Research Article

Comparative Evaluation of Curcumn Nano Particles of Casein and Chitosan

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ABSTRACT

Curcumin is a poly phenolic compound derived from turmeric possesses anti-inflammatory, antioxidant, ant proliferative and antigenic activities but exhibits poor bioavailability. Curcumin exhibits poor bioavailability, the reason for this was poor absorption, rapid metabolism and rapid systemic elimination. The main objective of the study is to overcome the above limitations by curcumin delivery through nanotechnology to formulate curcumin nanoparticles by using casein and chitosan and to carry out its comparative evaluation. The curcumin casein nanoparticles are prepared by using PH conservation method and curcumin chitosan nanoparticles are prepared by using ionic gelation method. In the present study 10 formulations were formulated. Five of curcumin and chitosan (CCH1- CCH5) five of curcumin and casein (CCN1-CCN5) by keeping the drug constant and by varying the polymer ratio. The prepared nanoparticles are smooth and free flowing and are subjected to evaluation like FT-IR, Particle size, Zeta potential, Surface morphology, Drug content, Entrapment efficiency and In-vitro release. The prepared nanoparticles possess zeta potential ranging from -16.3± 0.4mV to -39.07 ± 0.2mV for (CCN) and -11.14± 0.4mV to -32.75± 0.53mV for (CCH) and particle size ranging from 110±5.04nm to 248±10.7nm (CCN) and 121±8.2nm to 242±4.3nm (CCH), Drug content ranging from 75.0±0.706% to 94.5±0.706%(CCN) and 85.0±0.706% to 92.5±0.706%(CCH), In-vitro release studies were conducted for curcumin nanoparticles exhibited drug release in the range of 76.98 to 92.78 (CCN) and 83.42 to 96.75 (CCH). The present study conclusively demonstrates that curcumin nanoparticles prepared by using chitosan are more stable feasible and effective than prepared by using casein. Hence chitosan encapsulating curcumin nanoparticles form potential controlled release drug delivery system.

Keywords: Curcumin, Nanoparticles, Casein, Chitosan, FT-IR etc.

INTRODUCTION

Nanoparticles are sub-nanosized colloidal structures composed of synthetic or semi synthetic polymers. Nanospheres are solid core spherical particulates which are nanometric in size. They contain drug embedded within the matrix or adsorbed on to the surface.[1]

Nanoscience is an emerging field that deals with interactions between molecules, cells and engineered substances such as molecular fragments, atoms and molecules. In terms of size constraints, the National Nanotechnology Initiative (NNI) defines nanotechnology in dimensions of roughly 1 to 100 nanometers (nm), but in boarder range it can be extended up to 1000 nm.[1]

There is great interest in developing new nano delivery systems for drugs that are already on the market, especially cancer therapeutics. Ideally, nanodelivery systems will allow for more specific targeting of the drug, thereby improving efficacy and minimizing side effects. By using nanotechnology in drug design and delivery, researchers are trying to push nanomedicine to be able to deliver the drug to the targeted tissue, release the drug at a controlled rate, be a biodegradable drug delivery system, and to be able to escape from degradation processes of the body.[2]

Nanoparticles of different sizes have different biomedical purposes the potential use of polymeric nanoparticles as drug carriers has led to the development of many different colloidal delivery vehicles. The main advantage of this kind of systems lie in their capacity to cross biological barriers, to protect macromolecules such as peptides, proteins, oligonucleotides and gene form degradation in biological media.[3]

The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen.[4]

Curcumin an active compound derived from mostly curcuma species (*curcuma longa*) is a natural hydrophobic polyphenolic which has been used in Asian countries like Indonesia, India and china to cure various diseases from centuries. It's an Indian spice known for its yellow food coloring in addition to its long history of use in Ayurvedic medicine.[5,6]

Curcuma long rhizome has been traditionally used as antimicrobial agent as well as an insect repellant. Several studies have reported the broad-spectrum antimicrobial activity for curcumin including antibacterial, antiviral,

antifungal, and antimalarial activities. It exhibits antioxidant, anti-inflammatory and anti-carcinogenic activities. Additionally, the hepato and nephroprotective, thrombosis suppressing, myocardial infarction protective, hypoglycemic and antirheumatic effects of curcumin are well established.[2,5,6,]

MATERIALS AND METHODS

Materials

Curcumin, Casein, Glutaraldehyde, Acetone, Sodium hydroxide pellets, Sodium chloride, Hydrochloric acid was obtained from Lobachemie Pvt. Ltd. Mumbai. Methanol, Glacial acetic acid, Potassium dihydrogen phosphate was obtained from S.D fine chemicals limited, Mumbai, India.

Pre-formulation studies

Preformulation is defined as the phase of research and development process where physic chemical 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.

Identification of pure drug

The selected drug was subjected for investigation of physical characterization parameters such as:

IR Spectroscopy

Compatibility study using FT-IR

FT-IR spectroscopy was carried out to check the compatibility between drug and polymer. The FT-IR spectra of drug with polymers were compared with the standard FT-IR spectrum of the pure drug.[7]

Solubility analysis

Pre-formulation solubility analysis was done to select a suitable solvent system to dissolve the drug as well as various excipients used for formulation of nanoparticles.[7]

Melting point determination

The melting temperature of drugs was determined using capillary method.[8]

Analytical method used in the determination of curcumin

The UV spectrophotometric method was developed for the analysis of the drug using Shimadzu (UV-2500) spectrophotometer.[8]

Standard curve for curcumin

Determination of λ max for curcumin

10mg curcumin in 10ml methanol was prepared (1° stock solution) and 2^0 standard solution of 100mcg/ml was prepared by suitable dilution containing the concentration 10mcg/ml was prepared in methanol and UV spectrum was taken using shimadzu (UV-2500) double beam spectrophotometer. From the scanning of drug, it was concluded that the drug had λ max of 425 nm which was nearer to 420 nm as reported.[8]

Preparation of standard calibration curve of curcumin

Standard calibration curve for curcumin was prepared using methanol solution.

Calibration curve in methanol

Calibration curve for curcumin was developed by UV spectrophotometric method. 10mg curcumin in 10ml methanol was prepared (1^0 stock solution) and 2^0 standard solution of 100 mcg/ml was prepared by suitable dilution from this 0,1,2,4,6,8 mcg/ml working standards were prepared and measured at 425nm.[8]

Formulation of curcumin nanoparticles of Casein and Chitosan

Method of preparation of curcumin chitosan nanoparticles

Drug loaded chitosan nanoparticles were prepared by the method based on the ionic gelation of Chitosan with TPP anions. Chitosan was dissolved in acetic aqueous solution (1.5% v/v) at various concentrations such as1.0, 2.0, 3.0, 4.0 and 5.0 mg/ml. 10 mg of drug (curcumin) was dissolved in 5 ml of 2 % w/v tween 80 solutions, which was added to the chitosan solution. Under magnetic stirring at room temperature, 5 ml and 10ml of 0.25 % sodium tripolyphosphate (TPP) aqueous solution was added drop wise into drug and polymeric mixture, respectively. The stirring was continued for about 20–25 min. The obtained nanoparticle suspension was centrifuged at 12000x rpm for 30min. The formation of the particle was a result of the interaction between the negative groups of the TPP and the positively charged amino groups of chitosan.[9]

Method of preparation of curcumin casein nanopartiles

A 10ml solution of casein was prepared at a concentration of10 mg/mL by using NaoH solution at ph of 7.4 to obtain micellar solution of casein. A calculated amount of curcumin was dissolved in 95% ethanol was then added drop wise to casein solution under moderate magnetic stirring for 1h and a maintained temp of 60^0C. few drops of glutaraldehyde solution is added for rigidisation. The prepared colloidal solution was centrifuged for 10000 rpm for half an hour, the supernatant is removed and dried and dried nanoparticles were stored in a desiccator at 25 ºC till further analysis.[10]

Evaluation studies

Particle size analysis

Curcumin nanoparticles were diluted with water to ensure that the signal intensity was suitable for the instrument. Particle size was determined by dynamic light scattering (DLS) using Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK). Nanoparticles were diluted with water (viscosity (cp) 0.8872) and intensity scattered light was detected at a scattering angle of 173°

to an incident beam at a temperature of 25°C. The poly dispersity index range was comprised between 0 and1 and the measurements were done in triplicate.[11]

Particle size distribution

The particle size distribution of the nanoparticles was determined by photon correlation spectroscopy. The nanoparticle dispersions were added to the sample dispersion unit containing stirrer and stirred to reduce the aggregation between the nanoparticles. The average volume-mean particle size was measured after performing the experiment in triplicate.[11]

Zeta potential

The prepared nanoparticles were subjected to zeta potential analysis to determine the surface charge of the nanoparticles and to find out the aggregation behaviour.[8]

Process yield

Process yield was calculated as the weight of nanoparticles recovered from each batch in relation to the sum of starting material. Process yield (Y) was calculated by using following formula,[12]

$$Y = \frac{W1 \times 100}{W2}$$

Where, W1 = Weight of dried Nanoparticles

W2 = Initial dry weight of starting material

Drug content

The drug content of curcumin present in nanoparticles was determined by centrifugation method. The nanoparticles suspension was centrifuged at 15,000 rpm for 30 min at 15°C. The supernatant solution was separated and the free drug concentration of curcumin in the supernatant solution was determined by UV – Vis spectrophotometer at 420nm.[13,14]

$$\text{Drug Content (\%)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 100$$

Evaluation of curcumin encapsulation

The curcumin loaded nanoparticles were separated from the aqueous suspension medium by centrifugation at 16,000 rpm and 25°C for 30 minutes. The amount of free curcumin was measured in the clear supernatant by UV measured at a wavelength of 420 nm. The curcumin loading capacity (LC) of the nanoparticles and their % loading capacity (%LC) were calculated.[15]

Drug entrapment efficiency

The entrapment efficiencies of prepared systems were determined by measuring the concentration of free drug in the dispersion medium. The curcumin loaded nanoparticles were separated from the aqueous suspension medium by centrifugation at 16,000 rpm and 25°C for 30 minute. The supernatant was separated and then filtered through 0.45 µm Millipore (Millipore Filter). The amount of free curcumin was measured in the clear supernatant by UV measured at a wavelength of 420 nm. The amount of free drug was detected in the filtrate and the amount of incorporated drug was determined as a result of the initial drug minus the free drug.[16]

The entrapment efficiency was calculated using the following equation.

$$\text{Entrapment Efficiency} = \frac{\text{W initial drug} - \text{W free drug} \times 100}{\text{W initial drug}}$$

Scanning electron microscopy (SEM)

Surface morphology of nanoparticles was done by Scanning Electron Microscopy (JSMT6360A, JEOL). SEM has been used to determine surface topography, texture and to examine the morphology of fractured surface. Small volume of nanoparticulate suspension was placed on an electron microscope brass stub. The stubs were placed briefly in a drier and then coated with gold in an ion sputter. Pictures of nanoparticles were taken by random scanning of the stub. The shape and surface morphology of the nano- particles was determined from the photomicrographs of each batch.[16]

Differential scanning calorimetry (DSC)

The DSC analysis was carried out to identify the compatibility between the drug and excipients. The DSC analysis of pure drug, 1:1 physical mixture of drug excipient was carried out using DSC 60, Shimadzu, Japan. Samples (2- 8 mg) were accurately weighed into an aluminium pan, which was crimped non-hermetically and heated in sealed aluminium pans at a rate of 10oc/min between 0-300^{O} C temp ranges under nitrogen atmosphere.[13]

***In-vitro* drug release study**

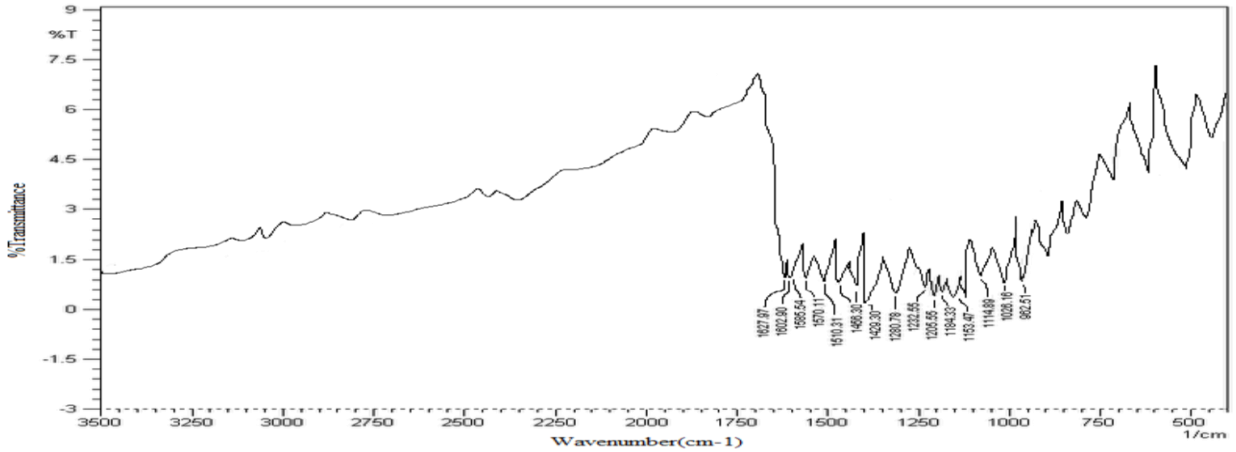
The *in-vitro* release studies were carried out by using dialysis membrane. The curcumin nanoparticles equivalent to 5 mg of curcumin and 10 ml of phosphate buffer pH 7.4 was added to the dialysis tubes and immersing the dialysis tube to the receptor compartment containing 250 ml of phosphate buffer pH 7.4. The medium was agitated continuously using a magnetic stirrer and the temperature was maintained at 37±0.5°C. The sample of 5 ml was taken at various intervals of time over a period of 24 hrs and fresh buffer was replaced during each sampling. The amount of curcumin released was determined by UV- spectrophotometer at 420 nm.[13,17]

Stability Studies

Stability studies are done to understand how to design a product and its packaging such that product has appropriate physical, chemical and microbiological properties during a defined shelf life when stored and used. The optimized formulation was subjected for two months stability study according to ICH guidelines**.** The selected formulations were packed in aluminum foil in tightly closed container. They were then stored at 30ºC 65% RH and 40ºC / 75% RH for two months and evaluated for their post-compression studies.

RESULTS AND DISCUSSION

The compatibility studies revealed both drugs and excipients were compatible after FTIR studies, the results are shown in Figure 1.



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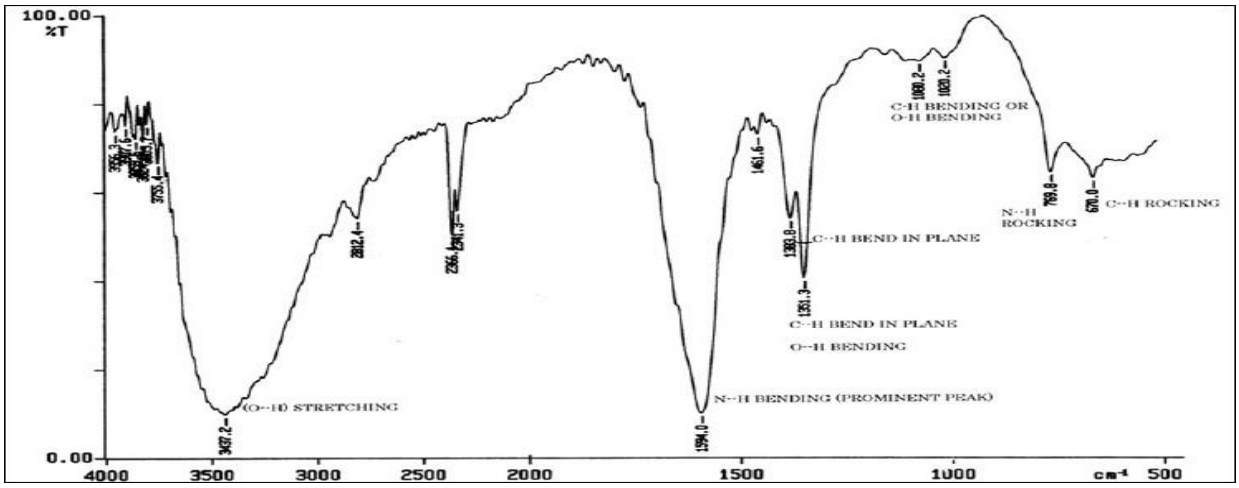

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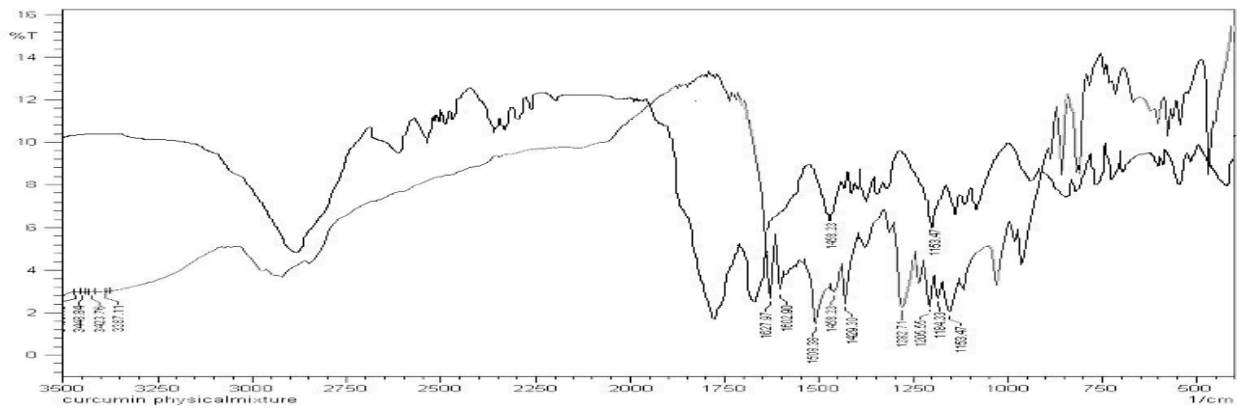

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Figure 1: FT-IR of a) Pure curcumin drug b) Curcumin chitosan nano particles (CCH) c) Curcumin casein nanoparticles(CCN)

Table 1: Formulation chart of curcumin chitosan nanoparticles (CCH)

Formulation code	Chitosan mg	TPP solution 0.25 %(ml)	Curcumin mg	Glacial acetic acid%	Drug: carrier ratio
CCH1	30	10	30	1.5	1:1
CCH 2	60	10	30	1.5	1:2
CCH 3	90	10	30	1.5	1:3
CCH 4	120	10	30	1.5	1:4
CCH 5	150	10	30	1.5	1:5

Table 2: Formulation chart of curcumin casein nanoparticles (CCN)

Formulation code	Casein mg	NAOH	Drug in mg	Glutaraldehyde in ml	Drug:carrier ratio
CCN1	30	0.05	30	0.1	1:1
CCN 2	60	0.05	30	0.1	1:2
CCN 3	90	0.05	30	0.1	1:3
CCN 4	120	0.05	30	0.1	1:4
CCN 5	150	0.05	30	0.1	1:5

Particle size distribution

The particle size and polydispersity (size distribution) optimized batch of curcumin nanoparticles measurement was performed by using Zetasizer (Malvern Instruments, UK) by dynamic light scattering technique. It showed drug loaded nanoparticles were found to be 200-500 nm. The characteristics of the chitosan/casein particles prepared with different concentrations of chitosan or casein were studied. The results indicated that the particle size increased with increasing the concentration of either chitosan or casein. The particle size of curcumin casein nanoparticles is in the range of 110±5.04 to 248±10.7nm and for curcumin chitosan nanoparticle its 121±8.2 to 242±4.3nm. The results are shown in Table 3 and 4.

Table 3: Profile of average particle size of curcumin chitosan nanoparticles(CCH)

Batch code	Drug: carrier ratio	Entrapment efficiencya (%)	Particle size (nm)
CCH1	1:1	58.32 + 0.02	121 + 8.2
CCH2	1:2	64.13 + 0.08	125 + 5.4
CCH3	1:3	70.83 + 0.03	140 + 9.6
CCH4	1:4	64.62 + 0.02	220 + 5.8
CCH5	1:5	59.96 + 0.04	242 + 4.3

Table 4: Profile of average particle size of curcumin casein nano particles (CCN)

Batch code	Drug: carrier ratio	Entrapment efficiency (%)	Particle size (nm)
CCN1	1:1	52.75 ± 0.23	110 ± 5.04
CCN2	1:2	54.38 ± 0.56	130 ± 4.2
CCN3	1:3	60.8 ± 0.58	170 ± 8.9
CCN4	1:4	60.8 ± 0.58	230 ± 10.5
CCN5	1:5	63.85 ± 0.36	248 ± 10.7

Zeta potential

Zeta potential is a measure of the magnitude of electrostatic or charge repulsion between the particles and is one of the fundamental parameters to affect the stability. The zeta potential increased suddenly as the cur cumin nanoparticles mass ratio increased from 1:1 to 1:2, this increase may be due to aggregation of the nanoparticles. The increase in zeta potential is due to the decrease in drug concentration the mean zeta potential is found to -16.3± 0.4mV to -39.07 ± 0.2mVforcurcumin casein nanoparticles and -11.14± 0.4mV to -32.75± 0.53mV for curcumin chitosan nanoparticles. The results are shown in Table 5 and 6.

Yield of production

Total amount of nanoparticles obtained was weighed individually for each batch and the percentage yield was calculated taking into consideration the weight of drug and polymer. Yields of production of different formulations were calculated and are found to be in the range of 60.3 to 61.2 for curcumin casein nanoparticles and 64.76 to 66.65 for curcumin chitosan nanoparticle

indicating curcumin chitosan nanoparticles have better yield compared to curcumin casein nanoparticles.

Table 5: Zeta potential of curcumin chitosan nano particles

Formulation code	Zeta potential (mV)
CCH1	-11.14± 0.40
CCH2	-16.75± 0.43
CCH3	-22.23± 0.32
CCH4	-28.75± 0.12
CCH5	-32.75± 0.53

Drug content uniformity

Data of curcumin estimation in nanoparticles are determined spectrophotometrically at 420nm. Percentage of drug content in the formulation range from 75.0±0.706% to 94.5±0.706% in CCN and 85.0±0.706% to 92.5±0.706% in CCH. The results are shown in Table 7 and 8.

Table 6: Zeta potential of curcumin casein nano particles

Formulation code	Zeta potential (mV)
CCN1	-16.3± 0.4
CCN2	-20.6 ± 1.3
CCN3	-27.04 ± 1.2
CCN4	-32.07 ± 0.2
CCN5	-39.07 ± 0.2

Table 7: Drug content uniformity curcumin chitosan nano particles (CCH)

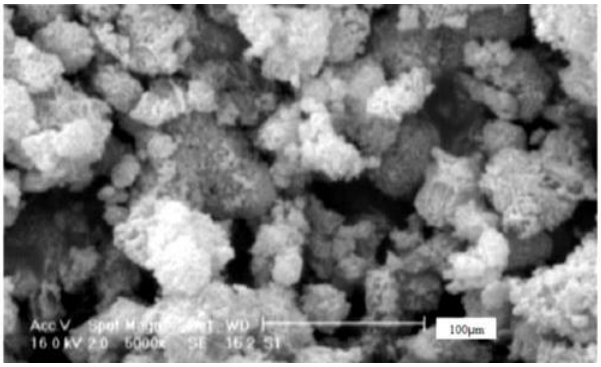
S.No	Formulation code	Drug content in mg mean SD	% of Drug content mean SD
1	CCH1	165±1.04881	85.0±0.70616
2	CCH2	172±1.04881	87.5±0.70616
3	CCH3	168±1.04881	89.0±0.70616
4	CCH4	172±1.04881	87.5±0.70616
5	CCH5	165±1.04881	92.5±0.70616

Table 8: Drug content uniformity curcumin casein nanoparticles (CCN)

S.No	Formulation code	Drug content in mg mean SD	% of Drug content mean SD
1	CCN1	170±1.04881	75.0±0.70616
2	CCN2	165±1.04881	87.5±0.70616
3	CCN3	188±1.04881	89.0±0.70616
4	CCN4	185±1.04881	87.5±0.70616
5	CCN5	189±1.04881	94.5±0.70616

Determination of particle size by SEM

SEM studies of pure curcumin and solid dispersions were carried out in order to analyze surface morphology of pure drug as well as solid dispersions. Pure drug particles were spherical in shape while nanoparticles obtained from hot melt method was plane and uniform indicating that the drug is dispersed in casein and chitosan and converted into amorphous state which might be the reason for improvement of solubility. Scanning electron photomicrographs of the formulations shown in Figure 2.



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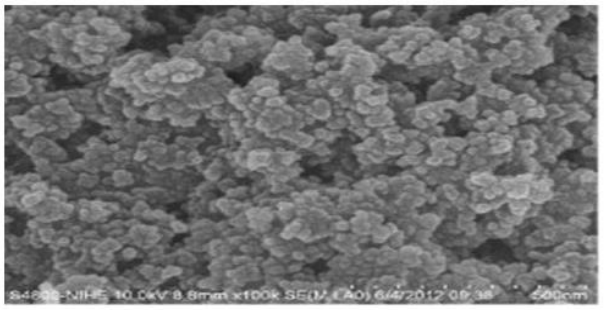

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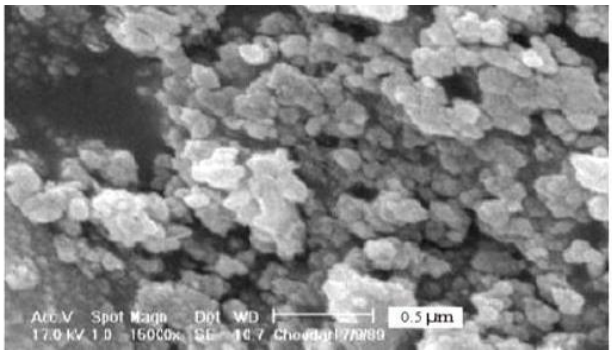

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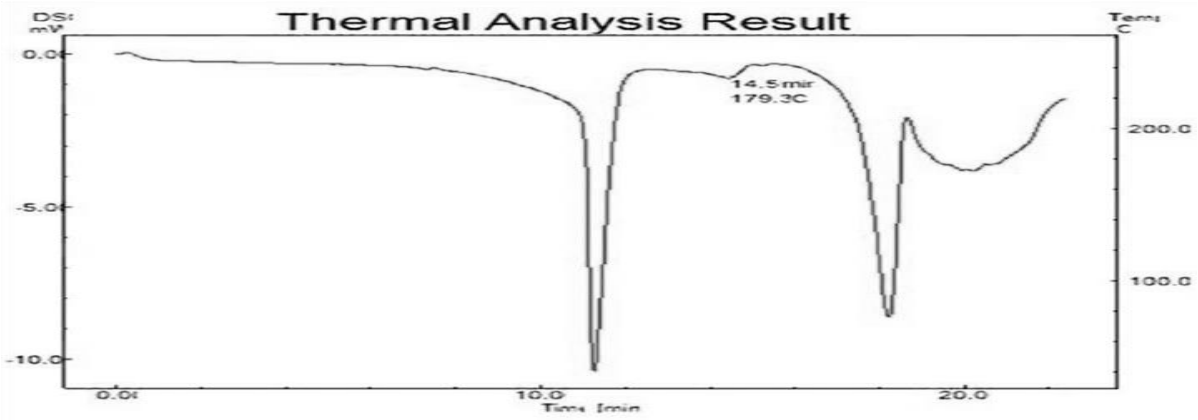
Figure 2: SEM of a)Pure curcumin drug b)Curcumin chitosan nanoparticles(CCH) c)Curcumin casein nanoparticles(CCN)

Differential scanning calorimetry (DSC)

The DSC thermograms of curcumin, casein, chitosan, and nanoparticles of curcumin were illustrated. The DSC curve of pure curcumin showed a single sharp endothermic peak at 180^0 C. All solid dispersion systems showed no endothermic peaks of curcumin. These findings may be due to the formation of an amorphous solid solution which has been known to cause an increase in drug dissolution.Differential scanning calorimetryof the formulations are shown in Figure 3.



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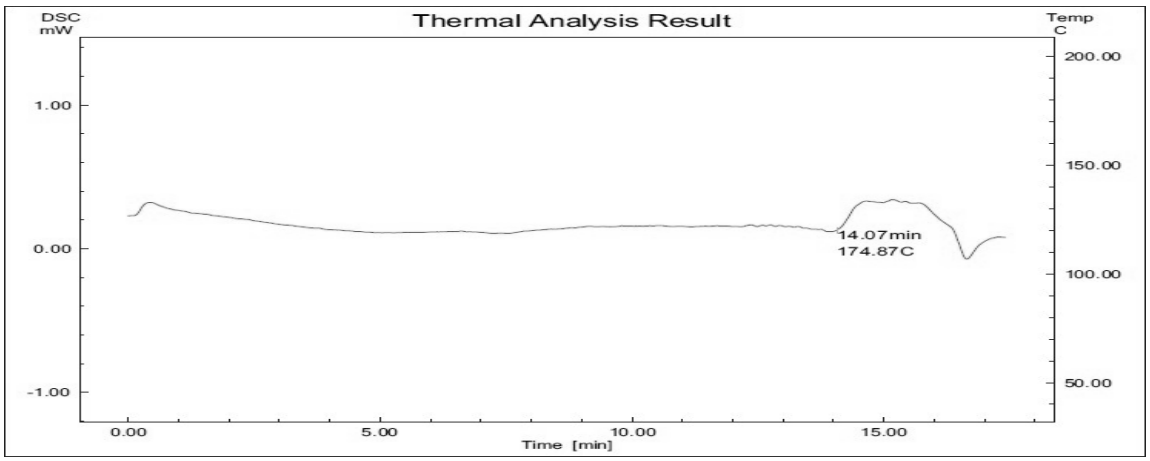

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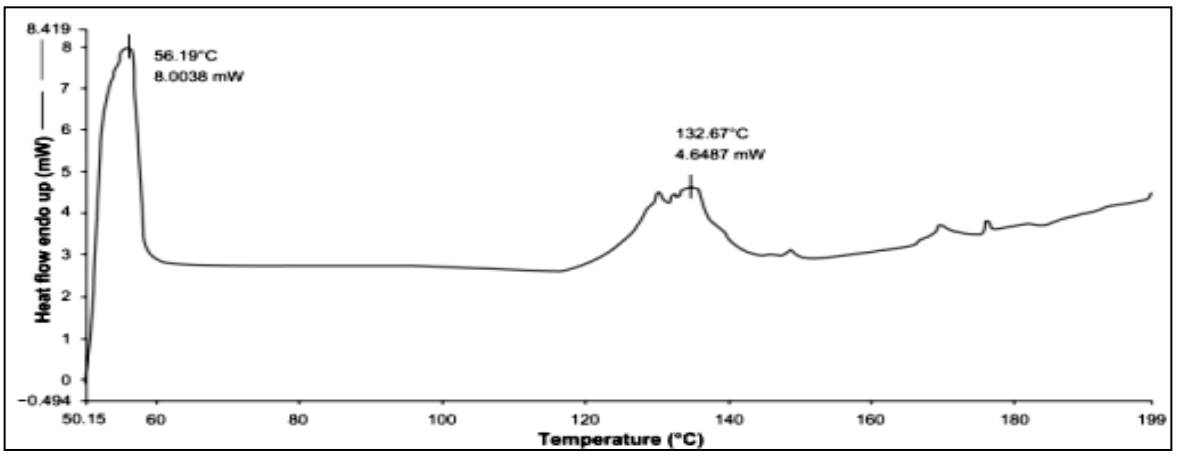

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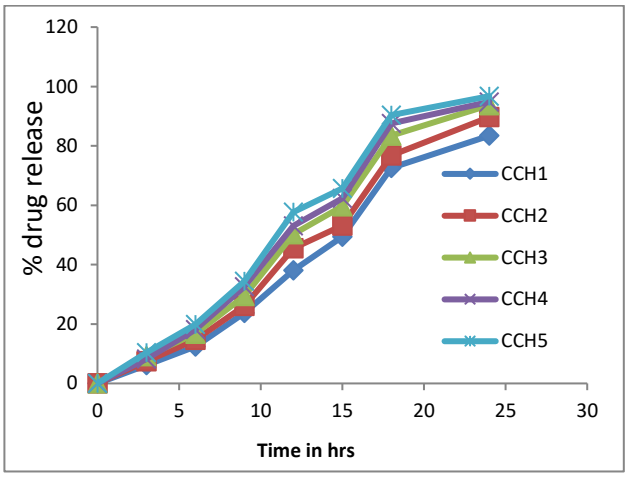
Figure 3: DSC of a) Pure curcumin drug b) Curcumin chitosan nanoparticles (CCH) c) Curcumin casein nanoparticles(CCN)

Drug release study of curcumin nanoparticles

All the formulation of both the nanoparticles have shown good release after 24hrs. But the CCN5 of curcumin casein nanoparticles and CCH5 of curcumin chitosan nanoparticles show greater release of 92.78% and 96.75%. The *in-vitro* drug release data of different formulations are shown in Table 9 and 10andFigure 4.



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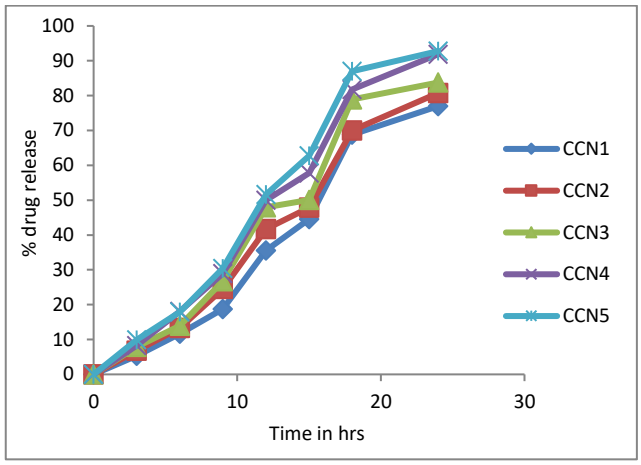

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Figure 4: a) Drug release study of curcumin chitosan nanoparticles (CCH) b) Drug release study of curcumin casein nanoparticles (CCN).

Table 9: Drug release study of curcumin casein nanoparticles (CCN)

S.No	Time in hours	% of drug release CCN1	% of drug release CCN2	% of drug release CCN3	% of drug release CCN4	% of drug release CCN5
1	0	0.00	0.00	0.00	0.00	0.00
2	3	5.34	6.78	7.89	8.27	9.89
3	6	11.67	13.34	13.99	18.12	17.89
4	9	18.78	24.56	26.78	28.98	30.43
5	12	35.56	41.67	47.98	49.98	51.67
6	15	44.55	47.87	50.09	57.78	62.78
7	18	68.78	69.98	78.90	81.76	86.98
8	24	76.98	80.76	83.76	91.90	92.78

Table 10: Drug release study of curcumin chitosan nano particles (CCH)

S.No	Time in hours	% of drug release CCH1	% of drug release CCH2	% of drug release CCH3	% of drug release CCH4	% of drug release CCH5
1	0	0.00	0.00	0.00	0.00	0.00
2	3	6.15	7.44	9.15	8.27	10.37
3	6	12.45	14.74	16.75	18.12	19.92
4	9	23.79	26.13	29.42	32.54	34.38
5	12	38.14	45.52	50.28	53.07	57.76
6	15	49.45	53.17	59.62	62.38	65.72
7	18	72.62	76.78	83.42	87.52	90.35
8	24	83.42	89.69	93.73	94.84	96.75

Stability studies results

The formulations subjected to the stability studies and the evaluation parameters performed after the study period was no significant changes with respect to the initial observations. Hence prepared formulation was physiochemically stable throughout study period.

CONCLUSION

In this present research work we had prepared curcumin nanoparticles of casein and chitosan as polymers with an objective of improving its bioavailability. Curcumin nanoparticles are prepared by using pH conservation and by using ionic gelation method. The prepared nanoparticles were evaluated for the number of parameters like particle size, zeta potential, drug

entrapment efficiency, drug content, *in-vitro* release, drug excipient interaction study, Scanning electron microscopy (SEM), Differential scanning calorimetry (DSC). In Preformulation study, estimation of curcumin was carried out by shimadzu UV spectrophotometer at λ max at 420 nm using methanol as solvent, which have a good reproductivity. The FT-IR spectrographs of pure, excipients and nanoparticles were taken which indicated no interaction of curcumin with carriers. The particle size of curcumin casein nanoparticles is in the range of 110±5.04nm to 248±10.7nm and for curcumin chitosan nanoparticle its 121±8.2nm to 242±4.3nm. Zeta potential is found to -16.3± 0.4mV to -39.07 ± 0.2mV for curcumin casein nanoparticles and -11.14± 0.4mV to -32.75± 0.53mV for curcumin chitosan nanoparticles. Drug content in the formulation range from 75.0±0.706% to 94.5±0.706% in CCN and 85.0±0.706% to 92.5±0.706% in CCH. The yield of production in the range of 60.3 to 61.2 for casein nanoparticles and 64.76 to 66.65. The *in-vitro* release studies suggest that release rate was related to drug: polymer ratio. Increase of drug release was observed as a function of drug: polymer ratio. The results were concluded that CCN5 and CCH5 can be considered as an optimized formula for sustaining the release of drug for over 24 hours and the formulation can be considered as best alternate to sustained release.

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