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Formulation and evaluation of curcumin embedded casein nanoparticle by micellar technique

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Abstract
The aim of this work is to develop and evaluated curcumin embedded casein Nanoparticle. The incorporation of curcumin inside a nanoglobules to improve curcumin stability and permeability. A nanoparticle was prepared by micells technique. using an casein, tween80 and NAOH. Evaluation of the nanoparticle included analysis of particlesize, Zeta potential, free drug content, Drug loading, % encapsulation, Invitro release studies, and stability study. Particle size range of 385.9-90.96. and 617.7 - 116.6 of BY and CY formulation respectively. Zeta potential was and it was found to be -2.6 mV and -2.9 mV of BY and CY formulation respectively. The percent of drug loading range 0.08 to 4.0 mg. The percentage entrapment efficiency was found to be 2.6% to 46.5%. Curcumin in a nanoparticle was more stable than free curcumin. The *invitro* release kinetic of curcumin shows maximum release 96.05
±0.98 in CY formulation and changed zero order to a Higuchi release profile. Overall, the developed nanoparticle system improved curcumin permeability but also protected the curcumin from chemical degradation.

Keywords: curcumin, nanoparticle, tween 80, NAOH

Introduction
Nanoparticles are defined as solid particles with the size in the range of 10- 1000nm. Last few decades have withered tremendous research fascination in particulate delivery systems as carriers for small and large drug molecules exploited. Particulate systems like nanoparticlulate have been used as a physical approach to alter and improve the pharmacokinetic and pharmacodynamics properties of various types of drug molecules. Polymeric nanoparticles have been extensively studied as particulate carriers in the pharmaceutical and medical fields, because they show promise as drug delivery systems as a result of their controlled and sustained release properties, subcellular size, biocompatibility with tissue and cells, biodegradability and green approach [1]. Nanoparticle is constituted nanospheres and nanocapsules. Nanospheres possess a matrix type of structure. In which drugs is absorbed the sphere surface or encapsulated within the particle. Nanocapsules are vesicular systems where in the drug is embedded to a cavity consisting of an inner liquid core enveloped by a polymeric membrane [2].

Production of Nanoparticles
Although any particle of a size <1µm diameter is a nanoparticle, several national initiatives are encouraging the development of particles <100 nm as they might exhibit some unique physical properties, and hence potentially different and useful biological properties. However, achieving sizes <100nm is more readily feasible with hard materials compared to drug and polymer molecules, which are soft materials. For hard materials, such as silica, metal oxides, and diamonds with melting points above 1000 °C, nanoparticles in the 1–100nm size range have been prepared. However, for drugs that are usually soft materials with melting point below 300°C particles in the 1-100nm size range are more difficult to prepare. For this reason, it is a reasonable goal to aim at <300nm particles for drug and polymer materials. There are several success stories for pharmaceutical materials in this size range. Production of nanoparticles of soft materials is much more challenging than that of hard materials because of the high stickiness of the former. The bulk pharmaceuticals are available in solids of large sizes (e.g., 1-mm-diameter powder), which can be often easily solubilized in solvent to obtain molecular size. Hence, there are two extremes of sizes: molecular size and large size [3]

The technologies to formulate drug nanocrystals are
1. Bottom Up and.
2. Top Down technologies.

The bottom up technologies starts from the molecules which are dissolved and precipitate them by adding the solvent to a non-solvent. The top down technologies are a disintegration method that means various types of wet milling. Examples for precipitation techniques are the hydrosols developed by Sucker (company Novartis), the product Nanomorph by the company Abbott and a number of other precipitation techniques differing in precipitation details such as use of certain stabilizers4, 5, 6.
Scaling up is relatively easy possible by using static blenders. High pressure homogenisation with different homogenizer types/ principles
The second most frequently used disintegration method is milling by high pressure homogenisation. The two homogeniser types applied are:

a. Microfluidisation is a jet stream principle, the suspension is accelerated and passes with a high velocity in an specially designed homogenisation chamber. In the Z type chamber, the suspension changes a few times the direction of its flow leading to particle collision and shear forces. In the second type of chamber, Y- type, the suspension stream is divided into

two streams which then collide frontally.

b. Piston-gap homogeniser In the knowledge of the potential problems associated with pearl/ball milling and the use of the microfluidisation principle, as an alternative a drug nanocrystal technology based on piston-gap homogenizers was developed in the middle of the 1990s. Based on principle the piston gap homogenizer developed into following techniques:

1. Based on homogenisation of particles in pure water
2. Homogenisation of drug particles in non-aqueous media.

Precipitation is the traditional approach to produce Nano sized drug material, but having the problem of potential growth of drug nanocrystals to drug microcrystals. The company Baxter introduced a combination technology called NANOEDGE. Precipitation is followed by a second high energy step, typical high pressure homogenisation. Table 1.2 gives an overview on which the various homogenisation processes are based [9].

c. High pressure homogenisation (piston-gap) with different principles for drug nanosuspension production [10, 11]. Homogenisation in water (Disso Cubes)

Many years cavitation was considered as the most important effect to small particles in a piston-gap homogeniser. In this homogeniser types, the dispersion (emulsion or suspension) passes a very thin gap with an extremely high velocity. Prior to entering the gap, the suspension is contained in a cylinder with a relatively large diameter compared to the width of the following gap. In the APV LAB 40, the diameter of the cylinder is about 3 cm, it narrows to about roughly 25 mm (varies with applied pressure) when the suspension enters this homogenization region.

Drug and Polymer

Curcumin

Curcumin is history goes back over 5000 years, to the heyday of Ayurveda (which means the science of long life). Turmeric derived from the rhizome of the plant Curcuma longa has been used by the people of the Indian subcontinent for centuries with no known side effects, not only as a component of food but also to treat a wide variety of ailments [7]. Extensive research within the last half a decade has revealed that curcumin has potential against a wide variety of diseases, both malignant and non-malignant. The potential of curcumin, however, has not been systematically examined through the modern multicenter, randomized, double- blind, placebo-controlled clinical trial. Its potential in humans is indicated either through preclinical studies, some pilot studies in humans, anecdotal studies in patients, or epidemiological studies. Curcumin has been shown to exhibit activity against numerous inflammatory diseases, includ-ing pancreatitis, arthritis, inflammatory bowel disease (IBD), colitis, gastrititis, allergy, and fever possibly through the down regulation of inflammatory markers, as indicated earlier. The effect of curcumin against various autoimmune diseases has also been demonstrated; they include scleroderma, psoriasis, multiple sclerosis, and diabetes. Again, these effects of curcumin are through the regulation of pro-inflammatory signaling. Although once thought to be distinct, the molecular targets for both the prevention and therapy of cancer are now considered the same. Numerous lines of evidence suggest the potential of

Casein

Casein is a protein that is found inmilk and used independently in many foods as a binding agent. Techni cally, it is part of a group called phosphoproteins, collections of proteins bound to something containing phosphoric acid. Casein may also be called caseinogen, particularly in European foods. Casein comprises about 94% protein and 6% low molecular weight compounds collectively called colloidal calcium phosphate. Mainly four casein phosphoproteins, αS1-, αS2-, β-, and κ-casein, exist approximately in proportions of 4:1:4:1 by weight respectively.

Materials and Mehods

Materials

Chemicls and Reagents: curcumin, NaOH, ethyl alcohol, methyl alcohol (loba chemicals, India)

Preformulation studies

Preformulation is defined as the phase of research and development process where physicochemical and mechanical properties of a new drug substance is characterized alone and in combined with excipients, with objective to develop stable, safe, effective, elegant and economical dosage form or delivery system

Solubility

Solubility of curcumin was determined in water, methanol, ethanol and castor oil, coconut oil, tween 80, Arachies oil etc.

Melting point determination

Melting point determination of was done by open capillary method.

Drug was taken in glass capillary whose one end was sealed by flame. The capillary containing drug was dipped in liquid paraffin inside the melting point apparatus, temperature at which melting was obtained was recorded. Melting point is a good first indication of purity of the sample since the presence of relatively small amount of impurity can be detected by lowering as well as widening in the melting point range.

Determination of λ max (UV Spectroscopy):

10 mg curcumin in 10ml methanol was prepared(1^0 stock solution) 2^0 standard solution of 100micro gm/ml was prepared by suitable dilution containing the concentration 10 microgram/ml was prepared in methanol and UV spectrum was taken using Shimadzu (UV-2550) double beam spectrophotometer. The solution was scanned in the range of 200-800nm. It shows λ_{max} at 425nm.

Compatibility studies by FTIR-Spectroscopy

FT-IR Spectroscopy data facilitates to ascertain the compatibility between drug and polymer. The FT-IR spectra of drug with polymers in physical mixture and dosage form were compared with the standard FT-IR spectrum of the pure drug. Spectroscopic Studies

Calibration Curve in Methanol

Calibration curve for Curcumin was developed by UV Spectrophotometric method. 10 mg curcumin in 10ml

methanol was prepared (10 stock solution) 20 standard solution of 100micro gm/ml was prepared by suitable dilution. From this 0, 1, 2, 4, 6 and 8 micro gm/ml working standards were prepared and measured at 425nm.

Preparation of Curcumin loaded Casein polysorbate 80 nanoparticles using 3^2 Factorial design Preparation of stock solution 8% casein containg Methyl Paraben

Eight gm of casein was dissolved in 100 ml of 0.1N NAOH using Magnetic stirrer for 1h whose PH was found to be 7.5 to form a sodium casinate. 0.1 gm of Methyl Paraben added and stirring continued for additional 15 min.

- **Preparation of stock solution 4% Polysorbate 80 solution containg 0.1% Methyl Paraben**

Two gm of Polysorbate 80 dissolved in 50 ml of water, Stirred for 10 min. added 0.1gm of Methyl Paraben and continued stirring another 10min.

- **Preparation of stock solution Ethanolic solution of curcumin**

Weighed 150mg curcumin dissolved in ethanol and made volume up to 150ml.

Formulation Design

Table 1: Preparation of Curcumin loaded Casein polysorbate 80 nanoparticlate disperion of Curcumin were prepared casein 1%, ploysorbate 80% for three formulations as per Formulation Chart.

Sl No	Formulation code	Vol of 8% casein (ml)	Vol OF 4% Polysorbate 80 (ml)	Vol of Water (ml)	Total Vol of Polymeric Blend (ml)
1	AX	2.5	0	17.5	20
2	AY	2.5	5	12.5	20
3	AZ	2.5	10	7.5	20
4	BX	5	0	15	20
5	BY	5	5	10	20
6	BZ	5	10	5	20
7	CX	10	0	10	20

Composition chart of Nanoparticle Preparation Table 1 Formulation of Nanoparticle

Preparation of stock solution is done. From the stock solution different quantity of casein, Polysorbate 80, and volume made 20ml of polymeric blend. 20ml of polymeric blend was taken in 100ml beaker stirred magnetically added 16ml ethanolic curcumin dropwise added to obtain Nanodispersion by micellar technique.

Preparation of vaccum concentrate of Nanodispersion

Nanodispersion placed in eppendrof's tube and placed in ultracentrifuge. And run for 30min. Nanoplug was obtained then washed twice with water. Further ultracentrifuged and plug was subjected for further studies.

Nanoparticle size and Zeta potential [6]

Dynamic light scattering (DLS) was employed to assess the mean nanoparticle size and size distribution. DLS measurements were performed with a wavelength of 532 nm at 25 °C with an angle detection of 90 °C after suitable dilution with distilled water. Volume (1 ml) of the sample was kept constant to eliminate the stray radiation effect amongst samples. Zeta potential was recorded from the same instrument. The yield was calculated by dividing the weight of nanoparticles recovered by the total weight of the input materials, i.e., weight of Curcumin and Casein [6].

$$\% \text{ yield} = \frac{\text{Wt of nanoparticles}}{\text{Wt of Curcumin + Wt of Casein}} \times 100$$

Drug loading and encapsulation efficiency [6]

Ten mg of nanoparticulate formulation was triturated in a glass mortar, using aliquots of methanol, quantitatively made upto 10ml in a volumetric flask and shaken overnight on a mechanical shaker. The mixture was filtered through whatman filter paper, 0.05 ml of the filtrate diluted to 10ml with methanol. Absorbance was measured at 425 nm. Percentage drug loading was calculated using the formula, Percentage encapsulation efficiency is calculated using formula.

$$\% \text{ Encapsulation efficiency} = \frac{\text{Percent drug loading}}{\text{Percentage of drug added in the formulation}} \times 100$$

Dissolution profiles of nanoparticles formulations [8]

In vitro dissolution studies were performed for prepared formulations using USP dissolution XXIII apparatus, Type II using 900ml phosphate buffer solution (pH 7.4±0.1), temperature of 37±0.10C, 75 rpm. Nanoparticles equvivalent to 100 mg of curcumin were weighed and kept for dissolution. 5 ml of the sample withdrawn was filtered through Whatman filter paper No1. 1 ml of the filtrate was made up to 10 ml with methanol in 10 ml volumetric flask. Suitable dilutions were further made when required. The absorbance of the samples was read at 425 nm against blank

Stability studies

Stability studies were evaluated to find out stable product under storage. Micro particles can be stored in Blass bottles at elevated temperature i.e. 4±1ºC freezing temperature, 25±1ºC room temperature, & 50±1ºC hot temperature for a period of 30 days & observed for change in drug content & morphology.

Results

1. Preformulation Studies

a. Melting point determination: 180ºc
b. Determination of λ_{max} methanol 425.0nm

Evaluation of curcumin based casein nanoparticle

Drug polymer interaction (FTIR) study

From the spectra of physical mixture of Curcumin and polymer, it was observed that all characteristic peaks of IR were present in the combination spectrum, thus indicating compatibility Curcumin of the and polymer.

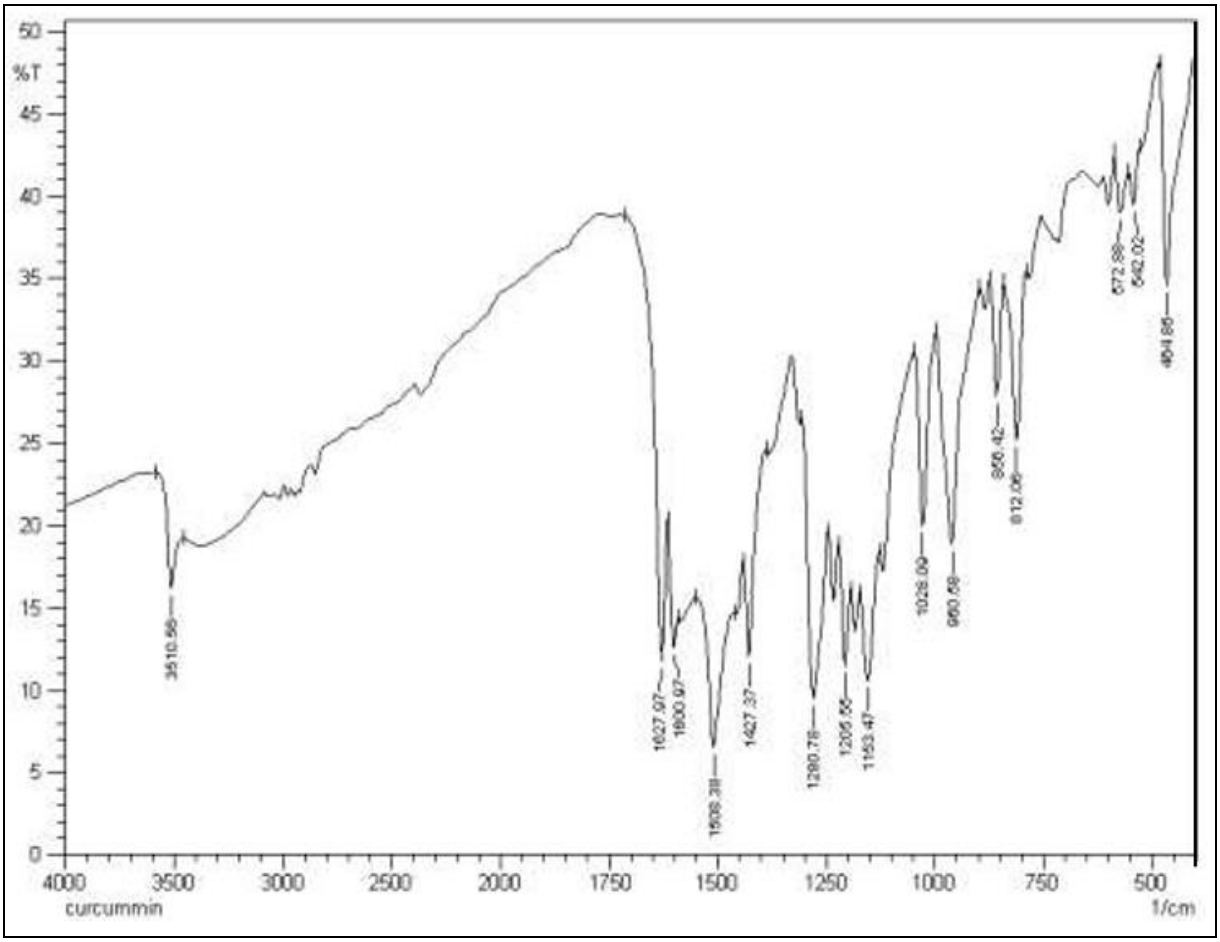

Fig 1: IR spectra of CURCUMIN

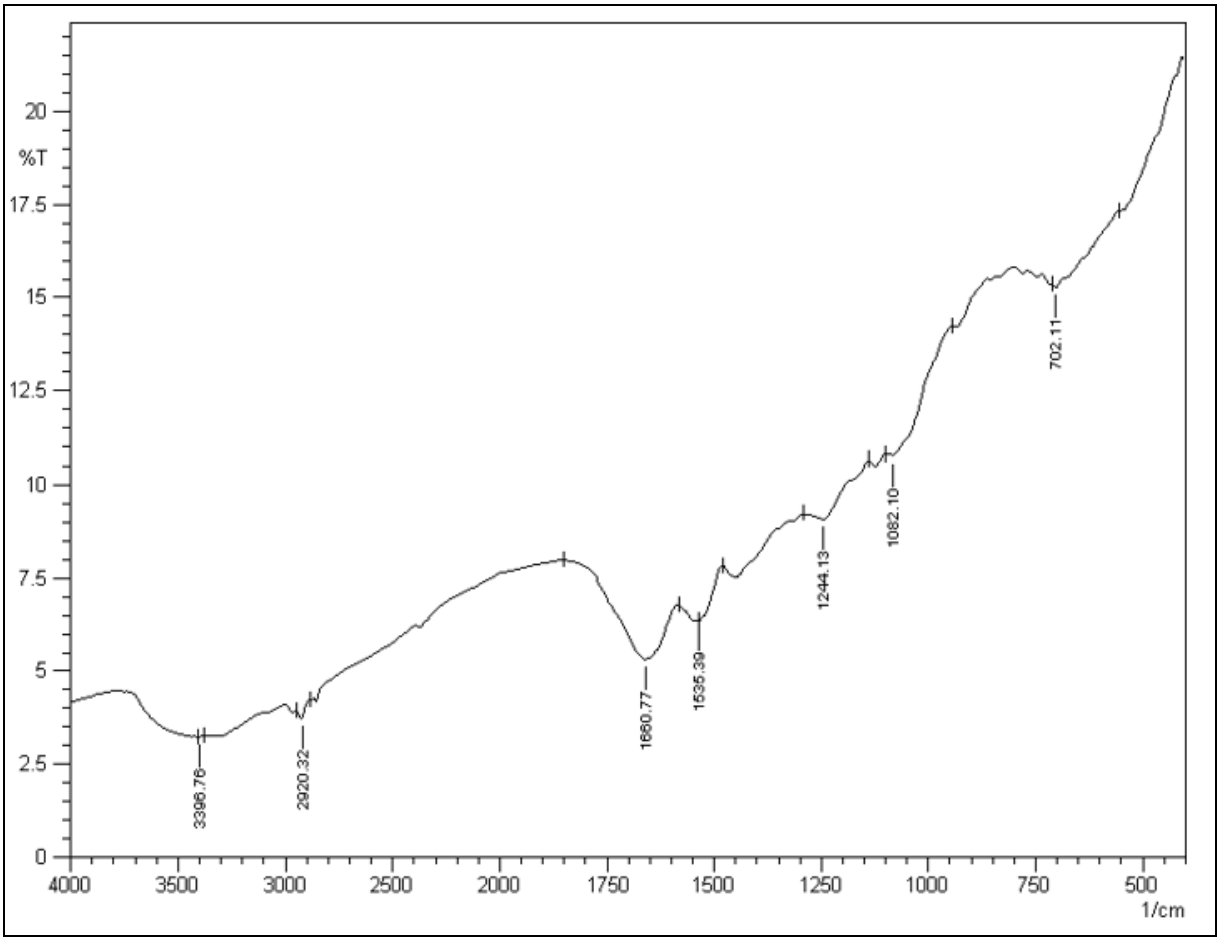

Fig 2: IR spectra of CASEIN

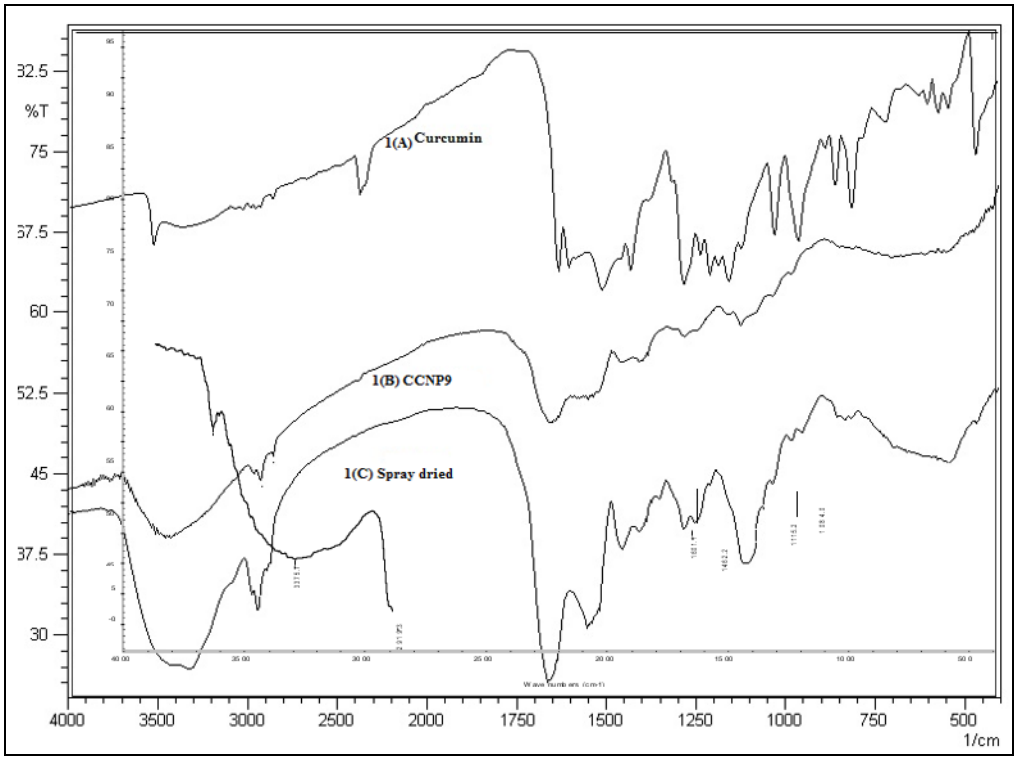

Fig 3: IR spectra Curcumin Embedded Nanoparticle (CY Formulation)

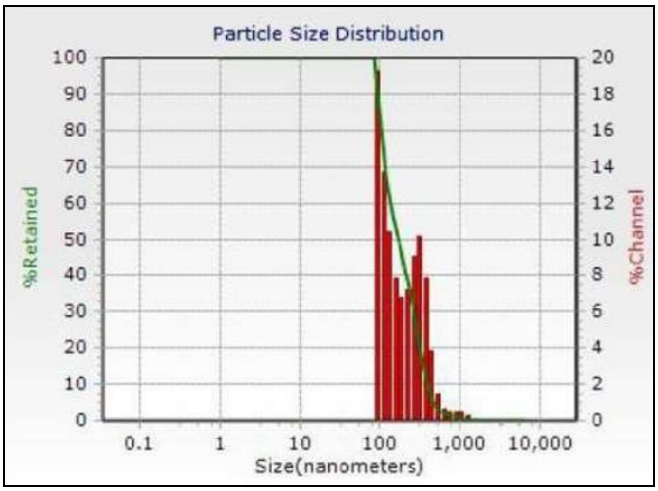

Fig 4: Particle size

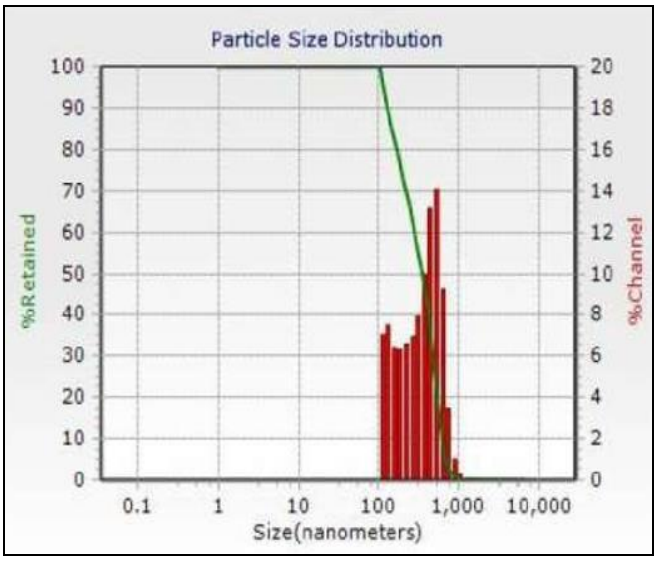

Fig 5: Particle size analysis of CY nanoparticles

Table 2: Particle size analysis of BY nanoparticles

Sl No	Percentiles	Size(nm)
1	10	385.9
2	20	318.7
3	30	267.8
4	40	215.3
5	50	167.0
6	60	135.9
7	70	116.4

Table 3: Particle size analysis of CY nanoparticles

Sl No	Percentiles	Size(nm)
1	10	617.7
2	20	536.5
3	30	475.4
4	40	417.2
5	50	353.0
6	60	283.8
7	70	220.1

Zeta potential of CY Formulation

Zeta Potential	
Mobility	-0.23u/s/V/cm
Zeta Potential	-2.9 mv
Charge	-0.01117 fC
Polarity	Negative
Conductivity	2,121 uS/cm
Field Strength	2.9 kV/m
Sample Information	
Fluid	
Viscosity	0.818
Temperature	28.83 C
Dielectric Const	79
Dispersant	
pH	7
Concentration	
Particle	
Concentration	0

Fig 6

Calibration curve
Development of calibration curve in Methanol:-fig 1.3.4

Table 4

Concentration in mcg/ml	Absorbance (±SD)
0	0.000±0.00
1	0.156±0.02
2	0.300±0.01
4	0.575±0.02
6	0.899±0.02

Calibration graph
Standard deviation, n = 3

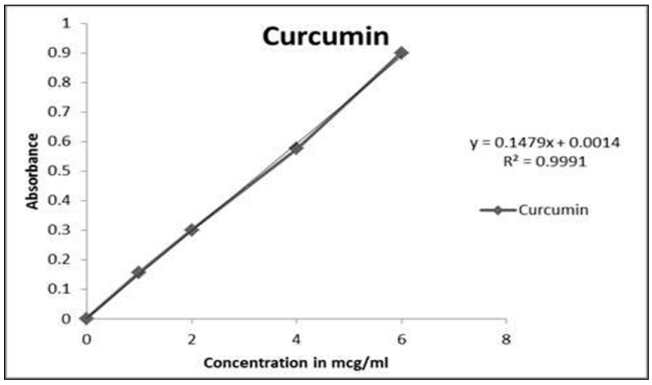

Fig 7: Standard calibration curve of Curcumin in Methanol at 425 nm.

Evaluation of nanoparticles

Table 5

Sl No	Formulation Code	Theoretical Yield (gm)	Practical Yield (gm)	Percentage Yield (gm)
1	AX	0.36	0.281	77
2	AY	0.36	0.333	91
3	AZ	0.36	0.290	80
4	BX	0.56	0.49	87
5	BY	0.56	0.542	96
6	BZ	0.56	0.501	89
7	CX	0.96	0.905	93
8	CY	0.96	0.951	98
9	CZ	0.96	0.912	94

Drug loading and encapsulation efficiency

Table 6

Sl No	Formulation Code	% Drug loading	% Encapsulation Efficiency
1	AX	0.08	2.6
2	AY	1.2	14
3	AZ	0.24	4.2
4	BX	0.12	3.9
5	BY	1.9	23.2
6	BZ	0.28	4.8
7	CX	0.15	5.1

***In vitro drug* release studies**

Table 7

Time (h)	AX (%)± SD	AY (%± SD	AZ (%)± SD	BX (%)± SD	BY (%)± SD	BZ (%)± SD	CX (%)± SD	CY (%)±SD	CZ (%)±SD
1	20.36	25.64	20.31	25.87	22.33	28.65	25.74	20.55	23.36
	±0.09	±0.13	±0.09	±0.13	±0.15	±0.09	±0.54	±0.09	±0.13
2	34.51 ±0.18	35.36 ±0.13	33.97 ±0.80	41.64 ±0.11	34.59 ±0.23	40.10 ±0.09	40.05 ±1.07	40.55 ±0.09	38.78 ±0.12
3	44.42	48.60	48.51	56.50	50.29	54.69	54.66	57.89	52.90
	±0.15	±0.18	±0.12	±0.11	±0.15	±0.10	±0.10	±0.04	±0.11
4									
	51.33	54.86	53.79	62.80	57.46	60.90	58.35	69.23	57.29
5	±0.20	±0.18	±0.15	±0.95	±0.13	±0.03	±0.36	±0.05	±0.05
	74.66	71.67	70.63	76.22	74.41	74.97	70.05	79.21	73.58
6	±0.13	±0.11	±0.13	±0.13	±0.14	±0.08	±0.88	±0.18	±2.22
7	82.82 ±0.38	81.01 ±1.22	77.73 ±1.69	86.27 ±2.16	83.38 ±0.62	80.64 ±0.63	82.44 ±0.62	88.95 ±0.20	82.66 ±4.30
8	85.52	82.14	79.75	89.56	84.34	81.78	84.04	93.65	81.02
	±0.18	±1.11	±0.80	±0.09	±0.18	±0.22	±1.04	±0.18	±1.85
	89.05	91.85	89.05	92.05	91.05	90.05	90.02	96.05	91.05
	±0.13	±0.18	±0.12	±1.02	±0.23	±0.96	±1.02	±0.98	±1.03

Stability study
Stability testing was also for a sample, once for initial and later for 1 month sample at 25±2 °C / 60%RH±5 and 40±2 °C / 75%RH±5. The formulation CY was found to be stable when exposed to long term stability conditions and accelerated stability conditions for 1 month, showed stablised in particle size, distrubution and free propofol content, so formulation CY is selected as optimized formulation.

Results and Discussion
The present research work was undertaken with a goal to optimize the process variables in the formulation of Nanoparticle. Nanoparticle of casein curcumin were prepared by Micellar technique by using tween 80 as a stablizer. Various evaluation parameters were assessed. Formulation development protocal is preceded by preformulation studies including analytical investigations, choice of the analytical methods, standardization and preliminary formulation trials. There is a need for selection of Polymers and excipient, which are compatible with the drug and among themselves and also physiologically safe and biocompatible. Preliminary information about the behavior of the dosage form formulated, using the prepared and selected ingredients and their singular and collective effect on the physicochemical and pharmaceutical properties of the dosage form also needs to be generated during this phase. Prepared Curcumin, casein formulations were subjected to FT-IR, particle size, Zeta potential, free drug content, Drug loading, % encapsulation, *Invitro* release studies, and stability study.

Preformulation Studies
Solubility of curcumin in water, different organic solvents,

aqueous solutions of stabilizers and the solubility of selected organic solvents in water were presented in Table 5.1 A drug which is a suitable candidate for nanoparticle preparation by Micellar technique should be very poorly soluble in water and well-soluble in the selected organic solvent. Curcumin is very poorly water-soluble and significantly better soluble in organic solvents, as recorded in this study. Curcumin is soluble in ethanol, which is water miscible and is therefore selected for the preparation of nanoparticles. Melting point was found to be 180^0 C.

Characterization of Nanoparticle Particle Size Analysis

Particle size determination was done from instrument which gave particle size range of 385.9 - 90.96. and 617.7 - 116.6 of BY and CY formulation respectively. Zeta potential was and it was found to be -2.6 mV and -2.9 mV. Zeta potential determines the physical stability of nanoparticle. Zeta potential is an indirect measurement of the thickness of diffusion layer, i.e. can be used to predict long term stability.

Percentage drug loading and entrapment efficiency

Entrapment efficiency increased with increase in the polymer concentration. From the results it can be inferred that there is a proper distribution of Curcumin in the nanoparticles and the deviation were within the acceptable limits. The percent of drug loading in the formulations were found to be in the range of 0.08 to 4.0 mg. The percentage entrapment efficiency was found to be 2.6% to 46.5%. and the CY formulation more in drug loading 4.0 mg and percentage entrapment efficiency 46.5%.

pH Determination

pH of the Nanoparticle was measured using lab pH metre and it was found to be 7.4

***In vitro* release studies**

All the formulation showed better release but formulation CY exhibited maximum release of 96.05 % at the end of 8 hrs % Drug release v/s time plot of CY showing zero order kinetics.as shown in Fig. 1.9.1 Log % drug remained v/s time plot of CY showing first order kinetics. as shown Fig. 1.9.2 % Drug release v/s square root of time plot of CY showing Higuchi's model as shown in Fig. 6.12. The slopes and regression co-efficient of determination (r2) were observed. The coefficient of determination indicated the release data was best fitted with zero order as well as first order kinetics. Higuchi equation explains the diffusion controlled release mechanism.

Stability Studies

Stability testing was also for a sample, once for initial and later for 1 month sample at 25±2 °C / 60%RH±5 and 40±2 °C / 75%RH±5. The formulation CY was found to be stable when exposed to long term stability conditions and accelerated stability conditions for 1 month, showed stablised in particle size, distrubution and free propofol content, so formulation CY is selected as optimized formulation.

Conclusion

The results obtained in this work indicate that developed NANOPARTCIE prepared by micellar technique. Formulation can be a successful carrier for Intravenous Delivery of Curcumin and might and shown better bio availability compared to oral administration of curcumin. From the results, we found in these studies the following conclusions can be drawn. The developed methods were found to be specific, accurate, reliable, and reproducible for quantitative estimation of the drug in vitro. Optimized curcumin and casein Nanoparticle composed of of tween80 and ethanol and water are selected. The particle size, Zeta potential, Drug loading, encapsulation, *In vitro* release studies, and free curcumin content were unaffected in studied stability conditions. In vitro release in phosphate buffer (7.4) revealed a release about 96.05 % at the end of 8 hrs. The formulation was found stable when exposed to long term room temperature conditions and accelerated stability conditions for 1 month.

Future Perspective

Long term and accelerated stability study would give better understanding on stability aspects of curcumin casein Nanoparticle.

Summary

In the present study curcumin embedded casein Nanoparticle were prepared by micells technique. The principle of the nanoparticle formulation was that curcumin was dissolved in the organic phase ethanol which was be added into polymeric blend containing casein, tween80 and NAOH. Magnetically stirred get nanodispersion the curcumin nanodispersion was subjected to preformulation studies such as solubility, melting point and compatibility studies which comply with the predetermined specifications. Mainly the compatibility studies which showed that there is no interaction between drug and excipients. Formulation of nanodispersion was done by optimization of batch formula and various process parameters like temperature, mixing speed. Around 09 trials were taken AX, AY, AZ, BX, BY, BZ, CX, CY, CZ. The obtained nanodispersion were subjected to characterization such as particlesize, Zeta potential, free drug content, Drug loading, % encapsulation, Invitro release studies, and stability study. It was found that the prepared curcumin embedded casein nanoparticle were having a particle size range of 385.9 - 90.96. and 617.7 - 116.6 of BY and CY formulation respectively. zeta potential of -2.6 mV and -2.9 mV of BY and CY formulation respectively. The stability studies indicated that were stable for a period of one month at 25±2 °C/60±5RH and 40±2 °/75±5RH. curcumin embedded casein nanoparticle

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