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FORMULATION AND EVALUATION OF SOLID LIPID NANOPARTICLES OF ETORICOXIB BY EMPLOYING GLYCERYL MONOSTEARATE AND GELUCIRE 48/16

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ABSTRACT

The aim of the present study is to formulate and evaluate solid lipid nanoparticles of Etoricoxib. Etoricoxib is a selective COX-2 inhibitor, these are a type of nonsteroidal anti-inflammatory drug (NSAID) that directly targets cycloxygenase-2, COX-2, an enzyme responsible for inflammation and pain. Etoricoxib is a poorly soluble drug. To overcome this problem an attempt was made to prepare Etoricoxib solid lipid nanoparticles which has ability to improve the solubility and enhance oral bioavailability. In the present study Etoricoxib loaded SLNs were prepared by Hot homogenization method. Different formulations of Solid lipid nanoparticles of Etoricoxib were prepared by employing Glyceryl Monostearate as a solid lipid, Soya lecithin as lipophilic surfactant and Gelucire 48/16 as hydrophilic surfactant. The evaluation studies like drug content, entrapment efficiency and drug release studies, Particle size analysis, zeta potential, were performed on the prepared formulations. Among all the prepared formulations E2 formulation was found to show better results.

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INTRODUCTION

Etoricoxib is a COX-2 selective inhibitor whichselectively inhibits isoform 2 of the enzyme cyclooxygenase(COX-2) which reduces the generation of prostaglandins (PGs)from arachidonic acid (Takemoto et al., 2008). It is a potentanalgesic, antipyretic and anti-inflammatory agent has beenapproved for significantly reduces joint inflammation, painintensity and the duration of morning stiffness and improved Handgrip strength (Gonzalez et al.,1994). An arthritic conditiondemands a controlled release drug delivery system for a prolong.

Glyceryl Monostearate is an organic molecule and it is used in food industries as a thickener, a preservative agent, an emulsifying agent for food and also for oils, waxes,and solvent in pharmaceuticals and cosmetics industries. Also used as a plastic lubricant.

Hot homogenization technique was used, the drug was incorporated into the melted lipid. The drug loaded lipidic phase was dispersed in a hot aqueous surfactant solution under continuous stirring to form a coarse o/w emulsion. It was then homogenized at the temperature above the melting point of the lipid using high pressure homogenizer (Panda Plus/GEA Niro Soavi, Parma, Italy) to form o/w nanoemulsion which was cooled to room temperature for solidification and formation of solid lipid nanoparticles.

MATERIALS AND METHODOLOGY:

Materials

Etoricoxib is obtained as gift sample from Aurabindo Pharma Ltd.

Glyceryl Monostearate, Soya Lecithin Obtained from BASF, Gelucire 48/16 was obtained from Gatteffose and Dialysis membrane from Hi media. And all other reagents used were of analytical grade.

Methodology

Solid lipid nanoparticles of Etoricoxib preparation

SLNs were prepared by hot homogenization method. In this method amount of solid lipid (Glyceryl Monostearate) taken in a china dish and melted, to this drug (Etoricoxib) was added and dispersed, then lipophilic surfactant (soya lecithin) was added, this is considered as oil phase. In a beaker 25ml of water and hydrophilic surfactant (gelucire 48/16) taken and kept under homogenizer and stirred at 2700rpm, this is aqueous phase. Oil phase is added to aqueous phase under high pressure homogenization and homogenized for 3 to 4 hrs. After homogenisation the SLNs were subjected to sonication for 30-40 mins. The obtained SLNs were collected and stored.

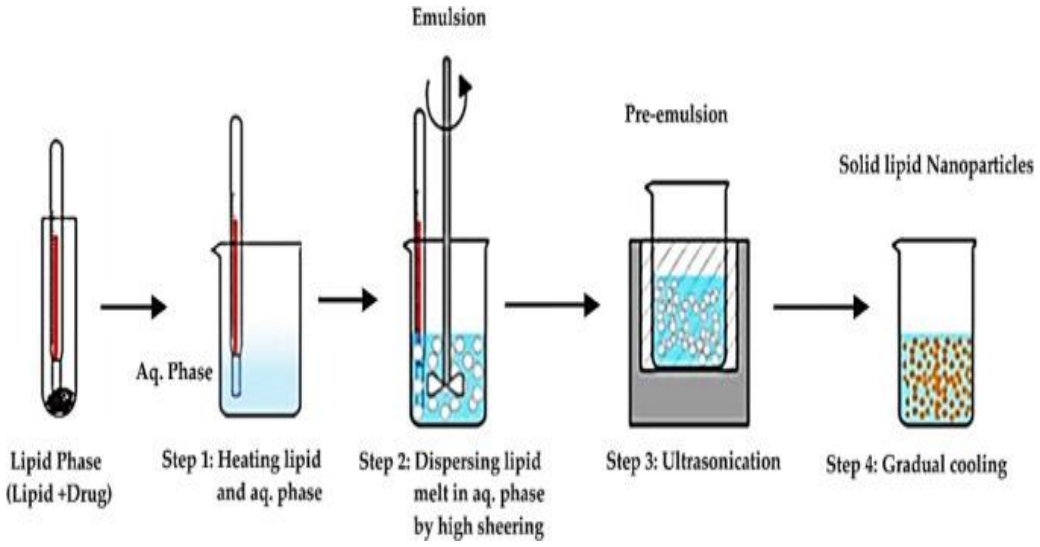

Fig 1: Model Flow Chart of Hot Homogenization Technique.

Table 1: Various solid lipid nanoparticles prepared for Etoricoxib using Gelucire 48/16.

S.No.	Formulation	Ratio Of Hydrophilic : Lipophilic Surfactant
1	E1	1:1
2	E2	1:2
3	E3	1:3
4	E4	2:1
5	E5	2:2
6	E6	3:1
7	E7	3:2

Evaluation of Solid lipid nanoparticles of Etoricoxib:

Drug content

1ml of the prepared Solid lipid nanoparticles of Etoricoxibsuspension was made to 10ml with methanol and was homogenously dispersed. The suitable dilutions were made with phosphate buffer saline of pH 7.4 and the concentration of the drug was analyzed using UV-visible spectrophotometer at 235nm

Entrapment Efficiency

The various formulations of Solid lipid nanoparticles of Etoricoxib was centrifuged at 20000 rpm for 25 min and the obtained supernatant of centrifuged was checked for absorbance by a spectrophotometer (systronics) at 235 nm. The loading efficiency was calculated using the following equation:

The entrapment efficiency was calculated using the following equation:

$$\text{Entrapment efficiency (\%)} = \frac{\textit{Total drug content} - \textit{Drug content in the supernattant}}{\textit{Total drug content}} \times 100$$

Determination of etoricoxib release profile from solid lipid nanoparticles:

The in vitro drug release of Solid lipid nanoparticles of Etoricoxib was determined by dissolution apparatus using USP type-II the SLNs equivqlent to 10mg of drug were taken in the dialysis bag andthen suspended into the dissolution basket containing 900ml of phosphate buffer saline solution of pH 7.4 at the temperature of 37± 5°C, and stirred at a constant speed of 50rpm. Aliquots were collected at the time intervals like 0.5,1,2,3,4,5,6 hours and the same was replaced with the fresh buffer. The drug content was determined spectrophotometrically (systronics) by measuring the absorbance at 235nm using the same buffer solution as the blank, and the amount of drug released from the nanoparticles were calculated.

Measurement of Particle Size

The mean diameter of Solid lipid nanoparticle in the dispersion was determined by Nano Partica Analyzer (Horiba SZ-100 series). Before the measurement one drop of sample was taken from each formulation and diluted in 10ml of dispersion medium (double distilled water). Dynamic Light Scattering (DLS), also known as Photon correlation spectroscopy, is a common technique for measuring the size of particles in sub-micron range

Measurement of Zeta Potential

The Zeta potential is a physical property, exhibited by all particles in the preparation. It is an important factor to be considered in understanding the electric double layer repulsion and it can be measured by phase analysis light scattering. When an electric field is applied across an electrolyte, charged particles in preparation are attracted towards the electrode of opposite charge while vicious force act on the particle tend to oppose the movement. When equilibrium is reached, the particles move with constant velocity, also known as electrophoretic mobility, and the zeta potential can be measured.

Stability Studies

Stability studies were carried out for finalized formulations by storing the formulations at two different temperatures, i.e., in refrigerated conditions and at room temperatures. The drug content was estimated at the end of every month for 3 months to find any changes in the entrapment efficiency and drug release of solid lipid nanoparticles.

Fitting data into various kinetic models

Zero Order Kinetics

A zero order kinetic could be used to predicted by the following equation:

$$\mathbf{At = Ao - Ko\ t \ldots\ldots\ldots\ (1)}$$

Where,

At = Drug release at time‘t’, Ao = Initial Drug Concentration, Ko = Zero order rate constant (hr^{-1}).

When, the data is plotted as cumulative percentage drug release versus time. If the plot is linear then the data obeys zero order Kinetics, with slope equal to Ko.

First order Kinetics:

A First order kinetic would be predicted by the following equation-2:

$$\mathbf{Log\ C = log\ Co\ Kt/2.303 \ldots\ldots\ldots\ldots\ (2)}$$

Where, C = Amount of drug remained at time‘t’, Co = Initial amount of drug, K = First order rate concentration (hr^{-1}).

When, the data is plotted as cumulative percentage drug remaining versus time yields a straight line, indicating that the release follows first order kinetics. The constant can be obtained by multiplying 2.303 with slope values.

Higuchi's Model

Drug release from the matrix devices by diffusion has been described by Higuchi's classic diffusion equation.

$$Q = [D\varepsilon/\tau \; X \; (2A-\varepsilon \; Cs \; t] \; \ldots\ldots\ldots\ldots \; (3)$$

Where,

Q = Amount of drug release at time't', Diffusion coefficient in the matrix , A = Total amount of drug in unit volume of matrix, Cs = the solubility of the drug in the matrix, ε = Porosity of the matrix,

τ = Tortosity, t = time in hrs.

The equation 3 may be simplified, if one assumes that D, ε, τ, Cs, and A are constant. Then the equation (3) becomes;

$$Q = Kt^{1/2} \ldots\ldots\ldots \; (4)$$

When, the data is plotted according to equation 4 i.e. cumulative drug releases versus square root of time yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to 'K'.

Korsemeyer and Peppa's Model

In order to understand the mode of release of drug from swellable matrices, the data were fitted to the following power law equation-5:

$$Mt/M\alpha = Mtn \; \ldots\ldots\ldots\ldots \; (5)$$

Where,

Mt/Mα = the fraction of the drug related time 't', K = Constant incorporating the structural and geometrical characteristics of thedrug/ polymer system. Diffusion exponent related to the mechanism of the release.

The equation 5 can be simplified by applying Log on both sides, we get

$$Log \; Mt/M\alpha = Log \; K + n \; Log \; t \; \ldots\ldots\ldots\ldots \; (6)$$

When the data plotted as log of drug released versus log time, yields a straight line with a slope equal to "n" and the "k" can be obtained from y-intercept.

For non-ficikian release the "n" value falls between 0.5 and 1.0 while for ficikian (Case-1 Diffusion) n = 0.5 and for zero order release (case-2 Transport) n = 1.0.

RESULTS AND DISCUSSIONS

Evaluation Studies of Solid lipid nanoparticles of Etoricoxib:

Drug content: The drug content of all seven formulations was evaluated.

Tab 2: Drug Content of Etoricoxib loaded solid lipid nanoparticles.

Formulation Code	%drug content
E1	43
E2	72
E3	68
E4	50
E5	56
E6	45
E7	62

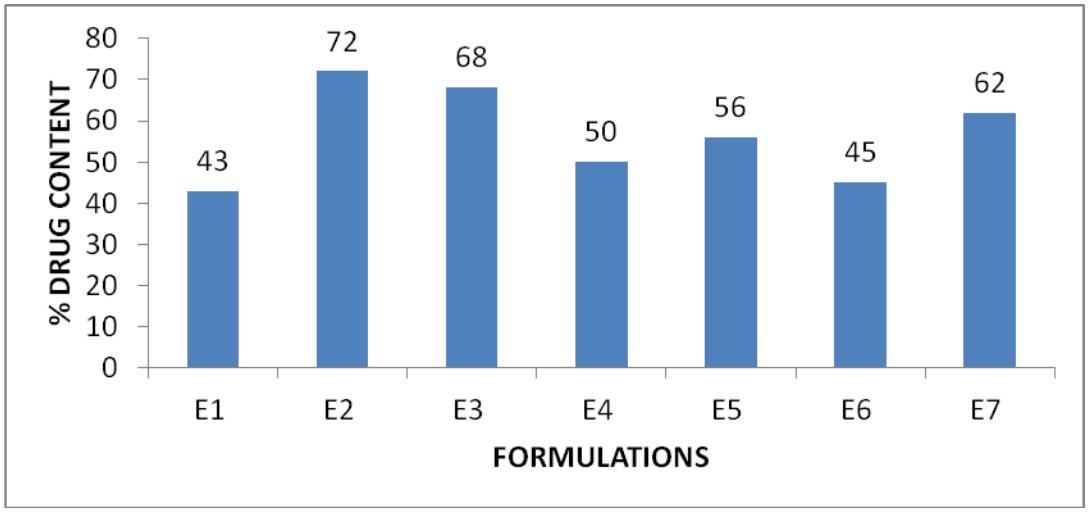

Fig 1: Drug Content of E1, E2, E3, E4, E5, E6, and E7 formulations of Etoricoxib loaded solid lipid nanoparticles.

Comparison of Entrapment Efficiencies of Etoricoxib loaded Solid Lipid Nanopartiocles

All the prepared formulations were evaluated for drug content. The values for all the formulations ranged from 43-72. Among all, E2 formulation has shown highest drug content of 72%

The Entrapment efficiency of Solid lipid nanoparticles of Etoricoxib

After the preparation of Solid lipid nanoparticles of Etoricoxib,this nano formulation were centrifuged and harvested. The amount of drug remaining in the supernatant of the solution was then measured by a spectrophotometer. The encapsulating efficiency was determined.

Tab 3: Entrapment efficiency of Etoricoxib loaded solid lipid nanoparticles.

Formulation Code	Entrapment efficiency
E1(1:1)	40.28
E2(1:2)	65.18
E3(1:3)	46.33
E4(2:1)	60.12
E5(2:2)	48.28
E6(3:1)	52.70
E7(3:2)	58.45

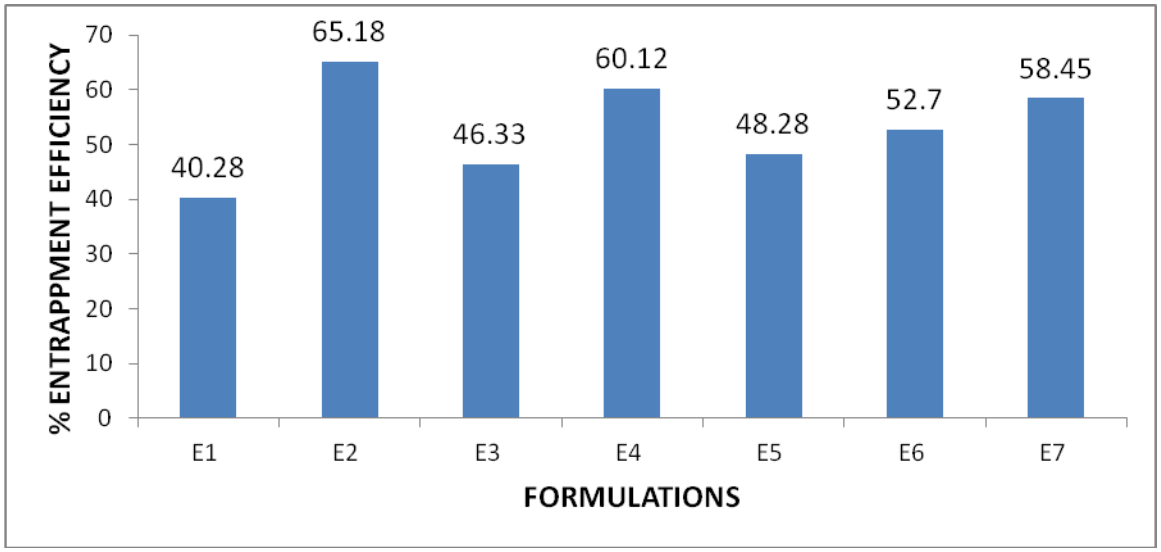

Fig 2: Comparison of entrapment efficiencies of E1, E2, E3, E4, E5, E6, and E7 formulations of Etoricoxib loaded solid lipid nanoparticles.

All the prepared formulations were evaluated for entrapment efficiency. The values for all the formulations ranged from 40-65. Among all, E2 formulation has shown highest drug content of 65.18% .

In vitro drug release studies:

The drug release studies of all formulations of Etoricoxib SLNs were conducted by means of dissolution apparatus for a time period of 6 hrs.

Tab 4: Invitro drug release of Etoricoxib loaded Solid Lipid Nanoparticles with pure drug.

TIME	E1	E2	E3	E4	E5	E6	E7	PURE DRUG
0	0	0	0	0	0	0	0	0
0.5	0.168	4.7	4.72	2.7	1.2	5.17	1.35	1.35
1	0.382	5.6	6.63	2.9	2.2	5.85	2.13	1.91
2	0.461	10.0	7.65	3.3	5.7	6.3	2.81	2.25
3	0.54	15.6	7.85	4.8	8.4	7.08	4.5	4.38
4	0.585	17.6	9.0	7.3	10.2	8.21	6.3	5.40
5	0.63	21.4	10.9	8.7	14.1	11.02	8.21	6.52
6	1.09	22.3	12.8	14.6	15	11.58	10.12	8.21

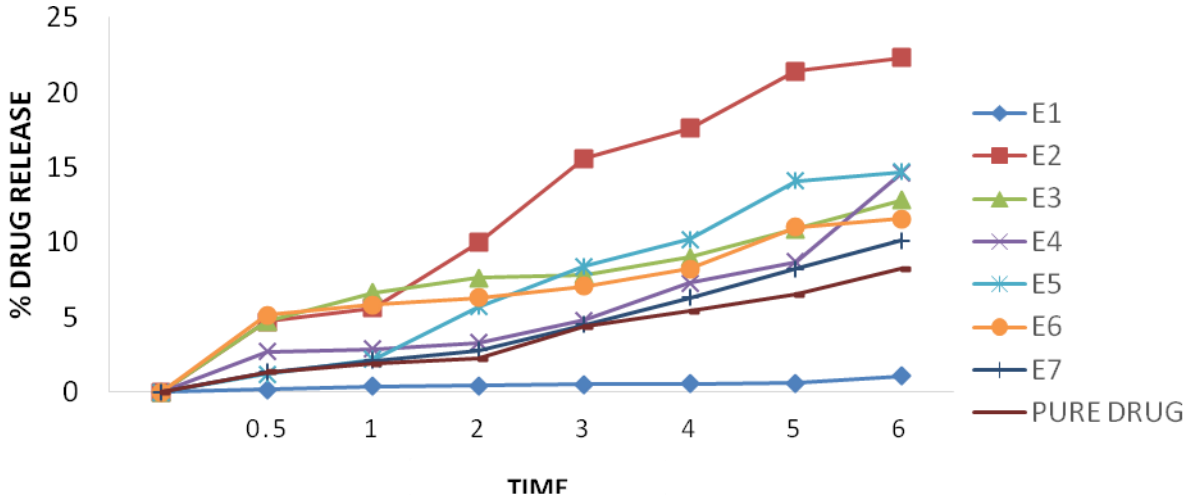

Fig 3: Comparison ofinvitro drug release of E1, E2, E3, E4, E5, E6 and E7 Formulations of Etoricoxib loaded Solid Lipid Nanoparticles with pure drug.

In-vitro drug release:

All the prepared formulations were evaluated for in-vitro drug release. The values for all the formulations ranged from 1-22. Among all, E2 formulation has shown highest drug release of 22.3% . By performing above studies with all seven formulations the E2 formulation had high drug content, entrapment efficiency and invitro drug release, so the formulation E2 is subjected for further evaluation studies.

DETERMINATION OF PARTICLE SIZE

Among the seven prepared formulations, by using Gelucire 48/16 formulation E2 was considered best formulation with the particle size of 127.9nm. Particle size analysis was determined by HORIBA SZ 100 Z nanoparticle analyzer. Thus it was observed that formulation was found to be in nano range.

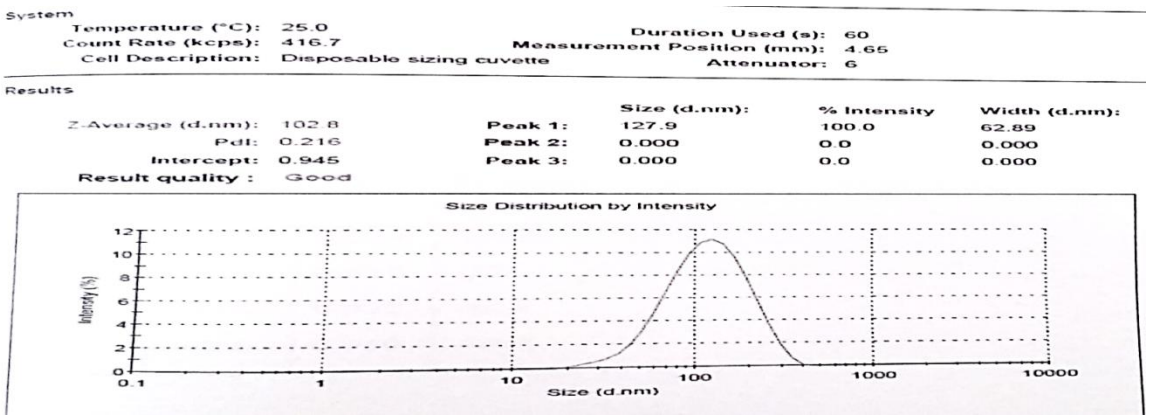

Fig 4: Particle size report of E2 formulation of Etoricoxib loaded SLNs.

DETERMINATION OF ZETA POTENTIAL

The zeta potential value indicates the stability of nanoparticles. It was determined by HORIBA SZ 100 Z nanoparticle analyzer. And the formulation E2 using Gelucire 48/16 was found -31.5Mv.The arbitrary value of zeta potential of nanoparticles is mV. Thus it was found that the formulation was stable.

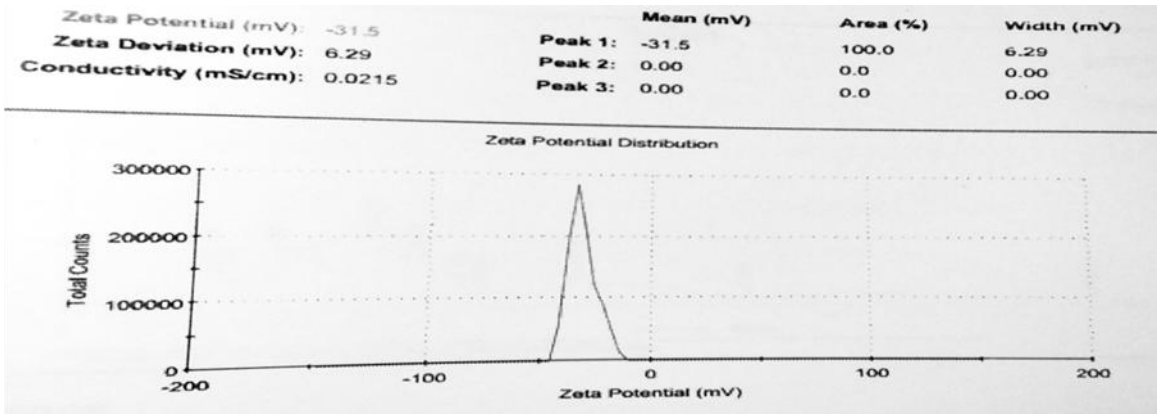

Fig 5: Zeta Potential report of E2 formulation of Etoricoxib loaded SLNs.

Fitting of data into kinetic plots of Etoricoxib loaded solid lipid nanoparticles.

The drug release data was fitted in various kinetic plots (Zero order, first order, Higuchi and peppas) in order to determine the order and mode of drug release.

Tab 5: Correlation coefficient of Etoricoxib loaded Solid Lipid Nanoparticles Gelucire 48/16.

Formulation code	Zero order (R^2)	First order (R^2)	Higuchi (R^2)	Peppas (R^2)
E2	0.992	0.994	0.9616	0.983

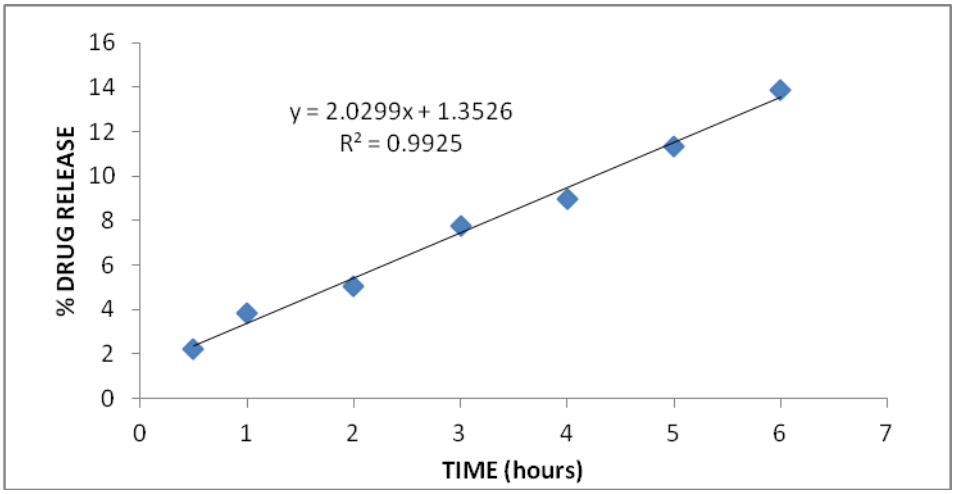

Fig 6: Zero order plot of E2 of Etoricoxib loaded Solid Lipid Nanoparticles.

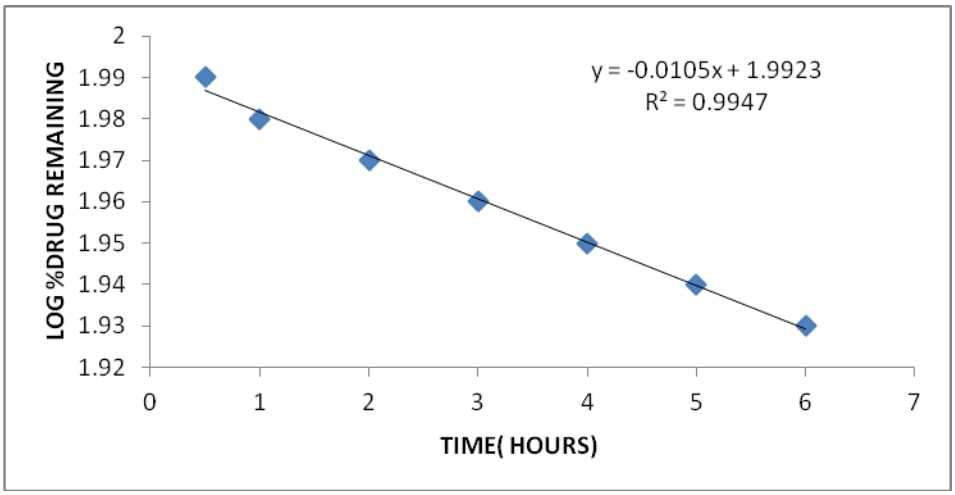

Fig 7: First order plot E2 of Etoricoxib loaded Solid Lipid Nanoparticles.

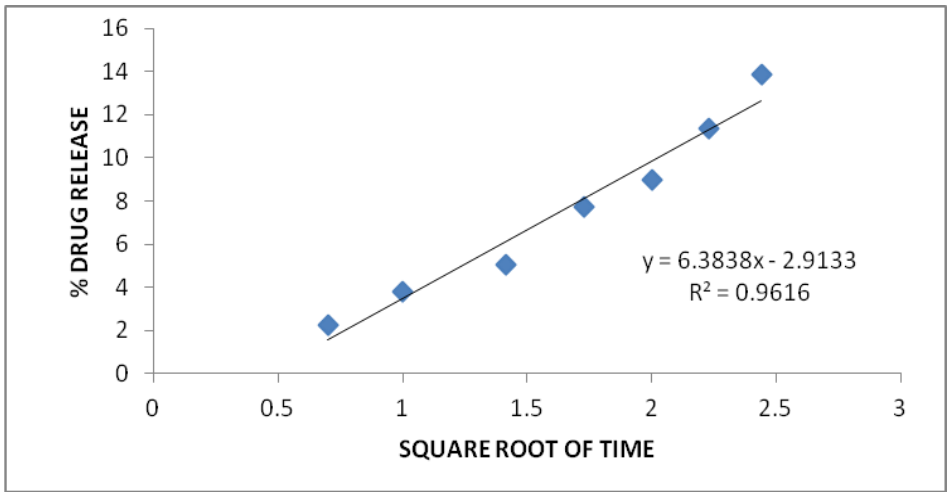

Fig 8: Higuchi plots of E2 of Etoricoxib loaded Solid Lipid Nanoparticles.

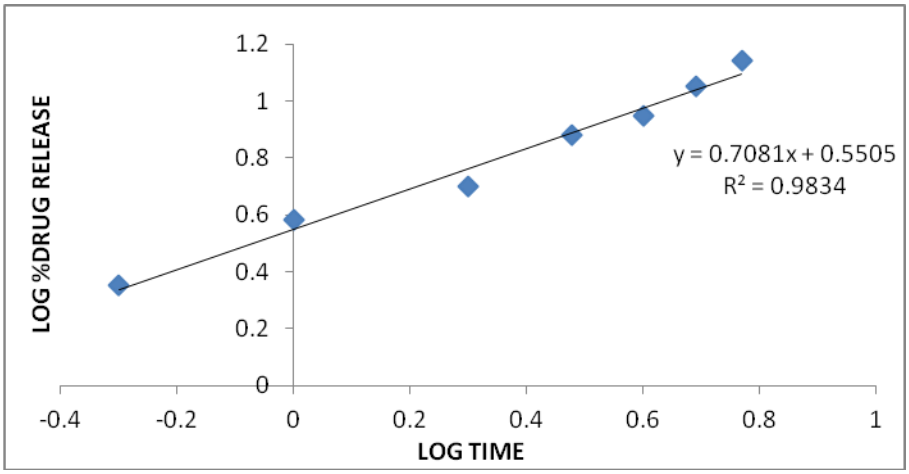

Fig 9: peppas plot of E2 of Etoricoxib loaded Solid Lipid Nanoparticles.

STABILITY STUDIES:

Tab 6: Stability studies for prepared SLNs of Etoricoxib (Entrapment efficiency at refrigerated temperature and room temperature).

Time	Refridgerated Temperature	Room Temperature
After 15 days	65.0 %	62.14%
After 1 month	62.24%	52.4%
After 2 months	55.40%	48.32%
After 3 months	45.32%	41.22%

The results have stated that the entrapment efficiency and drug release of SLNs which were stored in refrigerated condition was more when compared to the SLNs stored at stored at room temperature. This was may be due to more drug expulsion from lipid matrices at higher temperatures.

Tab 7: Stability studies for SLNs (drug release at refrigerated temperature and room temperature) Gelucire 48/16.

Time	Refridgerated Temperature	Room Temperature
After 15 days	22.0 %	19.12%
After 1 month	18.42%	15.0%
After 2 months	15.81%	10.24%
After 3 months	13.21%	7.89%

RESULTS AND DISCUSSIONS

SLNs of etoricoxib by employing GMS were prepared. Seven different formulations by varying the concentration of hydrophilic and lipophilic surfactants. All the prepared formulations were evaluated for drug content, entrapment efficiency, invitro drug release studies. The drug content values ranged from 43 to 72. With highest drug content value of 72% for E2 formulation. The engtrapment efficiency values ranged from 40-65 with highest entrapment efficiency value of 65.18% for E2 formulation. The percentage drug release values for 6 hrs are from 1.09 to 22.3 range. Based on the above results E2 formulations was subjected for particle size and zeta potential analysis. The results of particle size and zeta potential are also sync with the above studies.

The stability studies conducted on the finalizes formulation revealed that the SLNs were stable in refrigerated conditions.

The kinetic models suggested that the drug release from the finalized formulation was follows zero order and the mechanism of drug release is by non-fickian diffusion.

CONCLUSION

Etoricoxib can be successfully prepared in the form of Solid Lipid Nanoparticles by employing GMS, Gelucire 48/16 and soyalecithin. The prepared Solid Li[id Nanoparticles have shown good results in terms of Drug content 72%, Entraoment Efficiency 65.18% and release values 22.3%. The particle size of the solid lipid nanoparticles were in the range of 102.8 nm and zeta potential -31.5 mV which represents that the particles are in the nano range with good stability.

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