FORMULATION AND EVALUATION OF CURCUMIN LOADED SOLID LIPID NANOPARTICLES BY HOT HOMOGENIZATION METHOD BY EMPLOYING GLYCERYL MONOSTEARATE

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ABSTRACT: Curcumin was naturally occurring agent with poor solubility also with a bioavailability of 3%. It is useful in many potential pharmacological effects including anti-inflammatory, antibacterial, antioxidant and anticancer activities. It was also proved against cardiovascular disease, Alzheimer's disease, liver problems, rheumatic arthritis, diabetics, Parkinson's disease and neurological disorders. To enhance its solubility, permeability and bioavailability nanoparticle technology is very much useful.

In the present work attempts were made to prepare the SLNs of curcumin by employing Glyceryl monostearate (GMS) as solid lipid, Tween 80and soya lecithin as hydrophilic and lipophilic surfactants respectively. The five prepared formulations were evaluated for various parameters like entrapment efficiency, In Vitro drug release, particle size, Zeta potential and SEM analysis. Among all the Preparations G3 formulation was best in terms of Drug content of 85.5%, Entrapment efficiency of 72.2%, Drug release of 64.0%, Particle size of 393.1 nm with Zeta potential of -15.1 mV and was also in accordance with particle size in nano range by SEM analysis in Hot Homogenization. The present study conclusively demonstrated that the solubility of drug was improved by entrapment of drug into solid lipid carrier which led to prolongation of drug release.

KEYWORDS: Glycerol Monostearate (GMS), Solid lipid nanoparticles (SLNs), Hot Homogenization Method (HHM).

1. INTRODUCTION

The enhancement of solubility and oral bioavailability of poorly water soluble drugs remain one of the most challenging aspects of drug development. Now a days several novel techniques are been employed to improve these properties by overcoming the disadvantages of traditional ones.[1] A promising strategy to overcome these problems involves the development of suitable drug carrier system like solid lipid nanoparticles. Solid Lipid nanoparticles have ability to overcome the challenges associated with oral delivery of drugs that have low solubility, poor permeability, instability in the GIT and pre-systemic drug metabolism. The major advantages of nanoparticles are improved bioavailability by enhancing aqueous solubility, increasing resistance time in the body (increasing half-life for clearance/increasing specificity for its associated receptors and targeting drug to specific location in the body. Due to their unique size dependent properties, lipid nanoparticles offer the possibility to develop new therapeutics. The ability to incorporate drugs into nanocarriers offers a new prototype in drug delivery that could be site targeting. Therefore, these novel carriers improve the above problems by reaching to its target site without making any adverse effects to body and can carry the drug easily and safely to its destination. Hence, solid lipid nanoparticle hold great promise for reaching the goal of controlled and site specific drug delivery.[2]

Curcumin which has many potential pharmacological effects including anti-inflammatory, antibacterial, antioxidant and anticancer activities. It was also proved against cardiovascular disease, Alzheimer's disease, liver problems, rheumatic arthritis, diabetics, Parkinson's disease and neurological disorders. The oral bioavailability of curcumin is only 3% with the plasma half life of 7 hours.[3] Therefore, there is a need for some novel carriers which could improve the above problems by reaching to its target site without making any adverse effects to body and can carry the drug easily and safely to its destination.

In the current study, the curcumin loaded SLNs were prepared using GMS as lipid and surfactants like soya lecithin and Tween 80 by Hot homogenization method. The prepared SLN were characterized and evaluated.

MATERIALS AND METHODOLOGY

2.1 Materials
Curcumin was purchased from HiMedia laboratories, Glyceryl monostearate (HiMedia, M umbai), Soya lecithin (HiMedia laboratories), Tween 80 (S.D. Fine chemicals) and dialysis membrane (HiMedia, Mumbai). All other reagents used were of analytical grade.

2.2 Preparation of Curcumin loaded solid lipid nanoparticles
Curcumin loaded SLNs were prepared by Hot homogenization method followed by sonication.

Hot Homogenization Method

Hot homogenization method is best suited method for the preparation of solid lipid nanoparticles as it can be performed at elevated temperatures to that of lipids melting point. The reduction in the particle size is due to cavitations and turbulences during homogenization.[9] In hot homogenization technique the drug was dispersed in the lipid(GMS) and Soya lecithin (surfactant) by melting them above 5°C of their melting point. This is considered as oil phase. The aqueous phase was prepared by adding hydrophilic surfactant (Tween 80) in the distilled water and heated to the temperature of oil phase. The prepared oil phase was added to the aqueous phase drop by drop under continuous stirring at 2700 rpm for about 3hrs. The prepared formulation was further sonicated for half an hour and cooled to room temperature. At the room temperature the lipid recrystallizes and leads to formation of SLNs. Formulations prepared by Hot homogenization was coded as G1 to G5. The various formulations are shown in table 1.

Table 1: Composition of Curcumin loaded SLN formulations by hot homogenization.

Formulation Code	Ratio of lipid:lipophilic surfactant:hydrophilic surfactant
G1	1:2:1
G2	1:1:1
G3	1:1:2
G4	1:1:4
G5	1:2:2

In all SLN formulations, curcumin was equivalent to 10mg.

Evaluation of Solid Lipid Nanoparticles

Drug content: 1ml of the prepared curcumin loaded solid lipid nanoparticle suspension 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 430nm.

Entrapment Efficiency

Entrapment efficiency is an important parameter for characterizing solid lipid nanoparticles. This parameter gives us an idea of the drug that was entrapped in SLNs by the carrier. In order to attain optimal entrapment efficiency, the varying concentrations of lipid to lipophilic surfactant to hydrophilic surfactant ratio were used. The entrapment efficiency of prepared SLNs was determined by the centrifugation method. SLNs(containing equivalent to 10mg of drug) was centrifuged at 10000rpm for 40min in high speed research centrifuge to collect supernatant liquid. The collected liquid was filtered to measure amount of free drug concentration after suitable dilution with the fresh phosphate buffer saline of pH 7.4. The absorbance was measured at 430 nm in a UV-visible spectrophotometer to calculate the entrapment efficiency using the formula:

$$E.E= \frac{Amount\ of\ total\ drug\ -\ Amount\ of\ drug\ in\ aqueous\ phase}{Amount\ of\ total\ drug} X100$$

In vitro Drug Release

The in vitro drug release of Curcumin loaded SLNs was determined by dissolution apparatus using USP II. An accurate 1ml of Curcumin SLNs was taken into the dialysis bag and sealed. This sealed dialysis bag was then suspended into the dissolution basket containing 900ml of phosphate buffer saline solution of pH 7.4 at the temperature of 37± 2°C, and stirred at a constant speed of 100rpm. Aliquotes were collected at the time intervals like 0.5,1,2,3,4,5,6,7,8,9,10,11,12 up to 24 hours and the same was replaced with the fresh buffer. The drug content was determined spectrophometrically by measuring the absorbance at 430nm using the same buffer solution as the blank, to calculate the amount of drug released from the nanoparticles.

Measurement of particle size:

The mean diameter of solid lipid nanoparticle in the dispersion was determined by MALVERN Nano Particle analyzer. 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. It measures Brownian motion of particles, suspended in a liquid through the changes in the intensity of light scattered from particles through time. Consequently, the slower the motion the larger the particle will be, since smaller particles are more affected by interactions with the solvent. Considering this motion, and the temperature and viscosity of the sample throughout the analysis, DLS can calculate the hydrodynamic diameter of the particle.

Measurement of zeta potential:

The zeta potential is a physical property, exhibited by all particles in the preparation. It was analysed by MALVERN instrument. 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 viscous forces acting 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. The

magnitude of the zeta potential is large, the particles in preparation will tend to repel each other. Hence, there will be no tendency to agglomerate. Contrastingly, when zeta potential values are low, it means that there will be no force acting to prevent the particles coming together and agglomerate.

MORPHOLOGY OF NANOPARTICLES:

Scanning electron microscopy (SEM) is based on the incidence of a beam of accelerated electrons on the sample. This was analysed by Shimadzu Corporation, Japan.

Kinetic Studies: Mathematical models

Different release kinetic equations (zero-order, first-order, Higuchi's equation and Korsmeyer-peppas equation) were applied to interpret the release rate of the drug from matrix systems for the optimized formulation. The best fit with higher correlation (r^2) was calculated.

Fitting Data Into Kinetic Models

The obtained drug release data was fitted into various kinetic plots for the optimized formulation G3 (zero order, first order, Higuchi and Peppas) in order to determine the order and mode of drug release from the formulated SLNs.

RESUTS AND DISCUSSION

Drug content: The drug content of all the prepared SLN formulations by hot homogenization is shown in Table 2

Table 2: Drug content of curcumin loaded SLNs by hot homogenization

S.NO	FORMULATION CODE	% DRUG CONTENT
1.	G1	69.1%
2.	G2	71.4%
3.	G3	85.5%
4.	G4	68.2%
5.	G5	65.6%
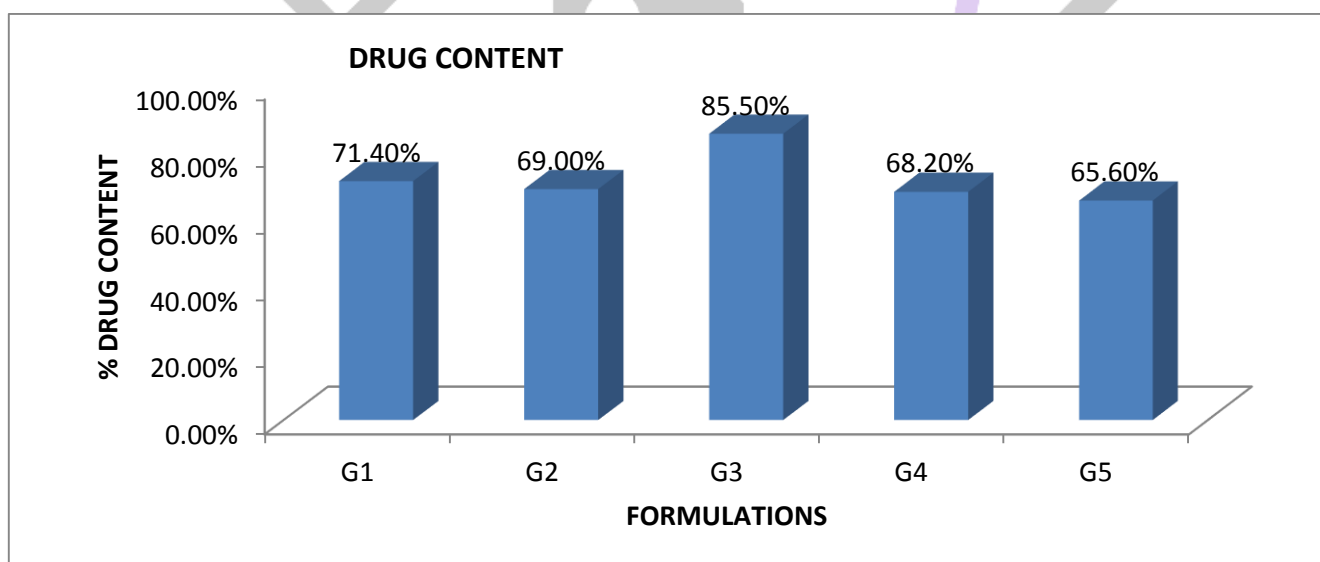
Fig 1: Comparison of drug content of curcumin loaded solid lipid nanoparticles
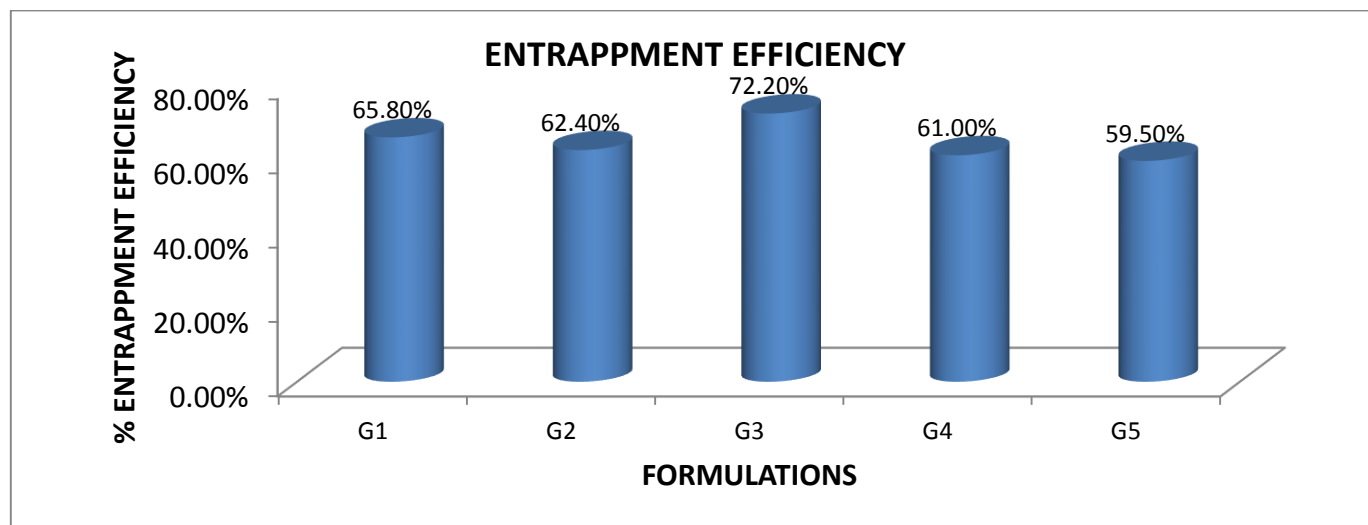
The drug content of all formulations were found to be 71.4%, 69.0%, 85.50%, 68.2% and 65.6%. Among all the formulations, G3 has shown greater drug content of 85.50%. As G3 formulation has the proportion of 1:1:2 for lipid to lipophilic surfactant to hydrophilic surfactant concentration, maximum amount of the drug content was found in this ratio.

Comparison of entrapment efficiencies of curcumin loaded solid lipid nanoparticles.

Entrappment efficiency defines the amount of drug that has been entrapped in the polymer matrix.

Table 3: Entrapment efficiency of Curcumin loaded SLNs by hot homogenization.

S.NO	FORMULATION CODE	% ENTRAPMENT EFFICIENCY
1.	G1	62.4%
2.	G2	65.8%
3.	G3	72.2%
4.	G4	61.0%
5.	G5	59.5%

Fig 2: Comparison of Entrappment efficiencies of curcumin loaded solid lipid nanoparticles

The entrapment efficiencies of all formulations were found to be 65.5%, 62.4%, 72.2%, 61.0% and 59.50%. Among all the formulations, G3 has shown greater entrapment efficiency. As G3 formulation has the proportion of of 1:1:2 for lipid to lipophilic surfactant to hydrophilic surfactant concentration, maximum amount of the drug was entrapped in the lipid. The lipid concentration was found to be sufficient to entrap the drug into it.

Invitro drug release studies of curcumin loaded solid lipid nanoparticles.

Invitro drug release studies were carried by dissolution apparatus using USP II (Paddle). Samples were collected at time intervals like 30min, 1, 2, 4, 6, 8,10, 12, 24 hours. Medium used for dissolution was pH 7.4 phosphate buffered saline, temperature $37\pm2°c$, rpm of 100 at wave length of 430nm.

Time interval (hr)	PERCENTAGE DRUG RELEASE					
	G1	G2	G3	G4	G5	P.D
0	0	0	0	0	0	0
0.5	11.3±0.02	11.6±0.06	6.3±0.02	11.1±0.03	9.2±0.02	0.1±0.08
1	14.8±0.01	12.6±0.01	10.6±0.05	12.1±0.02	10.4±0.01	0.5±0.01
2	15.6±0.06	14.2±0.04	13.8±0.03	15.8±0.05	12.8±0.05	5.3±0.03
4	17.4±0.05	15.2±0.02	22.8±0.01	16.3±0.08	14.3±.02	7.5±0.05
6	21.8±0.03	18.5±0.03	33.9±0.04	25.9±0.01	16.5±0.08	9.6±0.07
8	29.7±0.01	24.7±0.05	42.8±0.06	33.3±0.03	22.8±0.04	13.0±0.06
10	39.1±0.04	33.2±0.02	51.9±0.05	50.3±0.06	30.4±0.02	15.1±0.01
12	49.8±0.02	45.3±0.04	61.8±0.04	57.3±0.04	39.4±0.03	17.8±0.03
24	56.3±0.08	51.1±0.07	67.5±0.03	64.0±0.02	44.4±0.05	33.7±0.04

Table 3: Invitro drug release studies of curcumin loaded solid lipid nanoparticles with pure drug by hot homogenization.

In vitro release studies were performed for a period of 24hrs. The percentage drug release for the prepared formulations was calculated. The drug release of prepared formulations were found to be 56.3%, 51.1%, 67.5%, 64.0% and 44.4% Among all the formulations, G3 was found to be the best formulation as it controlled the drug release upto 24hours with 67.5%.

Fig. 3: Comparative drug release of all formulatios and pure drug
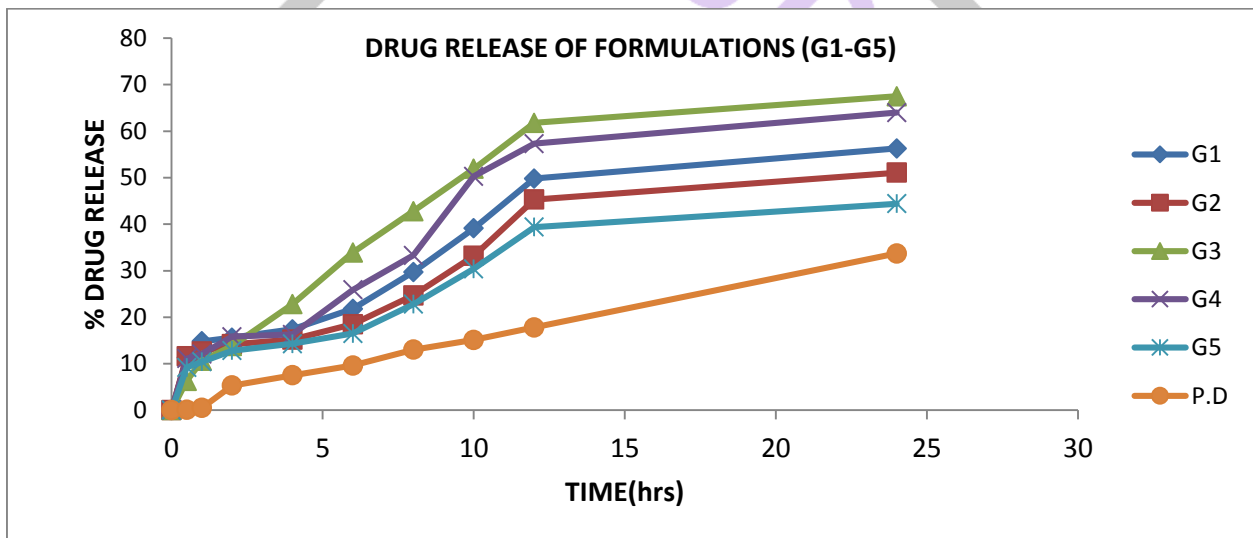
DETERMINATION OF PARTICLE SIZE OF FORMULATION G3

Among the five prepared formulations, the particle size of the G3 was considered as the best formulation with the particles of size of 393.9nm. Particle size analysis was determined by MALVERN nanoparticle analyser. Thus it was observed that formulation was fund to be in nano range.
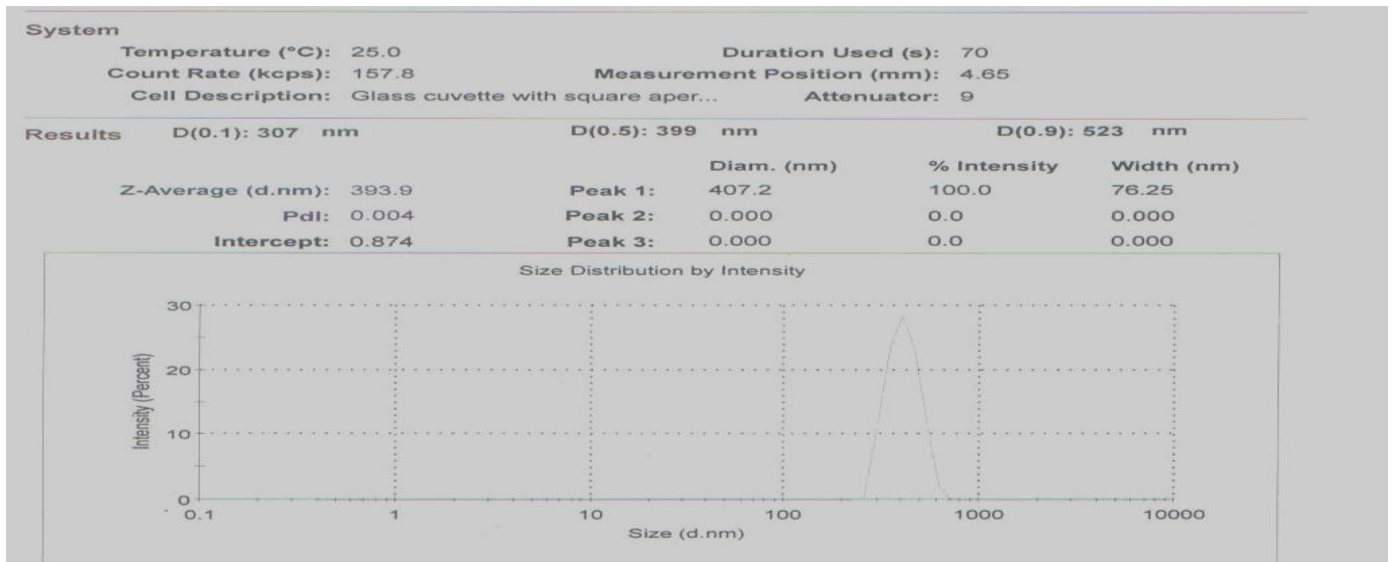
Fig 4: particle size report G3 formulation of curcumin loaded SLN by Hot homogenization method

DETERMINATION OF ZETA POTENTIAL OF FORMULATION G3

The zeta potential value indicates the stability of nanoparticles. It was determined by MALVERN nanoparticle analyzer. The formulation G3 showed the zeta potential value of -15.1mV which shows that the formulation is stable.
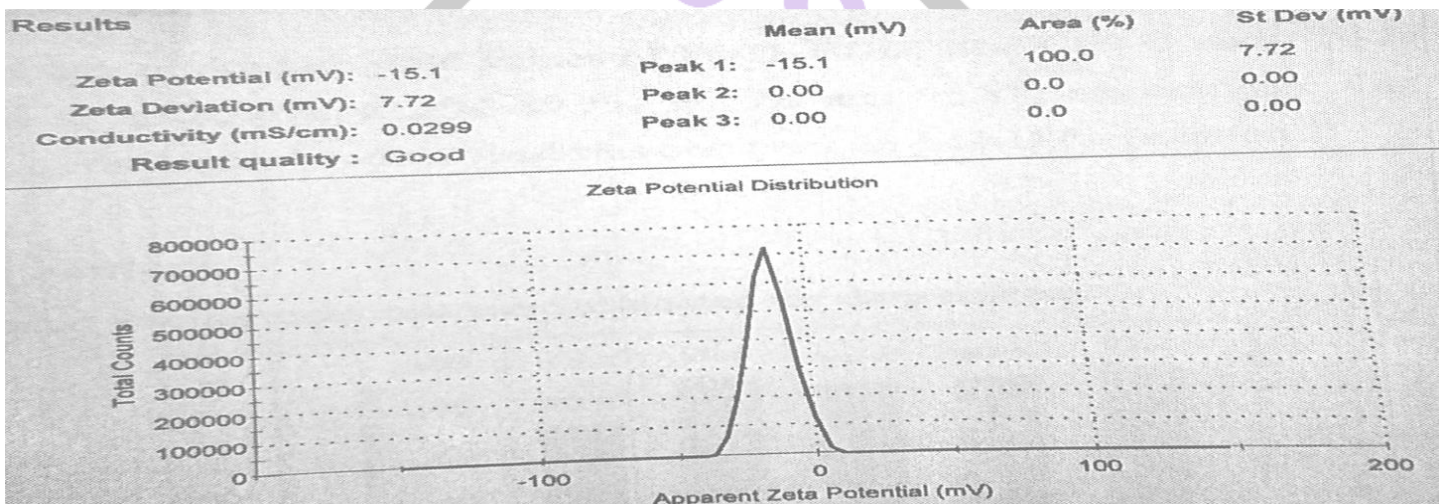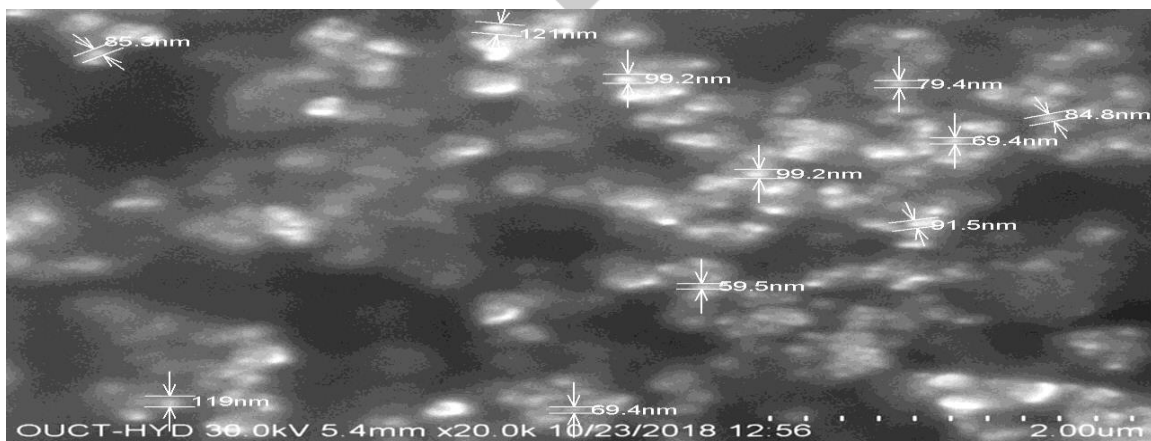

Fig 5: Zeta potential report G3 formulation of curcumin loaded SLN by Hot homogenization method
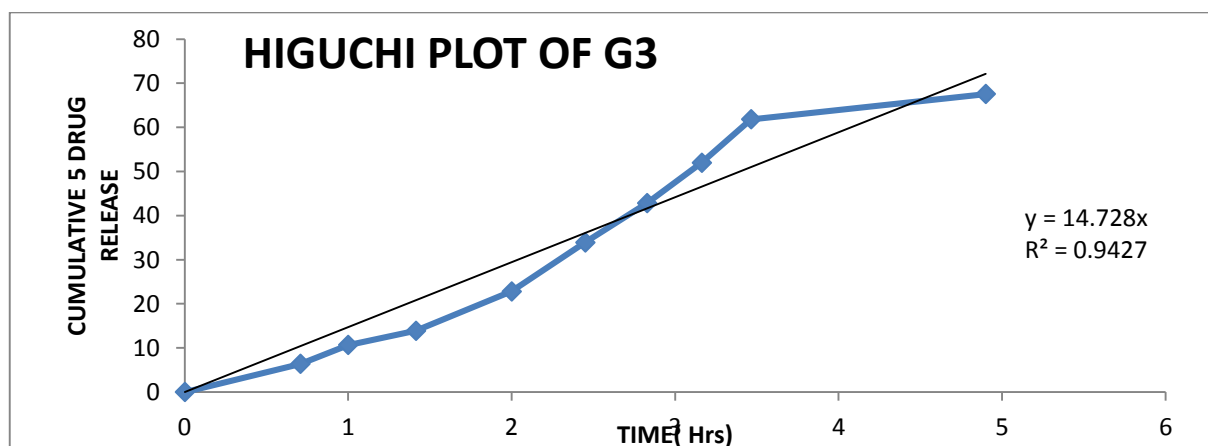
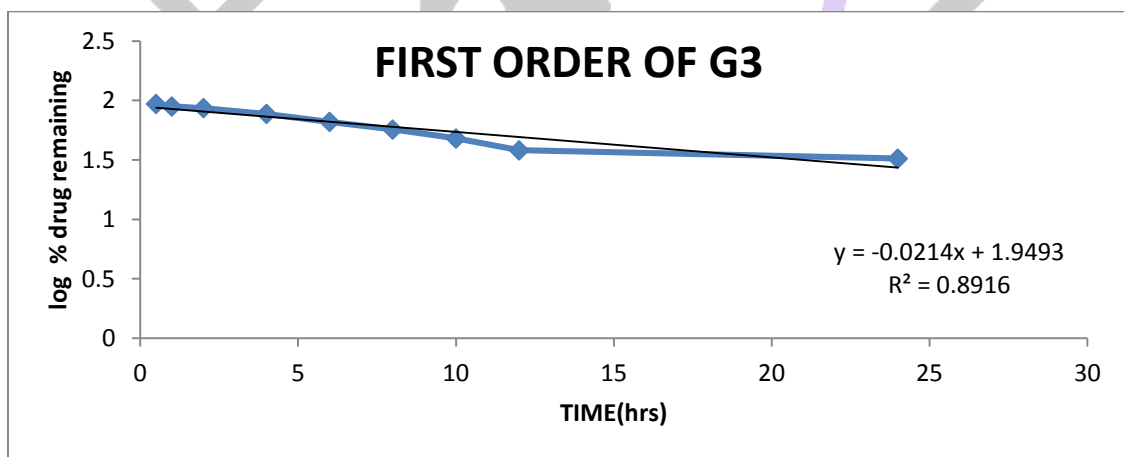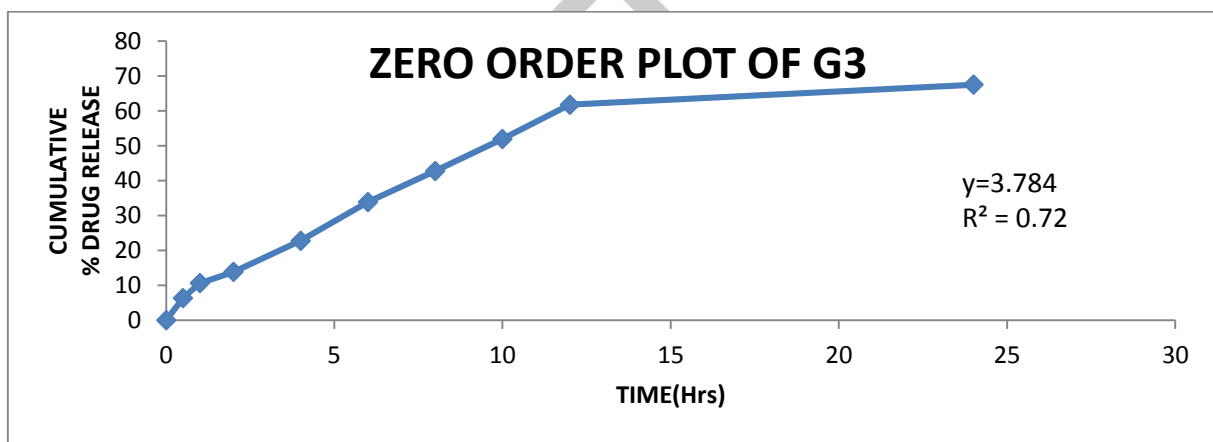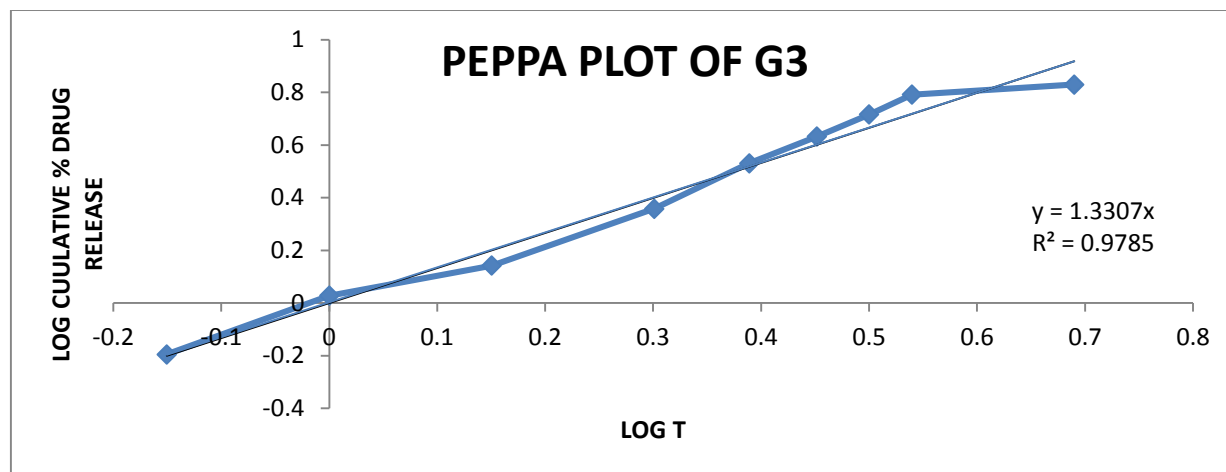## SCANNING ELECTRON MICROSCOPY ANALYSIS OF FORMULATION G3

Fig 6: SEM images of formulation G3

The sample was analysed for SEM to know the surface morphology. SEM results are also in accordance with particle size and zeta potential values.

KINETIC PLOTS OF FORMULATION G3

PEPPA PLOT OF G3

y = 1.3307x
R² = 0.9785

Table No 4 :- Kinetic release data for optimized formulation G3

Formulation	Zero order (R²)	First order(R²)	Higuchi plot(R²)	Peppas plot(n)
G3	0.72	0.891	0.942	1.330

According to the kinetic plots, the optimized formulation -G3 was following the First order release with non-fickian diffusion mechanism and supports super case II transport.

CONCLUSION

In the present research, the different formulations were prepared by using Cutina HR, by employing hot homogenization method. The entrapment efficiency and drug release profile were depended up on the concentration of lipid and surfactant mixture employed. The results of in-vitro drug release studies demonstrated significantly controlled release of Curcumin from prepared SLNs. Among all the Preparations C4 formulation was best in terms of drug content of 82.5%, Entrapment efficiency of 72.2 % and % Drug release of 64% in Hot Homogenization with a particle size of 393.1nm and a zeta potential of -15.1mV. SEM results were also in accordance with particle size and was observed in spherical shape. The drug release data revealed that a good regression was obtained for first order kinetics and Higuchi equation, which indicated that the formulation released drug in sustained release concentration dependent mode and drug release from lipid matrix was Higuchi diffusion. Release exponent, 'n' value of G3 formulation is greater than 0.5 indicating that release followed non fickian diffusion supporting super case II mechanism (r2 value was 0.891, n value was found to be 1.330 for G3 in hot homogenization method).

Hot homogenization was found to be the best method as particle size obtained was small, with high entrapment efficiency value which may be because of better association of surfactant with lipid particles. This method was found to be simple, cost effective, easy and suitable to produce SLNs. This method can be scaled up when compared with other preparations. Further it could be presumed that the obtained nanoparticles might increase oral bioavailability. Hence SLNs can be formulated Successfully by employing this Hot homogenization technique.

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