FORMULATION AND EVALUATION OF CURCUMIN LOADED SOLID LIPID NANOPARTICLES BY EMPLOYING CUTINA® HR AND CARBOPOL 934

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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 Cutina® HR as solid lipid, Carbopol 934 and soya lecithin as hydrophilic and lipophilic surfactants respectively. The five prepared formulations were evaluated for various parameters like drug content, entrapment efficiency, In Vitro drug release, particle size, Zeta potential, SEM analysis and Stability studies. Among all the Preparations C4 formulation was best in terms of Drug content of 83.1%, Entrapment efficiency of 67.2%, Drug release of 66.1%, Particle size of 796 nm with Zeta potential of -29.4 mV and was also in accordance with particle size in nano range by SEM analysis. 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: Solid Lipid Nanoparticles, Curcumin, Cutina® HR.

INTRODUCTION

Oral administration of the drug is most convenient way and commonly used route for the delivery of the drugs because its advantages like ease of administration, high patient compliance, cost effectiveness etc.[1] The major challenge in designing the oral dosage form lies with their poor bioavailability. The oral bioavailability depends on several factors like aqueous solubility, dissolution rate, first pass metabolism, drug permeability, pre systemic metabolism etc. But most often cause of the low oral bioavailability is due to poor solubility and low permeability.Thus to overcome this problem of oral bioavailability the solubility of drug must be enhanced.

There are several techniques to enhance solubility there by bioavailability of the poorly soluble drugs. Novel techniques possess advantages over traditional techniques. Various novel techniques that are used to enhance the solubility are like solid lipid nanoparticles, Self-emulsifying drug delivery systems, solid dispersions etc. Depending upon the problem associated with the drug the technique that is to be employed must be chosen. Here we chose solid lipid nanoparticles for the study of this drug Curcumin.

Curcumin is a natural compound obtained from *Curcuma longa* Linn which has wide therapeutic activities and practically insoluble in water and has poor bioavailability; it is a biopharmaceutics classification system (BCS) class-IV drug [2,3]. This class of drugs shows dissolution rate limited absorption and hence variable bioavailability [4,5]. To overcome these drawbacks, increasing the dissolution rate and aqueous solubility of curcumin is an important goal [6,7]. Possible methods to avoid first pass metabolism include transdermal, buccal, rectal and parenteral route of administration. But the oral route is considered as the natural, convenient and safest route of administration involving higher patient compliance with lesser complications.[8] The conventional preparations like solution, suspension or emulsion for drug delivery purpose has various boundaries like high dose and low availability, faster reach effect etc. The changes in blood plasma drug levels are also exhibited which do not provide sustained effect as well as reaching of drug to target site without any alteration in its physical and chemical properties.

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. Nanoparticles (NP) are a type of colloidal drug delivery system comprising particles with a size range from 10 to 1000 nm (diameter). 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). This is why nanoparticles are increasingly used in variety of applications that includes drug carrier systems and to pass organ barriers such as the blood-brain barrier, cell membrane etc. They are based on biocompatible lipid that provide sustained effect by either diffusion or dissolution.[4,5] Moreover it can be said that nanoparticles are now a day's acting as very prolific device for drug delivery system.

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.[6] Thus to overcome the problems that are associated

with drug Curcumin like low solubility and poor oral bioavailability, the Curcumin loaded solid lipid nanoparticles were prepared which are capable of improving above mentioned properties.

In the current study, the Curcumin loaded SLNs were prepared using cutina® HR as lipid and surfactants like Soya lecithin, Tween 80 and carbopol by hot homogenization followed by sonication. The prepared SLN were characterized and evaluated for various parameters like Drug content, entrapment efficiency and invitro drug release.

MATERIALS AND METHODS

2.1 Materials

Curcumin was purchased from HiMedia laboratories, Cutina® HR (BASF), Soya lecithin (HiMedia laboratories), Carbopol934 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(Cutina HR) 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 (Carbopol 934) 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 C1 to C5. The various formulations are shown in table 1.

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

Formulation Code	Ratio (lipid : lipophilic surfactant: hydrophilic surfactant)
C1	10:1:1
C2	5:1:1
C3	2:1 :1
C4	1:1 :1
C5	1:1:2

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:

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

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 spectrophotometrically 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 C4 (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

FORMULATION CODE	DRUG CONTENT(%)
C1	65.3
C2	69.2
C3	73.5
C4	83.1
C5	72.1

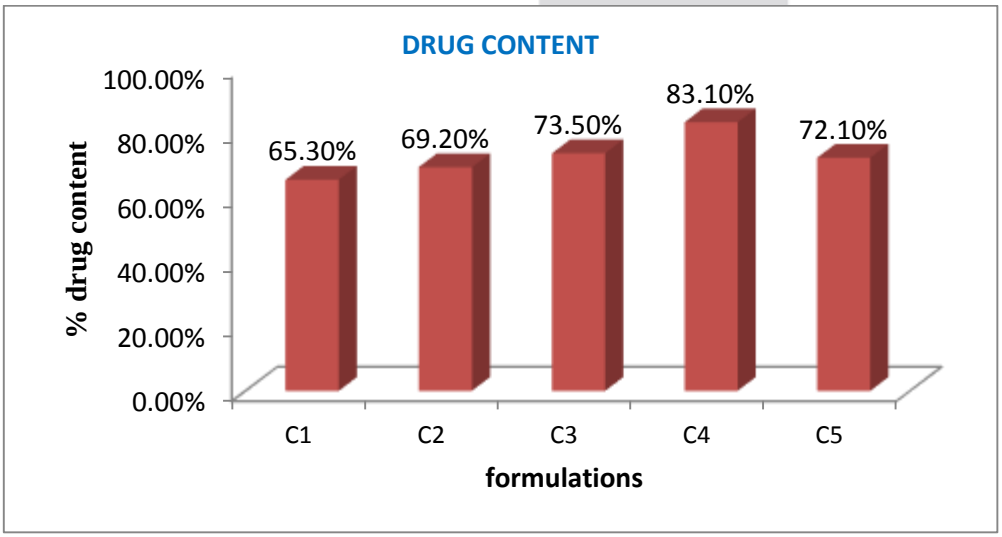


Fig 1: Comparison of drug content of curcumin loaded solid lipid nanoparticles

The drug content of all formulations were found to be 65.30%, 69.20%, 73.50% and 83.10%. Among all the formulations, C4 has shown greater drug content. As C4 formulation has the proportion of 1:1:1 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 SLN's

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

Entrapment Efficiency

The entrapment efficiency of all the prepared SLN formulations by hot homogenization is shown in Table 3. The entrapment efficiency of the prepared SLNs by hot homogenization was found to be in the range of 52.8 % to 67.2 %.

The best entrapment efficiency was found in C4 formulation with 67.2 % among all other formulation in hot homogenization method.

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

FORMULATION CODE	% ENTRAPMENT EFFICIENCY
C1	52.8
C2	58.6
C3	62.1
C4	67.2
C5	65.3

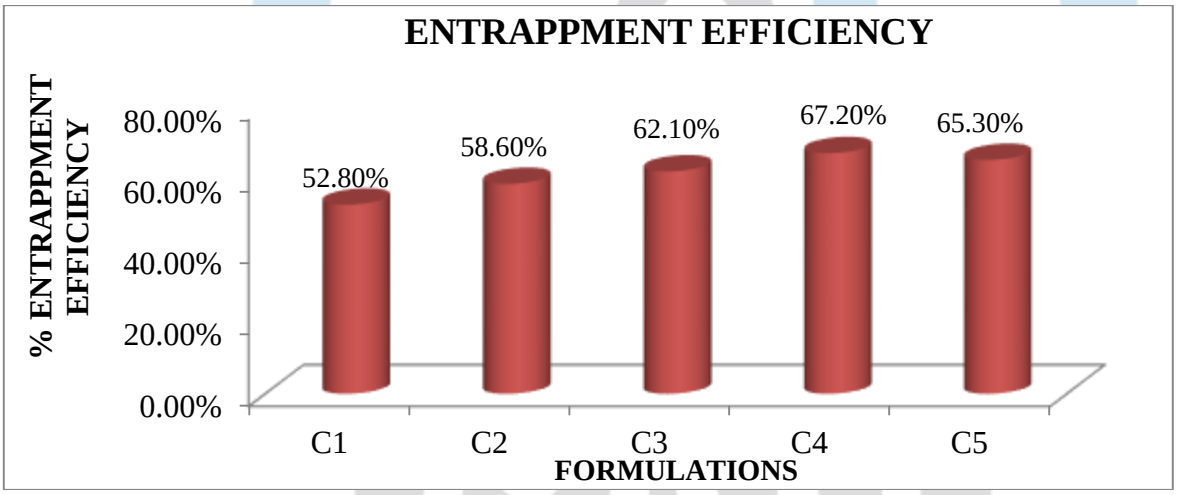


Fig 2: Comparison of Entrappment efficiencies of curcumin loaded solid lipid nanoparticles

The entrapment efficiencies of all formulations were found to be 52.8%, 58.65%, 62.10%, 67.20% and 65.30%. Among all the formulations, C4 has shown greater entrapment efficiency. As C4 formulation has the proportion of of 1:1:1 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±2ºc, rpm of 100 at wave length of 430nm.

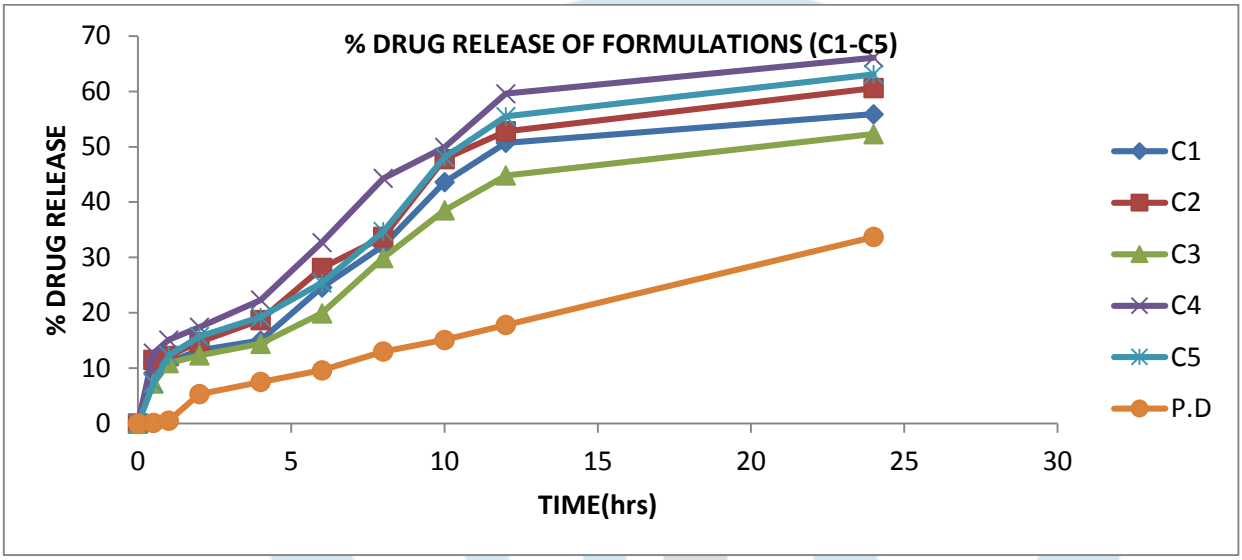
Table 3: Invitro drug release studies of curcumin loaded solid lipid nanoparticles with pure drug by hot homogenization.

Time interval (hr)	C1	C2	C3	**C4**	C5	P.D
0	0	0	0	0	0	0
0.5	9.1 ±0.02	11.5±0.04	7.2±0.06	12.7±0.02	7.3±0.07	0.1±0.08
1	11.1±0.07	12.2±0.09	10.9±0.08	15.1±0.04	12.3±0.03	0.5±0.01
2	13.2±0.09	14.7±0.04	12.3±0.07	17.4±0.06	15.7±0.02	5.3±0.03
4	15.0±0.04	18.7±0.02	14.4±0.03	22.3±0.03	19.2±0.06	7.5±0.05

6	24.6±0.01	28.1±0.01	19.9±0.09	32.7±0.01	25.4±0.03	9.6±0.07
8	32.1±0.02	33.7±0.03	29.9±0.04	44.3±0.05	34.7±0.04	13.0±0.06
10	43.6±0.05	47.8±0.07	38.5±0.02	49.9±0.07	48.1±0.01	15.1±0.01
12	50.7±0.08	52.8±0.06	44.8±0.06	59.6±0.02	55.5±0.01	17.8±0.03
24	55.9±0.03	60.6±0.01	52.3±0.02	66.1±0.05	63.1±0.08	33.7±0.04

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 55.9%, 60.6%, 52.3%, 66.1% and 63.1%. Among all the formulations, C4 was found to be the best formulation as it controlled the drug release upto 24hours with 66.1%.

Fig. 3: Comparative drug release of all formulatios and pure drug



CHARACTERISATION OF OPTIMISED FORMULATIONS FOR INSTRUMENTAL ANALYSIS

Among all the prepared formulations, the optimised formulation was found to be C4, formulation as it has shown better results for drug content, entrapppment efficiency and percentage drug release than other formulations.

It was further analysed by instrumental methods including particle size determination, zeta potential, Scanning electron microscope. These were further applied to the kinetic models.

PARTICLE SIZE DETERMINATION OF FORMULATION C4

Among the five prepared formulations, the particle size of the C4 was considered as the best formulation with the particles of size of 796.0 nm. Particle size analysis was determined by MALVERN nanoparticle analyser. Thus it was observed that formulation was found to be in nano range.

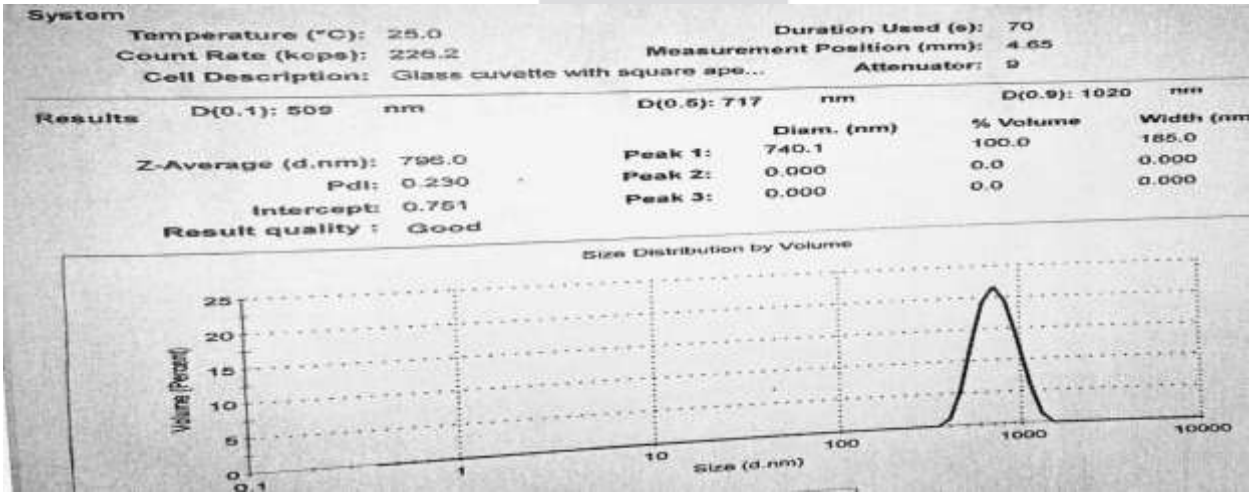


Fig 4: particle size report C4 formulation of curcumin loaded SLN by Hot homogenization method

DETERMINATION OF ZETA POTENTIAL OF FORMULATION C4

The zeta potential value indicates the stability of nanoparticles. It was determined by MALVERN nanoparticle analyzer. The formulation C4 has shown a zeta potential value of -29.4 mV which shows that the formulation is stable.

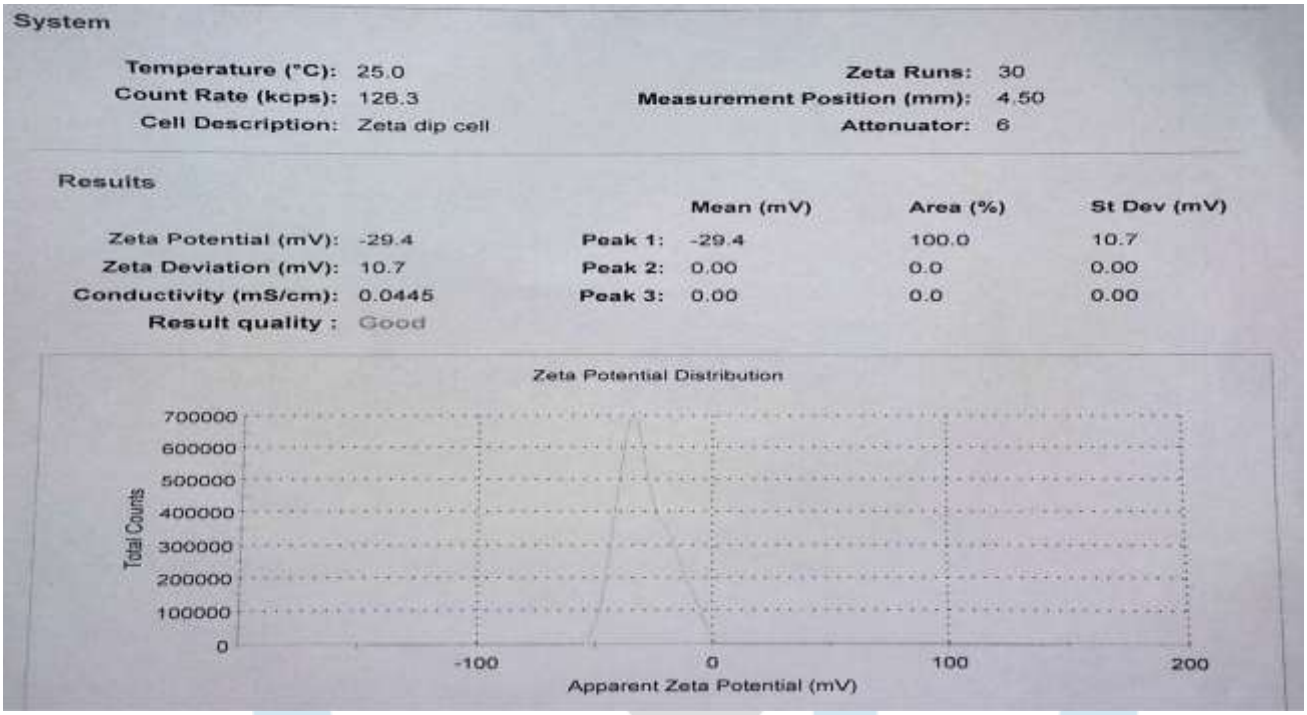
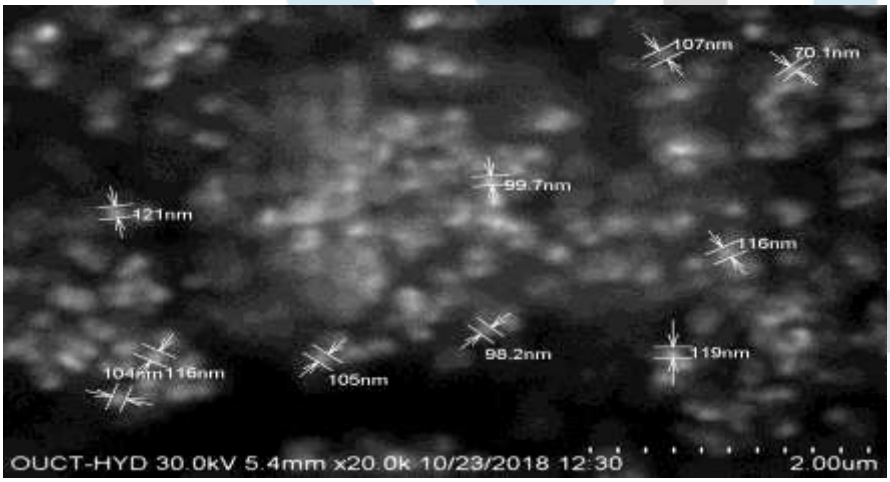


Fig 5 : Zeta potential report for C4 formulation of curcumin loaded SLN

SCANNING ELECTRON MICROSCOPEY(SEM) ANALYSIS OF FORMULATION C4



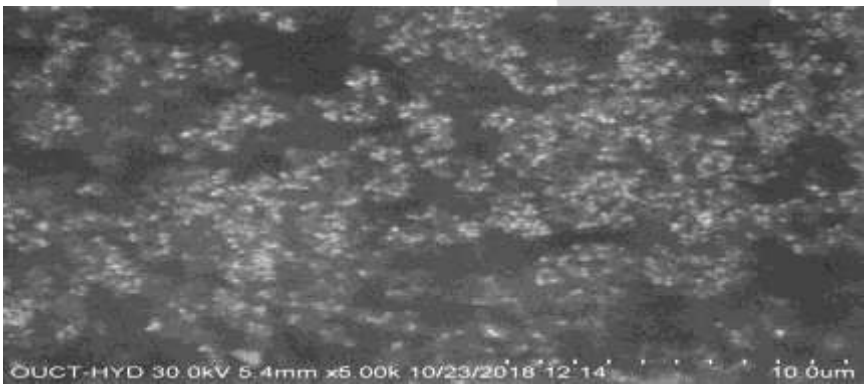


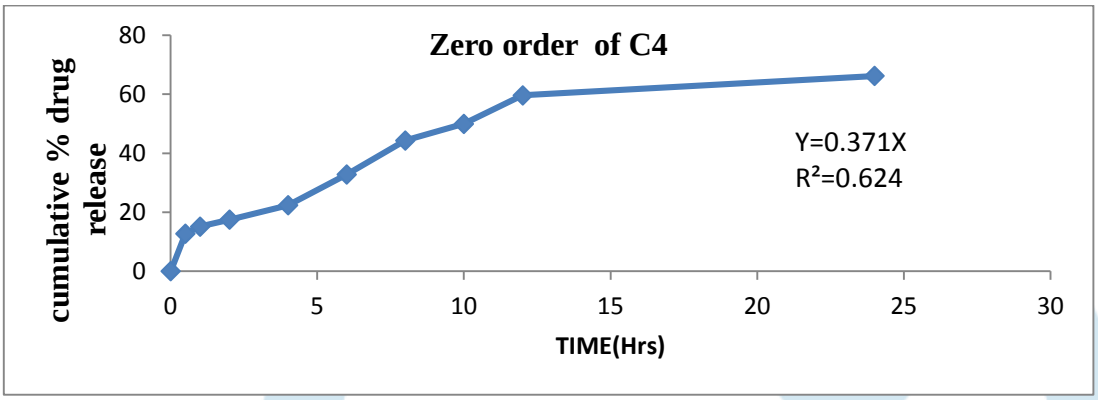
Fig 6: SEM images of formulation C4

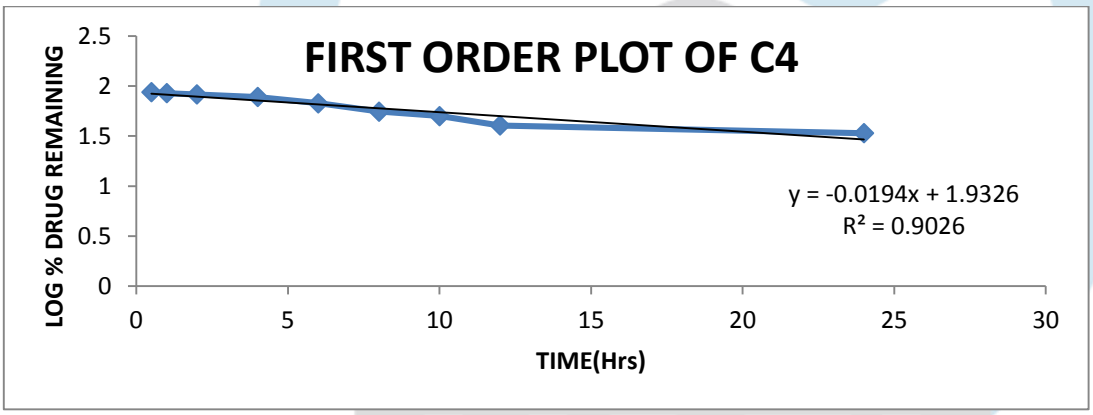
The sample was analysed for SEM to know the surface morphology. SEM results are also in accordance with particle size and was observed in spherical shape.

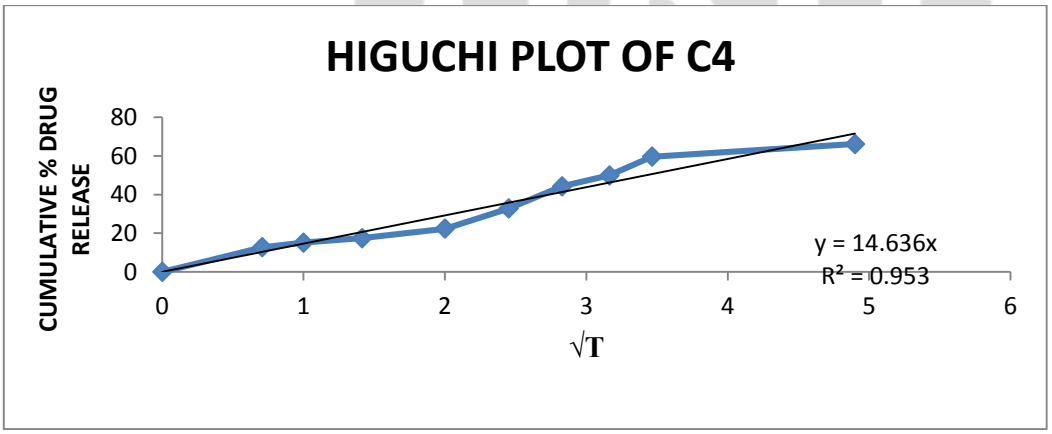
FITTING DATA INTO KINETIC PLOTS OF CURCUMIN LOADED NANOPARTICLES FOR OPTIMISED FORMULATION

The drug release data was fitted in various kinetic plots (Zero order, First order, Higuchi and Korsmeyer Peppas plots) which were drawn for the optimised formulation C4 in order to determine the order and mode of drug release.

KINETIC PLOTS OF FORMULATION C4







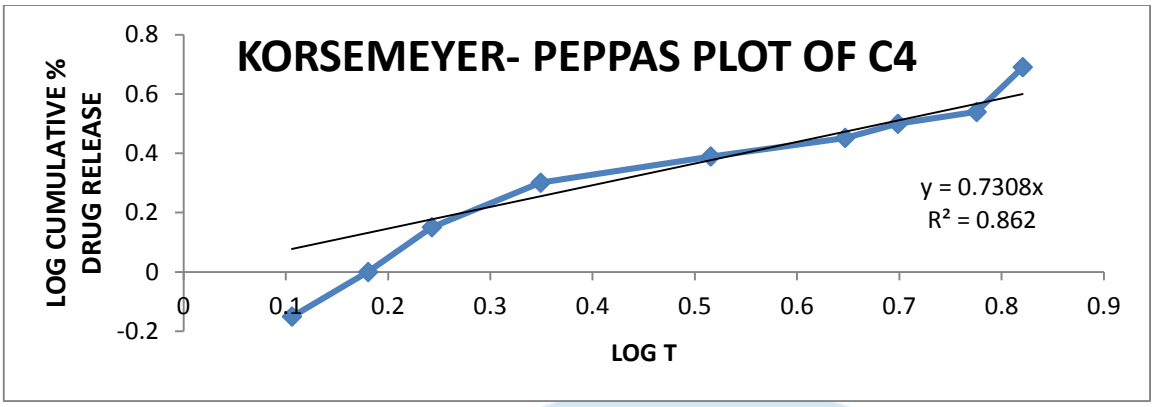


Table No 4:- Kinetic release data for optimized formulation C4

Formulation	Zero order (R^2)	First order(R^2)	Higuchi plot(R^2)	Peppas plot(n)
C4	0.624	0.902	0.953	0.730

According to the kinetic plots, the optimized formulation -C4 was following the First order release with non-fickian diffusion mechanism.

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 83.1%, Entrapment efficiency of 67.2 % and % Drug release of 66.1% in Hot Homogenization with a particle size of 796nm and a zeta potential of -29.4mV. 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 C4 formulation is greater than 0.5 indicating that release followed non fickian diffusion. (r2 value was 0.902, n value was found to be 0.730 for C4 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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