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PREPARATION AND EVALUATION OF NANOENCAPSULATED TOPICAL GEL OF CURCUMIN

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ABSTRACT

The objective of the present study is to development and evaluation of Curcumin nanoparticles prepared by ionic gelation technique using different ratios of drug and chitosan (1:1, 1:2, 1:3, 1:4, 1:5 and 1:6). From these 6 formulations the ideal formulations was selected and used for the curcumin nanoparticles bioadhesive topical gel. The obtained nanoparticles were discrete, spherical with free flowing properties and evaluated for process yield, loading efficiency, Zeta potential, particle size *in vitro* drug release, kinetic studies and stability studies. The drug carrier interactions were investigated by FT-IR spectroscopy and DSC. The optimized nanoparticles formulations FS-5 (drug: polymer ratio 1:5) were employed for topical gel preparation using stirring method in order to develop a controlled release. Nanoencapsulated curcumin containing bioadhesive topical gel (NCBTG) were characterized for pH, homogeneity, drug content, viscosity, spreadability, in vitro skin permeation and stability. The *in-vitro* experiments indicated a controlled release of drug and an acceptable bioadhesion quality for the formulation of topical gel (FTG)-3. Hence it can be concluded that the formulation has potential to deliver curcumin in a controlled manner for prolonged period over 12 hours than the other formulations and can be adopted for a successful delivery of curcumin for topical use.

KEYWORDS: Curcumin; Nanoparticles; Chitosan; Ionic gelation technique; Topical gel; Carbapol.

1. INTRODUCTION

Skin cancer is the most commonly diagnosed cancer. Every year, more than 1 million cases of SCC are discovered. According to the most recent statistics, more than 15,000 people die each year from squamous cell carcinoma of the skin, which is more than twice as many as melanoma. Every month, more than 5,400 people die from non-melanoma skin cancer around the world.[1] Natural products with chemo preventive and chemotherapeutic activities have been exploited for decades. Owing to their good safety profile and low-cost, researchers have been focusing on developing innovative drug formulations based on bioactive natural products as an effective alternative to the conventional chemotherapy which is usually associated with high toxicity and serious side-effects.[2] One of such interesting products is curcumin (diferuloylmethane) it is an active constituent of turmeric extract, which has gained a significant interest as a plant based compound along with anti-cancer activity.[3]

Curcumin has a variety of pharmacological activities such as antitumor, anti-inflammatory and antioxidation. Curcumin's application advancement has been hampered by its water insolubility, alkaline pH degradation, and low bioavailability.[4] So evade this difficulty, considerable efforts have been devoted to improve drug solubility. The development of drug nanocarriers for poorly soluble pharmaceuticals seems to be more promising.[5] Numerous studies have focused on preparing curcumin nanoformulations to enhance its pharmacokinetics and biopharmaceutical value in order to overcome these constraints. Among these nanoformulations are polymeric nanoparticles, which are usually prepared from biodegradable and biocompatible polymers such as chitosan which is a well-known semi-synthetic polymer and possesses some ideal properties of a polymeric carrier for nanoparticles such as biodegradability, non-toxicity, biocompatibility, and low cost.[6] Delivering the drug to its target site accurately and safely at the right time to achieve a controlled release and maximum therapeutic effect remains a benchmark in the design and development of newer drug delivery systems. Nanoparticles help to deliver targeted drug delivery, maintain drug effect in target tissue, improve oral bioavailability, and increase therapeutic agent stability against degradation.[7]

Topical delivery system is an important route of drug delivery for local and systemic disease. There have been

concerns about drug diffusion or release from the car and delivery through the skin with traditional topical dosage forms such as lotions, creams, ointments, and powder.[8] Because creams and lotions are quickly cleared from the skin and release the drug from the base, they also have low bioavailability. Non hydrophilic ointments are oleaginous, greasy and are non convenient to patients and also medicated powders for topical application have short residence time on the skin.[9] Gels are semisolid structures with a three-dimensional network of particles or solvated macromolecules in the dispersed phase limiting the movement of the dispersion medium. The semisolid state is caused by increased viscosity caused by interlacing and, as a result, internal friction. Also a gel may consist of twisted matted strands often tied together by stronger types of Vander Waals Forces to form crystalline and amorphous regions throughout the system.[10] The use of gel as a delivery system will extend the time that drugs spend on the skin, enhancing bioavailability. Gel delivery systems have several advantages such as the ease of administration, non greasy, patient compliance, high residence time on the skin and better drug release.[11]

2. MATERIALS AND METHODS

2.1. Materials

Curcumin was provided as a gift sample from Natural Remedies, Bangalore560100.

2.2. Preparation of Curcumin Nanoparticles[12-14]

Chitosan nanoparticles were made by ionic cross linking chitosan solution with TPP anions. Chitosan was dissolved in acetic acid (0.25v/v) aqueous solution at concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml. At room temperature, 5 ml of 0.84 percent (w/v) TPP aqueous solution was added drop by drop into 10 ml chitosan solution containing 10 mg of Curcumin using a syringe needle. 0.1 M NaOH was used to change the pH to 0.6. After that, the stirring was kept going for another 5 minutes. The nanoparticles suspensions were then homogenized at 8000rpm for 20 min. The interaction between the negative groups of the TPP and the positively charged amino groups of chitosan resulted in the nanoparticles formation. From all the 6 formulations the best and the ideal formulation was selected and used for the curcumin nanoparticles bioadhesive topical gel (CNBTG) preparation (Table 1).

Table no: 1 Preparation of Curcumin Nanoparticles.

Sl.No	Formulation code	Drug: Polymer ratio	TPP (ml)	RPM
1	FS-1	1:1	5	8000
2	FS-2	1:2	5	8000
3	FS-3	1:3	5	8000
4	FS-4	1:4	5	8000
5	FS-5	1:5	5	8000
6	FS-6	1:6	5	8000

2.3. Characterization of prepared nanoparticles[15, 16]

2.3.1. Fourier transform infra-red spectroscopy (FT-IR) analysis

The FT-IR spectra of pure Curcumin and chitosan nanoparticles loaded with Curcumin were recorded using Shimadzu IR spectrophotometer, Model 840, Japan, to check drug polymer interaction and stability of drug.

2.3.2. DSC Analysis

DSC thermograms were obtained using (Mettler, Switzerland). A small amount of dried nanoparticles powder was crimped in a standard aluminum pan and heated at a constant temperature of 10 °C/min from 25 to 400 °C.

2.3.3 Drug entrapment efficiency

Drug content was determined by centrifugation method. To separate the free drug in the supernatant, the redispersed suspension of nanoparticles was centrifuged at 15000 rpm for 40 minutes at 25°C.Concentration of Curcumin in the supernatant was determined by using UV-visible Spectrophotometer at 425 nm after suitable dilution. The drug entrapment efficiency (% EE) was determined using the relationship in equation 1: EE (%) = Experimental drug content x 100% / Theoretical drug content.

2.2.4 Surface morphology study

Scanning electron microscopy (SEM) of the nanoparticles of chitosan was performed to examine the surfaces morphology and particle size. The nanoparticles were mounted on metal wrappers, which could then be coated with conductive gold with using instrument's sputter coater. The photographs were taken using a Jeol scanning electron microscope under magnification of 11.6mm × 6.00K.

2.3.5 Particle size distribution

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

2.3.6. Zeta potential

Drug loaded nanoparticles was measured by Zeta sizer. To determine the zeta potential, nanoparticles samples were diluted with KCl (0.1 mm) and placed in electrophoretic cell where an electrical field of 15.2 V/cm was applied. Each sample was analyzed in triplicate.

2.3.7 *In vitro* release studies[17]

In vitro release studies were carried out by using dialysis tubes with artificial membrane. The prepared Curcumin nanoparticles were re-dispersed in 5 ml of phosphate buffer pH 7.4 and subjected to dialysis by immersing the dialysis tube to the receptor compartment containing 150

ml of phosphate buffer pH 7.4. The medium in the receptor was continuously agitated using a magnetic stirrer and the temperature was maintained at 37 ± 1˚C. 1ml sample of receptor compartment was taken at various intervals of time over a period of 12 h and each time 1 ml fresh buffer was replaced. The amount of drug released was determined spectrometrically at 427 nm.

2.3.8 Stability studies[18]

The stability study was carried out for the formulation FS-5. Formulation FS-5 was divided into 3 sets of samples and stored at 5^0± 3^0C in refrigerator, room temperature (30^0 ± 2^0C, 65% ± 5% RH) and 40^0 ± 2^0C, 75% ± 5% RH in humidity control ovens. Drug content of all samples were determined by the method as in drug content at 0 month, 3months and 6 months. *In vitro* release study of formulation FS-5 was also carried at 0 month, 3months and 6 months of storage.

2.4. Preparation of Curcumin Nanoparticles Topical Gel[19]

Selected batches of prepared Nanoparticles were then incorporated in gels prepared by mechanical stirring with polymer carbapol 934 with different concentrations and other formulation additives. The experimental design of the gel formulated was expressed in Table 2.

Table no 2: Experimental designs of Nanoencapsulated Topical Gels.

Nanoencapsulated Topical Gels compositions Amount taken in percentage (w/w)						
Formulation	**Nanoparticles**	**Carbopol 934**	**Triethanolamine**	**Alcohol**	**Propylene glycol**	**Distilled Water**
FTG1	1	0.2	0.5	20	10	q.s
FTG2	1	0.4	0.5	20	10	q.s
FTG3	1	0.6	0.5	20	10	q.s
FTG4	1	0.8	0.5	20	10	q.s

2.5. Characterization of Formulations[20-22]

2.5.1 Determination of pH

The pH of the carbapol gels were determined by a digital pH meter (Model MK- VI, Kolkata). 1g gel was dissolved in 25 ml of distilled water and the electrode was then dipped into gel formulation and reading was noted. The pH measurements of each formulation were replicated three times.

2.5.2 Viscosity Measurements

A Brookfield Rotational Digital Viscometer DV II RVTDV-II was used to measure the viscosity (in cps) of the gels. The spindle was rotated at 10 rpm. Samples of the gels were allowed to settle over a period of 30 min at the assay temperature (25 ± 1°C) before the measurements were taken.

2.5.3 Spreadability

It is measured in seconds the time it takes for two slides to detach from the gel and be put in between the slides under the impact of a particular load; the shorter the time it takes for two slides to separate from the gel, the greater the spreadability. Spreadability is calculated using the formula S = M. L / T, where M is the weight attached to the upper slide. L stands for the duration of the glass slides. T is the amount of time it took to separate the slides. The pH of each gel was determined using a pH meter that had been calibrated with standard buffer solutions at pH 4, 7, and 9 before each use. After that, the electrode was placed into the sample for 10 minutes until the reading was taken at room temperature.

2.5.4 Homogeneity

All the developed gels were tested for its homogeneity by visual inspection once after the gels have been set in the container. They were tested for their presence of any aggregates and appearance.

2.5.5 Drug Content Studies

By dissolving an appropriately weighted quantity of gel (about 100 mg) in about 50 ml of pH 6.4 phosphate buffer containing 20% v/v ethanol, the drug content of the gel formulations was calculated. The solutions were quantitatively transferred to volumetric flasks and dilutions were carried out using the same buffer solution. After that, the solutions were filtered through a 0.45 m membrane filter before subjecting the solutions to spectrophotometric analysis for CUR at λmax of 427 nm. Drug content was calculated from the linear regression equation obtained from the data of calibration study.

2.5.6 *In Vitro* Release and Release kinetics

The *in vitro* release studies were carried out by using Franz-diffusion cells apparatus from different formulations. An exact amount of formulations (1.0 g) was spread out on membrane between the donor and receptor compartments with an available diffusion area. The receptor compartment was filled with pH 6.8 phosphate buffer and continuously stirred with a small magnetic bar at a speed of 150 rpm during the experiments to ensure homogeneity and maintained at 37.2±0.5ºC. The samples were withdrawn at various interval of time and replaced with the same volume of phosphate buffer solution. Sink conditions were met in all cases. The samples were analyzed spectrophotometrically at 427 nm (Shimadzu UV-Visible-1800).

In order to study the exact mechanism of drug release from the nanoparticles-containing gels, drug release data

were analyzed according to zero order, first order, Higuchi square root, Korsmeyer-peppas equation.

2.5.7 Stability study

For the evaluation of stability studies, maintaining the formulations at an ambient condition over a period of three months. The physical appearance, rheological properties, pH value, drug content, drug release studies were determined periodically.

3. RESULTS AND DISCUSSION

3.1. Physicochemical characterization of nanoparticles

Spherical nanoparticles were formed spontaneously upon the incorporation of solution of TTP to the chitosan solution under magnetic stirring. Nanoparticles of chitosan were obtained by ionic gelation which is a simple process, where particles are formed by means of electrostatic interactions between the positively charged chitosan chains and polyanions employed as cross linkers.

The FTIR spectrum shows that there were no significant changes in the chemical integrity of drug and also indicates that the polymer and drug are compatible with each other (Fig. 1-3). SEM analysis revealed that nanoparticles prepared using the ionic gelation technique was discrete. (Fig. 4), their mean size distribution was found to be 56.4 nm.

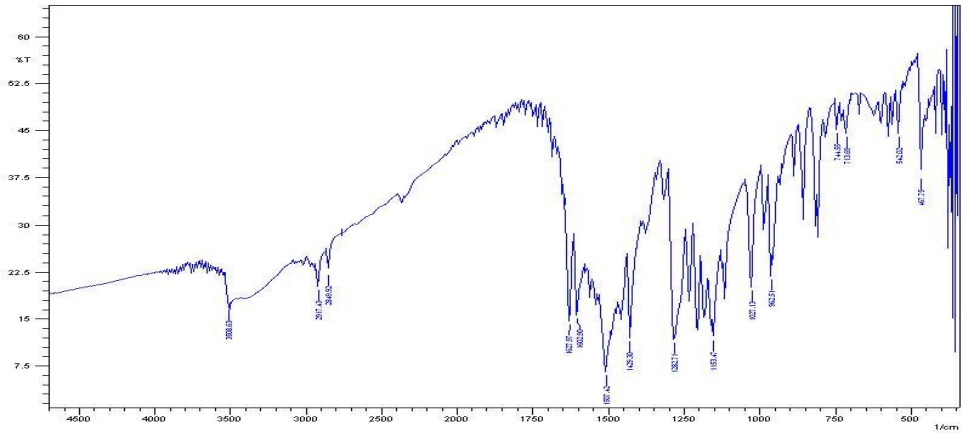

Figure 1: Fourier transform infra-red spectroscopy of Curcumin.

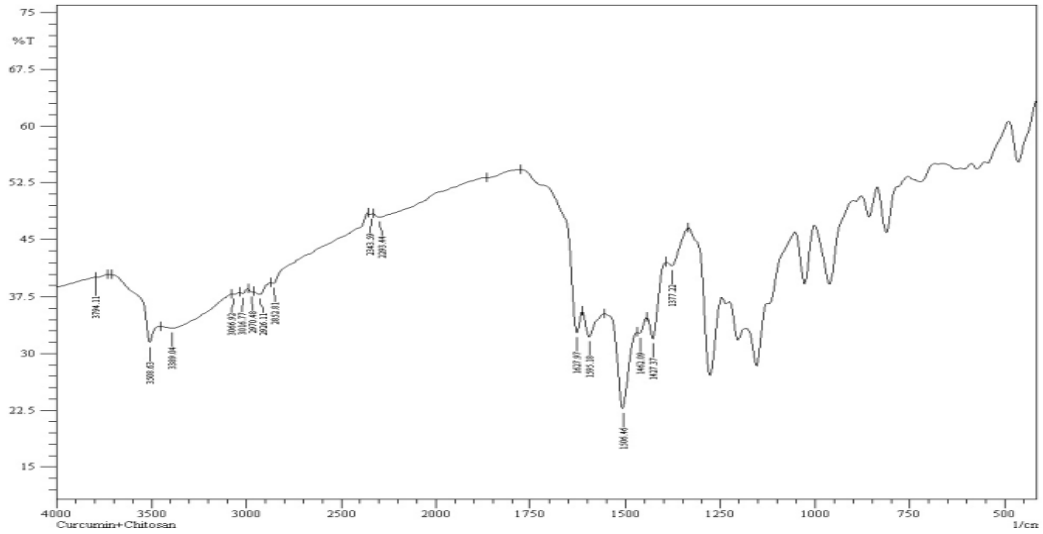

Figure 2: Fourier transform infra-red spectroscopy of Curcumin and Chitosan.

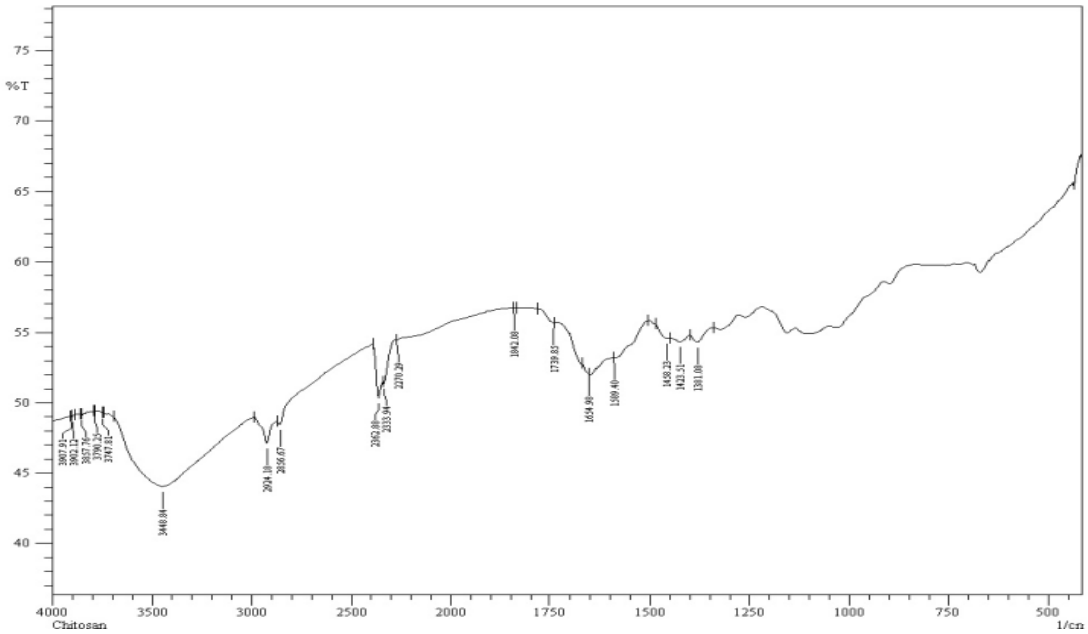

Figure 3: Fourier transform infra-red spectroscopy of Chitosan.

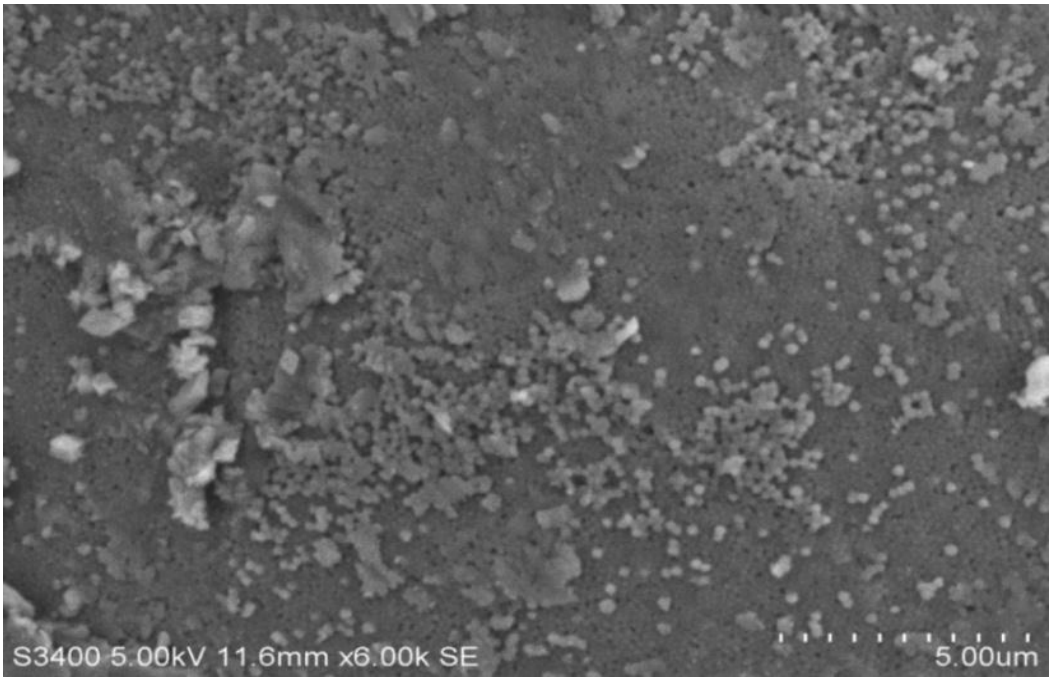

Figure 4: SEM images of Formulation FS-5.

3.2. Differential scanning colorimetry

When comes to the preparation of drug formulation, it becomes necessary to evaluate the interaction between the drug and the polymer. The DSC thermogram of pure curcumin shows a sharp endothermic peak at 182ºC which corresponds to its melting point. The thermogram of curcumin with chitosan shows sharp endothermic peak at 185ºC (Fig. 5, 6).

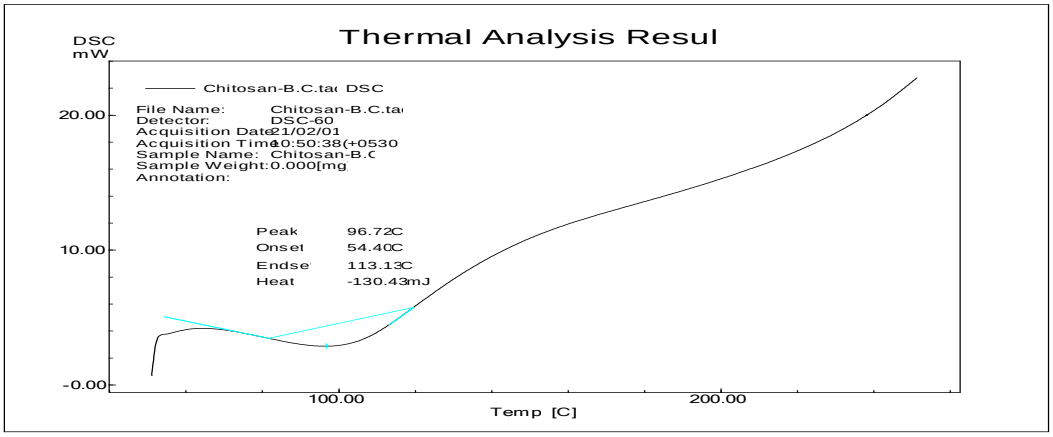

Figure 5: Differential scanning calorimetry thermograph of Chitosan.

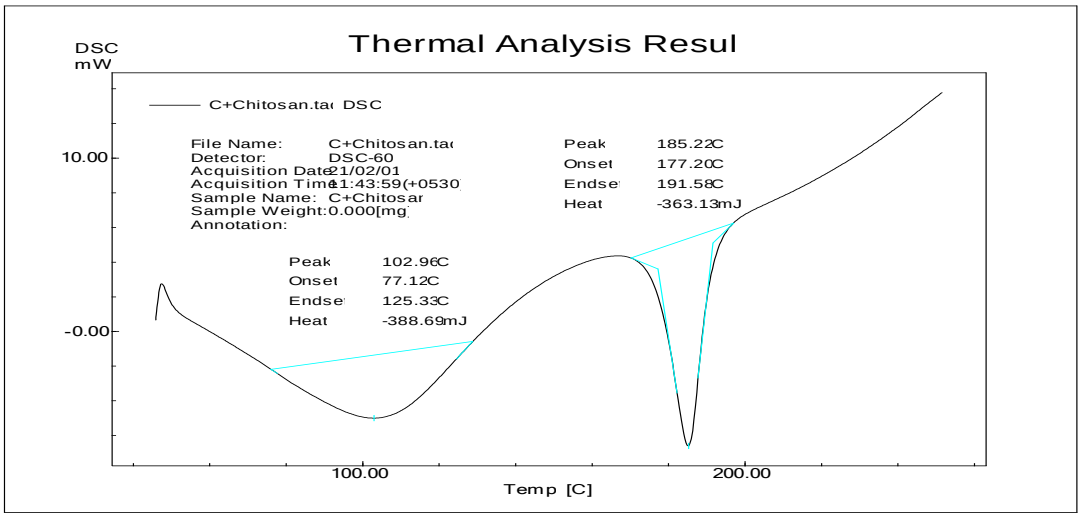

Figure 6: Differential scanning calorimetry thermograph of Curcumin + Chitosan.

3.3. Drug Entrapment Efficiency and Zeta potential

The drug entrapment efficiency of nanoparticles containing drug: polymer in various ratios of 1:1, 1:2, 1:3, 1:4, 1:5 and 1:6 were found to be 44.6%, 57.5%, 62.3%, 78.4%, 92.64% and 81.23% (Table 3). Hence there was a steady increase in the entrapment efficiency on increasing the concentration of polymer in the formulation. The formulation FS-5 registered highest entrapment of 92.64% (Fig. 7).The high entrapment efficiency is likely due to electrostatic interactions between the drug and the polymer. Zeta potential of FS-5 nanoparticles was in the range of 1.2 mV, and it shows good stability.

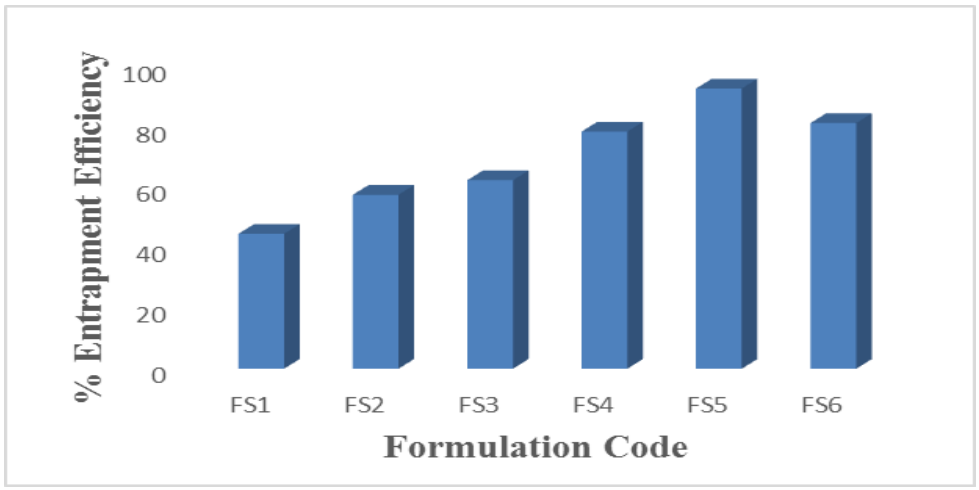

Figure 7: Percentage Entrapment Efficiency of Formulations FS1-FS6.

Table no 3: Percentage yield, drug content and entrapment of formulation FS1-FS6.

Formulation Code	%yield	%Drug content	%Entrapment efficiency
FS-1	49	46	44.6
FS-2	57.6	56	57.5
FS-3	62.8	57	62.3
FS-4	78	63.2	78.4
FS-5	90	89.5	92.64
FS-6	82	65	81.23

3.4. In vitro release of nanoparticles

Cumulative percentage drug released for FS-1, FS-2, FS-3, FS-4, FS-5 and FS-6 after 12 h were found to be 43.52%, 57.68%, 64.98%, 76.93%, 85.89% and 71.43% respectively. It was apparent that *in vitro* release of curcumin of FS-5 formulation showed maximum drug. And hence FS-5 formulation was considered as the ideal formulation of the study shown in Fig. 8

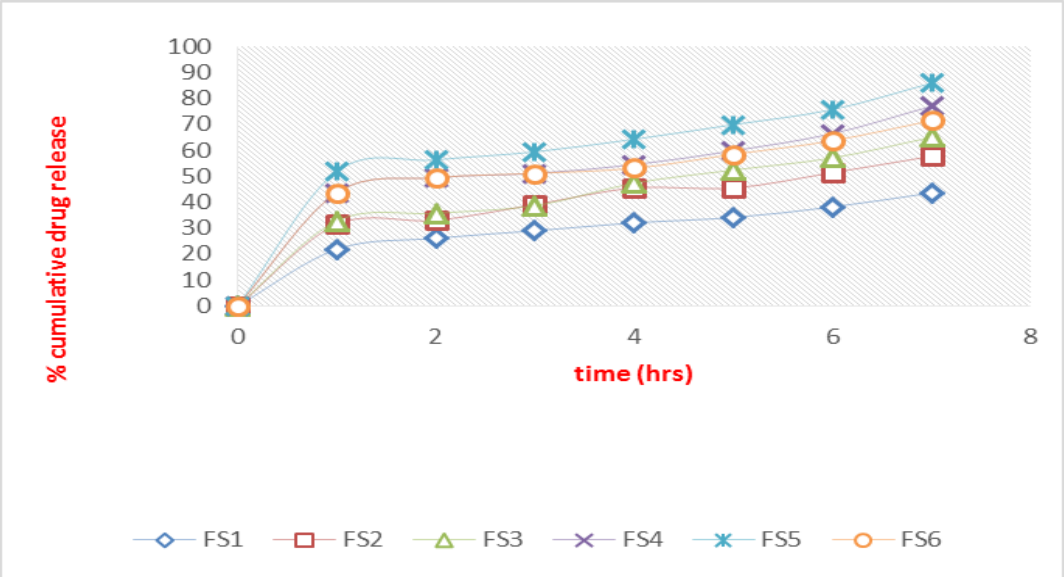

Figure 8: *In vitro* release profiles of Curcumin nanoparticles.

3.5. Stability studies

The results of drug content of ideal formulation FS-5 after 6 months of stability testing at different storage conditions were shown in (Table 4) *In vitro* release profiles for the same formulation was stored at different storage conditions. It was observed that there was a slight increase in drug content when the formulation was stored at $5^0\pm 3^0$C which showed 84.04% and at Room temperature it showed 83.35% and at 40 ± 2˚C/75% there was a decrease in the drug content which showed 79.68% (Fig: 9, 10).

Table no 4: Stability study comparison of drug content of formulation FS-5 at $5^0\pm 3^0$C, Room temperature and at 40 ± 2˚C/75% RH after 6 months.

Temperature in ºC	% Drug content
$5^0\pm 3^0$C	84.04
30 ± 2˚C	83.35
40 ± 2˚C/75% RH	79.68

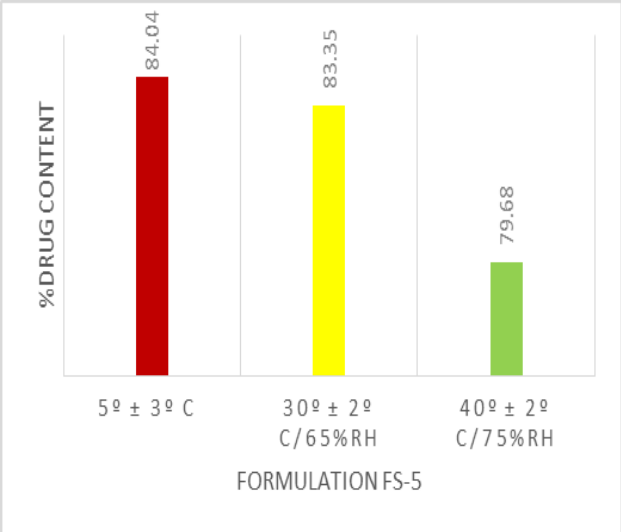

Figure 9: Stability study comparison of drug content of formulation FS-5 at $5^0\pm 3^0$C, Room temperature and at 40 ± 2˚C/75% RH after 6 months.

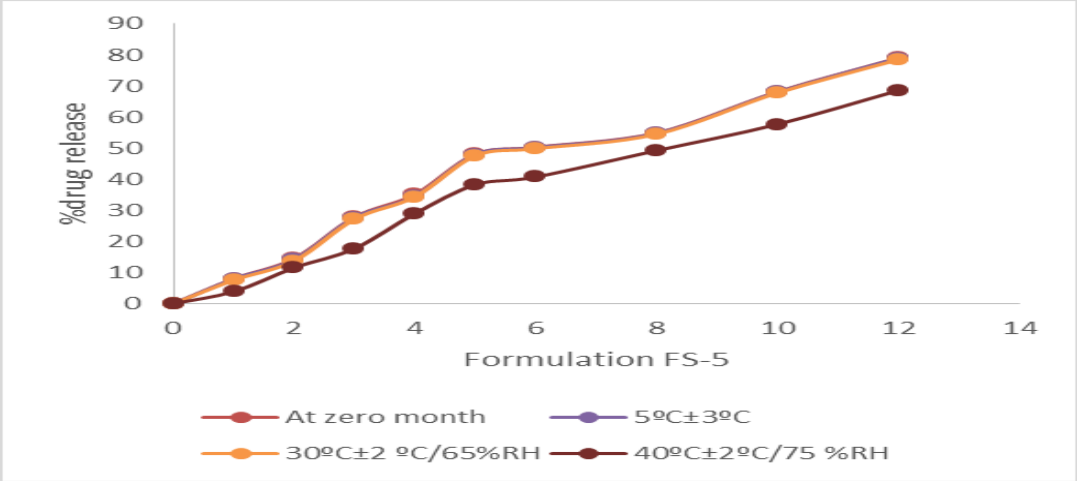

Figure 10: *in vitro* Drug Release of Formulation FS-5 after six month of storage at 5º± 3ºC, room temperature 30º ± 2ºC/65% ± 5% RH and 40 ± 2ºC/75% ± 5% RH.

Evaluation Parameters of Prepared Curcumin Nanoparticles Topical Gel

The gel formulations were uniformly smooth and homogeneous in appearance. The Carbopol curcumin gels were transparent, dark yellow gummy with smooth and homogeneous appearance. All preparations were spreadable easily, with good bioadhesion and fair mechanical properties.

3.6. pH

The pH values ranged from 6.8 to 7 for all the formulations which were in acceptable range to avoid skin irritation after application to the skin (Table 5)

3.7. Viscosity

Viscosity is an important physical parameter which will reflect the consistency in case of topical preparations and it also affects the rate of drug release. High viscosity is due to high polymeric entanglements; therefore, the resistance to deformation will be increased and it will lead to more rigid structure. The highest viscosity was found for FTG-1 formulation (11639.7) due to high polymer concentration and FTG-3 showed (6500.3) low viscosity (Table 5)

3.8. Spreadability

FTG-1 formulation showed the lowest spreadability, due to the high polymer concentration. The ability of the gel to spread has decreased due to high polymeric entanglements FTG-3 shows high spreadability (Table 5)

3.9. Extrudability

Extrudability of FTG-1 formulation was found good and extrudability of FTG-3 formulation was excellent (Table 5)

3.10. Drug content and Uniformity

All the formulations have satisfactory appearance, clarity and drug content in the range of 87.5 to 95.6. Formulation FTG-3 showed maximum drug content. Drug content data indicates the suitability of the applied method for semisolid system preparation (Table 5)

3.11. In vitro dissolution profile

In vitro dissolution profile of curcumin gels containing different concentration of carbapol are shown in Table 5 and Fig. 11. Curcumin release profiles across the egg membrane from various gel formulations revealed that drug release decreases as the concentration of the gelling agent increases. Drug release values were also found to be lower in formulations with a high polymer concentration.

Table no 5: Evaluation Parameters of Prepared Curcumin Nanoparticles Topical Gel.

Formulation code	FTG-1 (0.2)	FTG-2 (0.4)	FTG-3 (0.6)	FTG-4 (0.8)
Drug content (%) (X±S.D)	87.5	93.2	98.9	95.6
Drug content uniformity	*	**	***	**
pH	6.9	6.95	6.8	7
Extrudability	*	**	***	**
Spreadability(g.cm/sec)	5.9	6.1	8.5	7.3
Viscosity(cps)(X × 104)	11639.7	9040.67	6500.3	7900.07
In vitro drug diffusion(%)(12h study)	64.98±1.25	46.95±1.97	41.22±0.75	57.68±1.12
Bioadhesive strength (kg)	3.5	6.3	7.9	5.2
Bioadhesion Time(hr)	10-12	15-17	35-37	22-24

(Good), ** (Very Good), * (Excellent)*

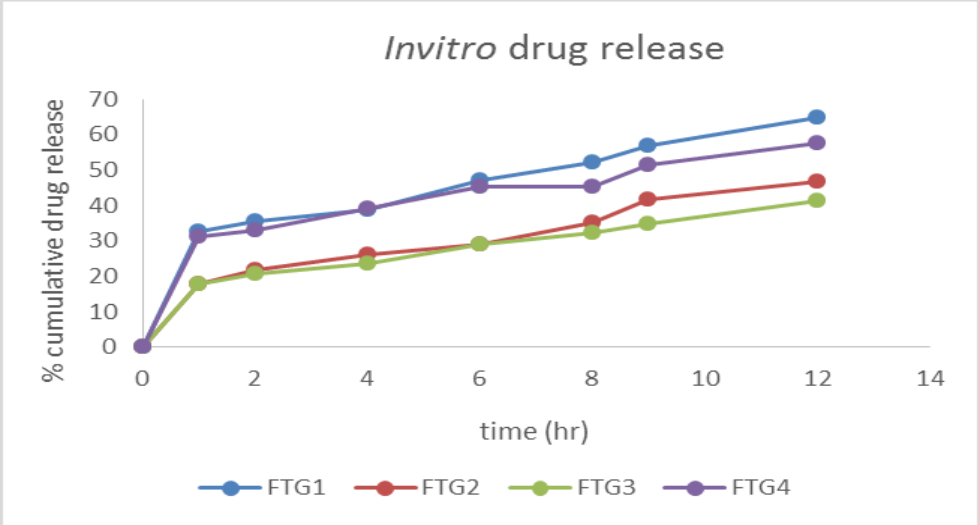

Figure 11: *In vitro* release profiles of Curcumin nanogel formulations.

3.12. Release kinetics

The *in vitro* drug release data of the formulation FTG-3 was fitted to various mathematical models, it showed linear nature between cumulative percentage drug released and time suggesting that it followed zero order kinetics. The best fit with higher correlation was found with zero order r^2 =0.92. The diffusion co-efficient data indicates that the formulation FTG-3 release the drug by diffusion which follows non-fickian transport mechanism (Table 6)

Table no 6: Mathematical model release kinetics of optimized batch.

MODEL NAME	r^2
Zero order	0.92
First order	0.90
Higuchi square root	0.99
Korsmeyer and peppas	0.51
N	0.85

3.13. Stability studies

The CUR nano gel was found stable upon storage over the period 3 months. The results of drug content of ideal formulation FTG-3 after 3 months of stability testing at different storage conditions were shown in Fig. 12. It was observed that there was a slight increase in drug content when the formulation was stored at $5^0\pm 3^0$C which showed 92.04% and at Room temperature it showed 88.35% and at 40 ± 2˚C/75% there was a decrease in the drug content which showed 56.23%. (Table 7)

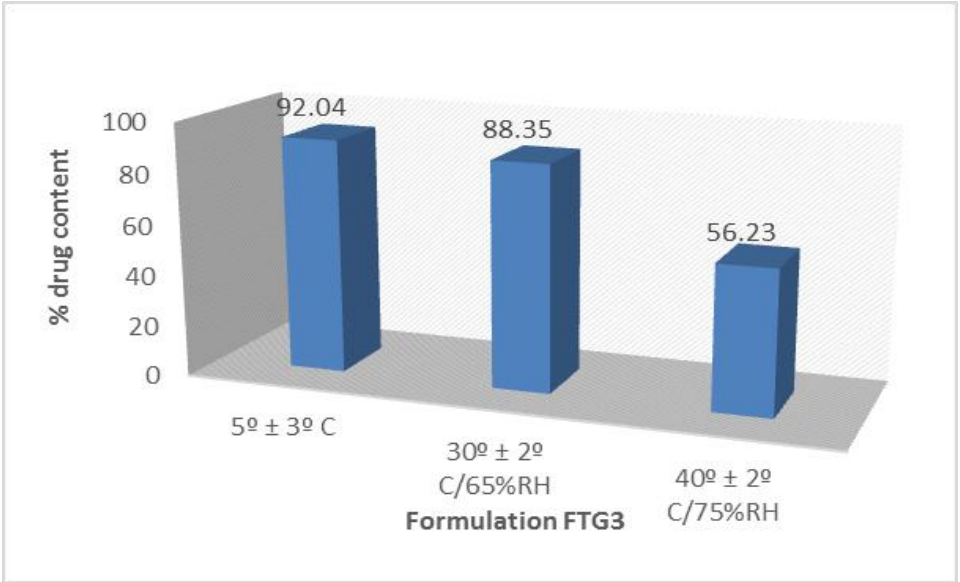

Figure 12: Stability study: comparison of drug content of formulation FTG-3 at $5^0\pm 3^0$C, Room temperature and at 40 ± 2˚C/75% RH after 3 months.

Table no 7: Stability study of nano gel formulation FTG-3 at $5^0\pm 3^0$C, Room temperature and at 40 ± 2˚C/75% RH after 3 months.

Temperature in ºC	% Drug content
$5^0\pm 3^0$C	92.04
30 ± 2˚C	88.35
40 ± 2˚C/75% RH	56.23

4. CONCLUSION

Chitosan nanoparticles have been used to encapsulate curcumin which enhances its solubility, and improve its efficacy against cancer cells. Based on drug content, drug entrapment efficiency, particle size morphology, zeta potential and *in vitro* release, formulation FS-5 was selected as an optimal formulation. Stability studies were carried out for the selected formulation FS-5. The stability studies showed the maximum content of the drug for FS-5 formulation stored at $5^0\pm 3^0$C, Room temperature and at 40 ± 2˚C/75% RH. Thus, with the combination of the prepared curcumin nanoparticles, it is possible to achieve higher damage to tumor cells using lower dosage of curcumin in a relatively brief time. Topical route of application has a great potential as an effective and safe way to administer curcumin. To overcome the side effects associated with Curcumin therapy and to have the benefits associated with topical therapy; Curcumin topical gels are prepared in this study. It showed that drug release was decreased with increasing in the concentration of gelling agent. The *in vitro* release showed controlled release of the drug over a period of 12 hours, therefore with an intention to keep the Curcumin at the site of action, and thus prolonging the time of absorption. Also topical application yields better patient compliance.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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