0.9919	0.7863	0.9946	Zero order

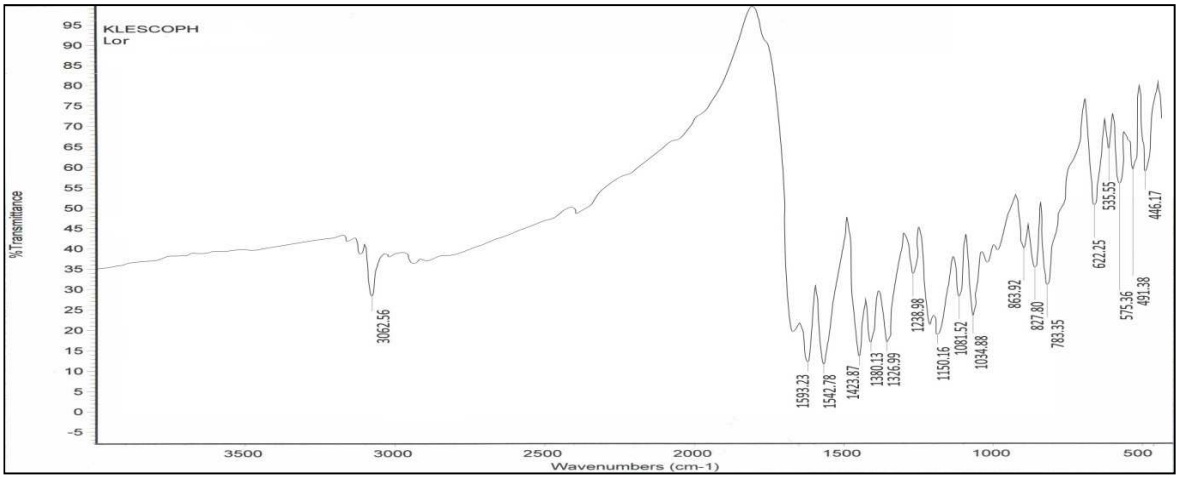

Figure No.1: FTIR spectra of Lornoxicam

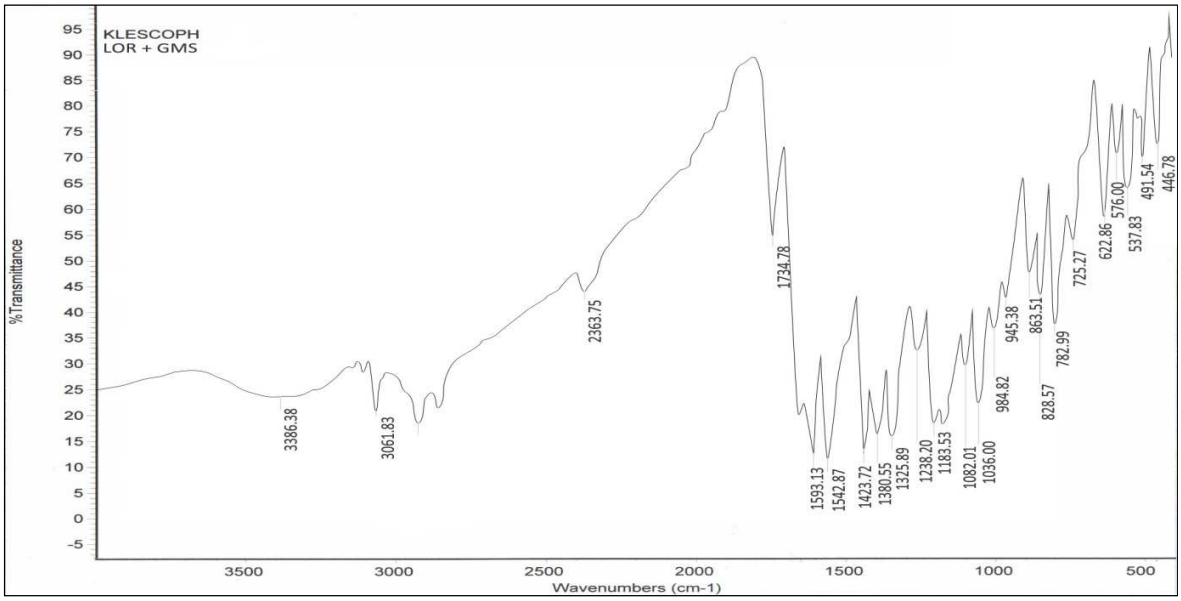

Figure No.2: FTIR spectra of LORG

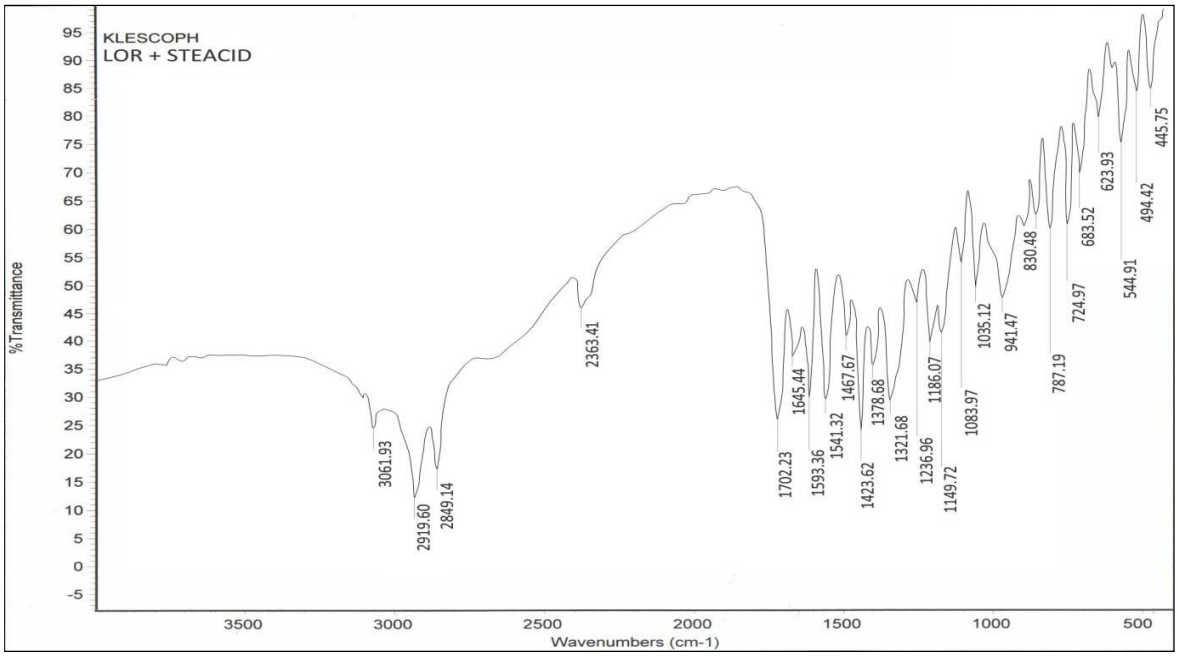

Figure No.3: FTIR spectra of LORS

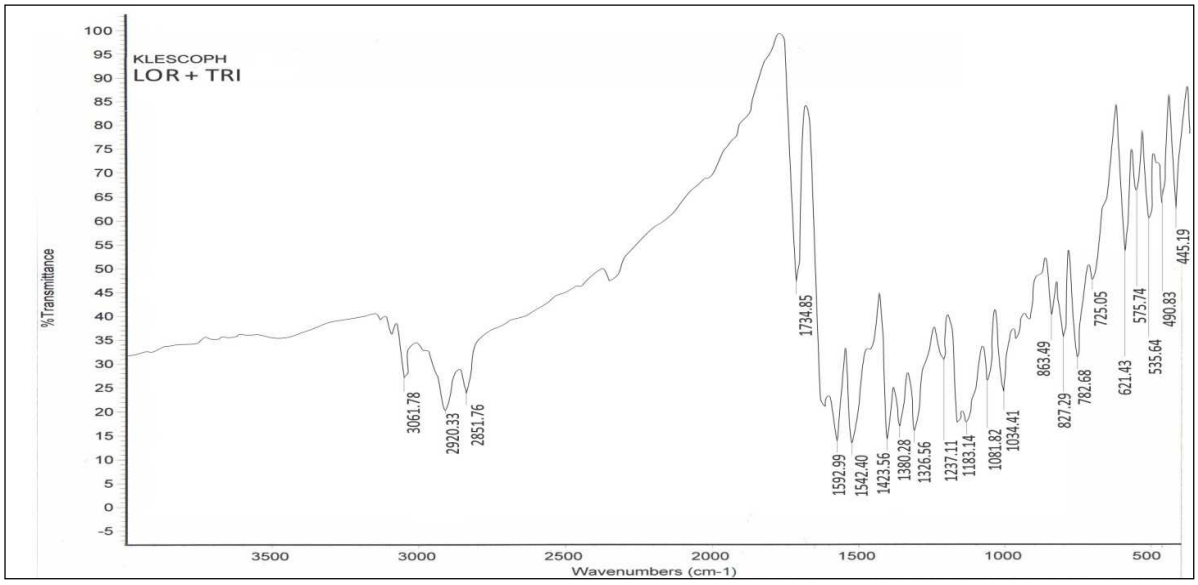

Figure No.4: FTIR spectra of LORT

LORG – 1 **LORS – 1** **LORT– 1**

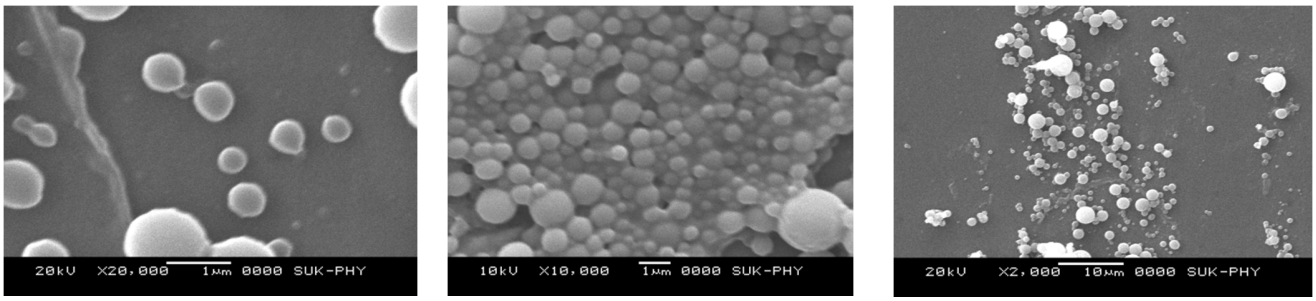

Figure No.5: Scanning Electron Microscopy

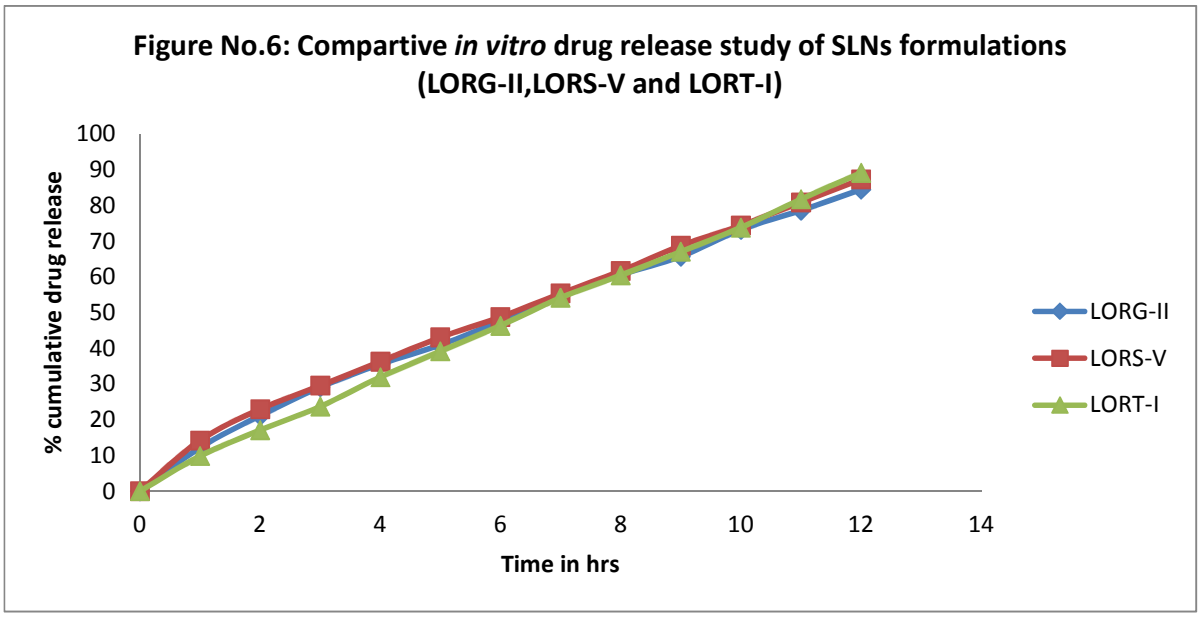

Figure No.6: Compartive *in vitro* drug release study of SLNs formulations (LORG-II, LORS-V and LORT-I)

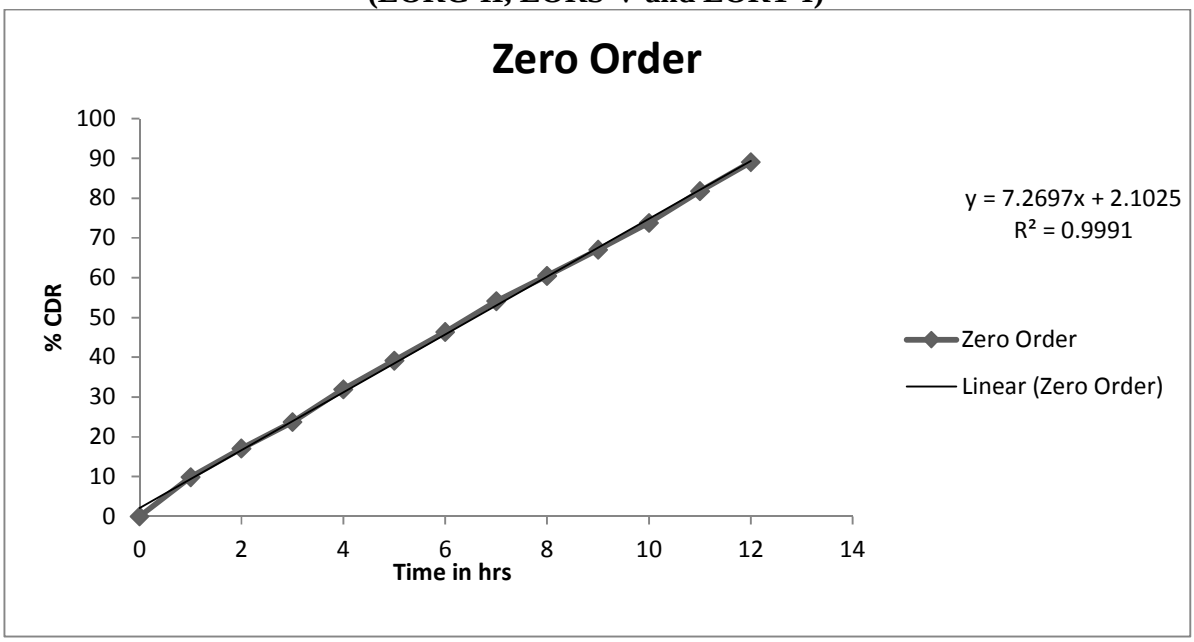

Figure No.7: Zero order plot of LORT-1

CONCLUSION

Solid lipid nanoparticles of lornoxicam were prepared with various colloidal carriers by hot homogenization technique. Tripalmitin had shown controlled release and maximum entrapment than others lipid carriers which can be attributed to the hydrophobic long chain fatty acids of the triglyceride that retain drugs and also increased accommodation of drugs. Thus, from the above studies it can be concluded that the current investigation illustrates the effect of lipid nature on the entrapment efficiency, *in vitro* release of drug.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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