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Preparation And Evaluation Of Chitosan Loaded Curcumin Nanoparticles

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Abstract

Natural compounds are emerging as an effective agent for the treatment of malignant diseases. 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. Curcumin is physiologically well tolerated, with negligible systemic toxicity. Despite Curcumin's superior properties as anti-cancer agent its applications are minimized due to its low cellular uptake and low solubility. The aim of the present study was to develop a method for the preparation of nanoparticles of curcumin with a view to improve its aqueous-phase solubility and examine the effect on its anticancer activities. The curcumin loaded nanoparticles were prepared by ionic gelation technique by using different ratios of drug and chitosan (1:1, 1:2, 1:3, 1:4, 1:5 and 1:6) by adding TTP (Tripolyphospahte anions) solution. Nanoparticles were formulated and evaluated for process yield, loading efficiency, Zeta potential, particle size in vitro drug release, kinetic studies and stability studies. The chitosan nanoparticles have a diameter of particle ranging approximately 56.4 nm and a Zeta potential 1.2 mV. The results showed that the water solubility and anticancer property of curcumin was improved by reduction of particle size up to the nano range and there was a steady increase in the entrapment efficiency on increasing the concentration of polymer in the formulations and thus chitosan- based delivery system opens new and interesting perspectives as drug carriers.

Keywords: *Nanoparticles, Chitosan, Curcumin, Ionic gelation technique.*

1. INTRODUCTION

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[1]. One of such interesting products is curcumin which is a natural polyphenolic compound found in the rhizomes of Curcuma longa. It has low intrinsic toxicity but a wide range of pharmacological activities including antitumor, antioxidant, and anti-inflammatory properties[2]. Curcumin has also been intensively investigated as a therapeutic or preventive agent against cancer. However, the effectiveness of curcumin is limited due to its poor instability at physiological pH, low bioavailability and water solubility. To evade this difficulty, considerable efforts have been devoted to improve drug solubility. The development of drug nano carriers for poorly soluble pharmaceuticals seems to bemore promising.[3] To overcome these limitations, numerous studies have been focusing on preparing curcumin nanoformulations to improve its pharmacokinetics and biopharmaceutical value. 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. It possesses a positive charge and exhibits an absorption enhancing effect. This characteristic can be employed to prepare cross-linked chitosan nanoparticles[4]. The aim of this work is to utilize chitosan to prepare curcumin loaded nanoparticles that have the ability to destroy cancer cells in an effective and selective manner without the need of additional modifications. Therefore, chitosan nanoparticles were prepared using ionic gelation method[5]. The prepared nanoparticles have been characterized and evaluated for their physicochemical characteristics such as size and surface charge, shape, zeta potential, drug loading capacity and in vitro release characteristics[6].

2. Methods and materials

2.1 Materials:

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

2.2 Preparation of Curcumin Nanoparticles[7-9]

Nanoparticles of chitosan were prepared by ionic cross linking of chitosan solution with TPP anions. Chitosan was dissolved in aqueous solution of acetic acid (0.25 v/v) at various concentrations such as 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg/ml. At room temperature under the magnetic stirring, 5 ml of 0.84% (w/v) TPP aqueous solution was added drop by drop using syringe needle into 10 ml chitosan solution containing 10 mg of Curcumin. pH was adjusted to 6.0 by adding 0.1 M NaOH. The stirring was then continued for about 5 minutes. The resultant suspensions of nanoparticles were then homogenized at 8000rpm for 20min. The formation of the nanoparticles was a result of the interaction between the negative groups of the TPP and the positively charged amino groups of chitosan (table 1).

2.3 Characterization of prepared nanoparticle[10, 11]

2.3.1 Fourier transforms 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 thermo grams were obtained using (Mettler, Switzerland). Small amount of powder of the dried nanoparticles was crimped in a standard aluminum pan and heated from 25 to 400 °C at a heating rate of 10 °C/min under constant heating.

2.3.3 Drug entrapment efficiency

Drug content was determined by centrifugation method. The redispersed suspension of nanoparticles was centrifuged at 15000 rpm for 40 min at 25˚C to separate the free drug in the supernatant. 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. 3. 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 a metal stubs and the stub was then coated with conductive gold with sputter coater attached to the instrument. 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[12]

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 425 nm.

2.3.8 Stability studies[13]

The stability study was carried out for the formulation FS-5. Formulation FS-5 was divided into 3 sets of samples and stored at $5^0 \pm 3^0$C in refrigerator, room temperature ($30^0 \pm 2^0$C, 65% ± 5% RH) and $40^0 \pm 2^0$C, 75% ± 5% RH in humidity control ovens. Drug content of all samples were determined by the method as in drugcontent 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.

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). Nanoparticles prepared by ionic gelation technique were found to be discrete and through SEM analysis (Fig: 4), their mean size distribution was found to be 56.4 nm.

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).

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 2). 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.

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 (Fig: 8).

3.5 Stability studies

The results of drug content of ideal formulation FS-5 after 6months of stability testing at different storage conditions were shown in (Table 3) *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).

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 optimum 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. The potential of these nano formulations for tumor therapy could be further realized by performing *in vivo* studies.

5. Declaration of interest

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

6. Acknowledgements

The authors are thankful to Natural Remedies for providing the drug as a gift sample for this work.

FIGURES AND TABLES

Table 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

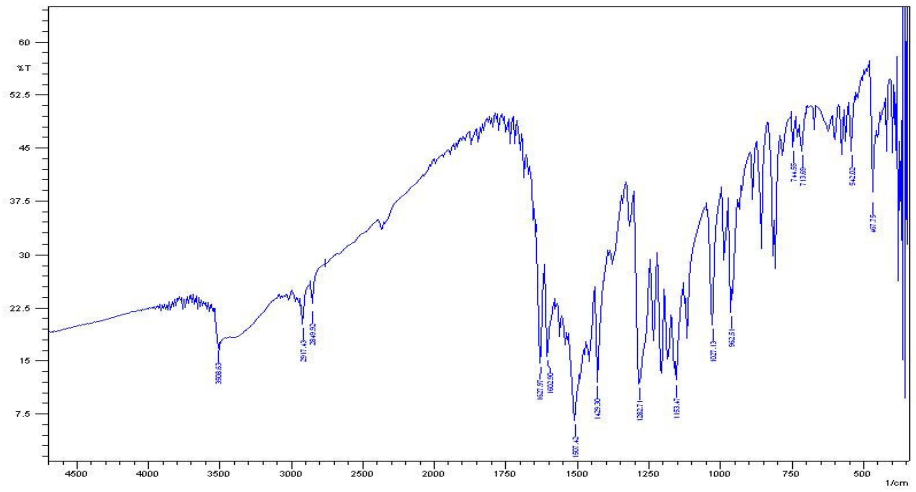

Figure 1: FTIR Spectrum of Curcumin

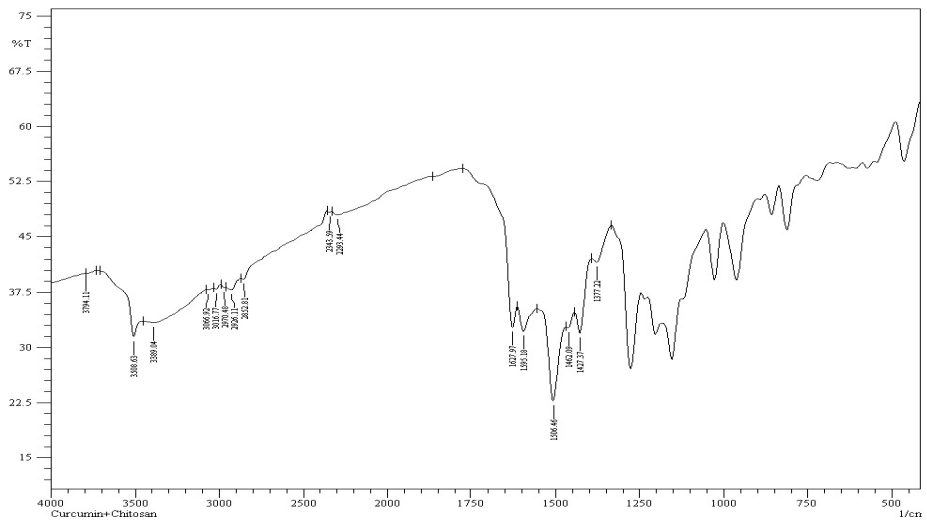

Figure 2:

FTIR Spectrum of Curcumin and Chitosan

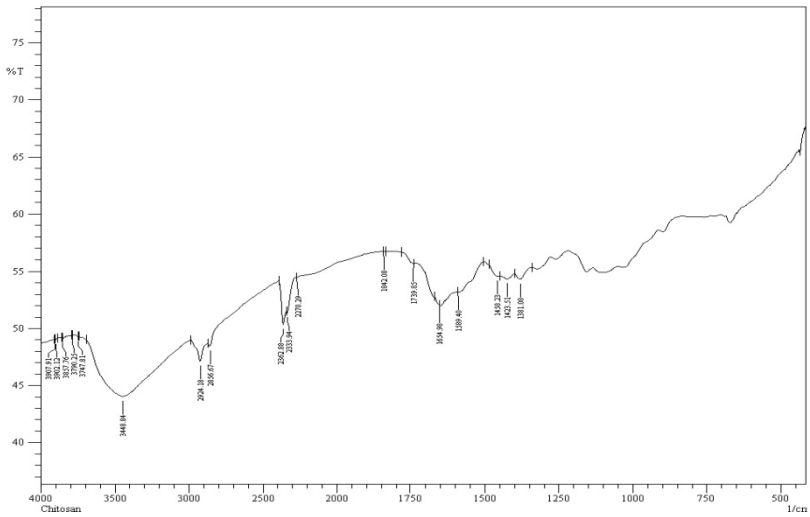

Figure 3: FTIR Spectrum of Chitosan

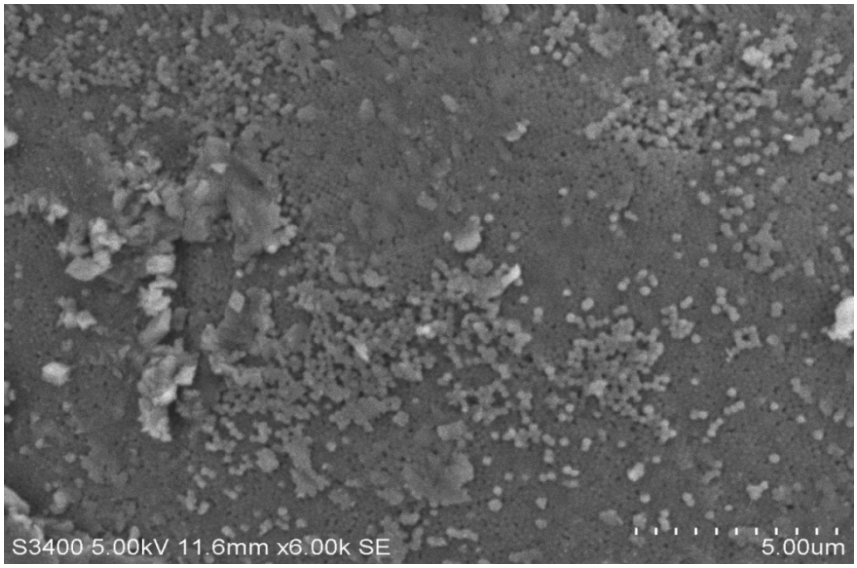

Figure 4: SEM images of Formulation FS-5

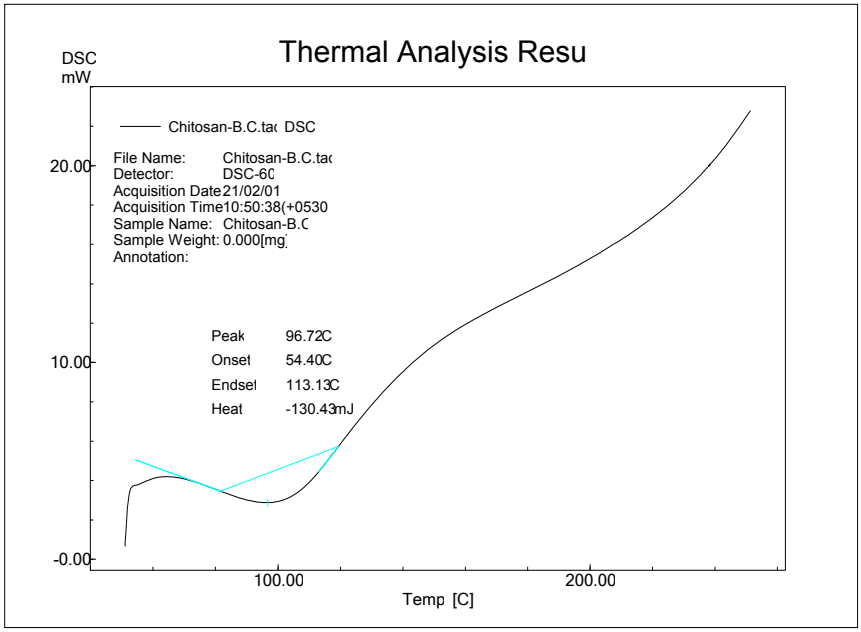

Figure 5: DSC Thermograph of Chitosan

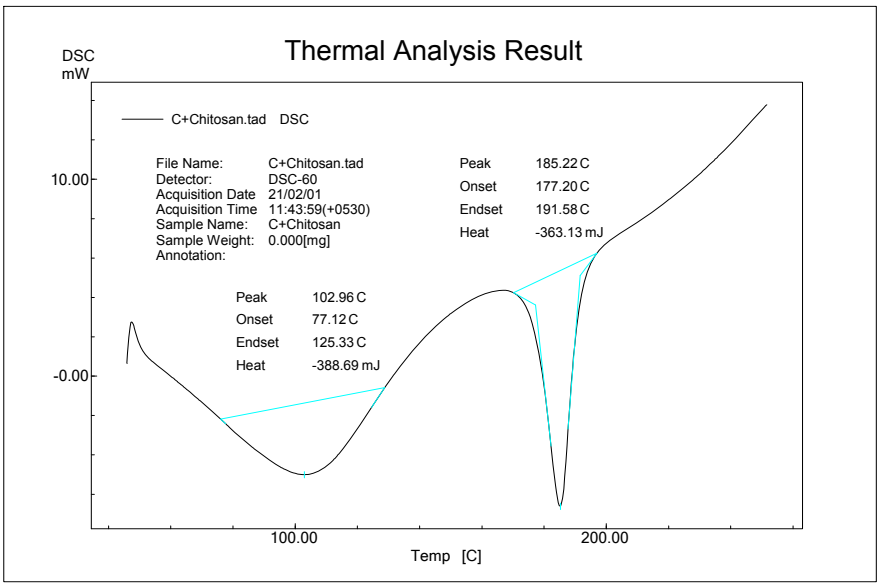

Figure 6: DSC Thermograph of Curcumin + Chitosan Nanoparticles

Table 2: 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

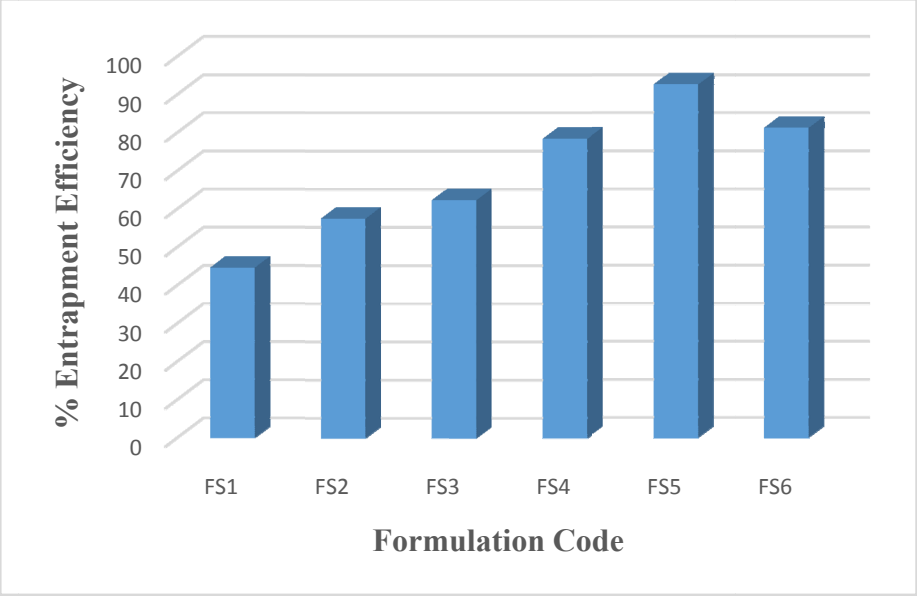

Figure 7: Percentage Entrapment Efficiency of Formulations FS1-FS6

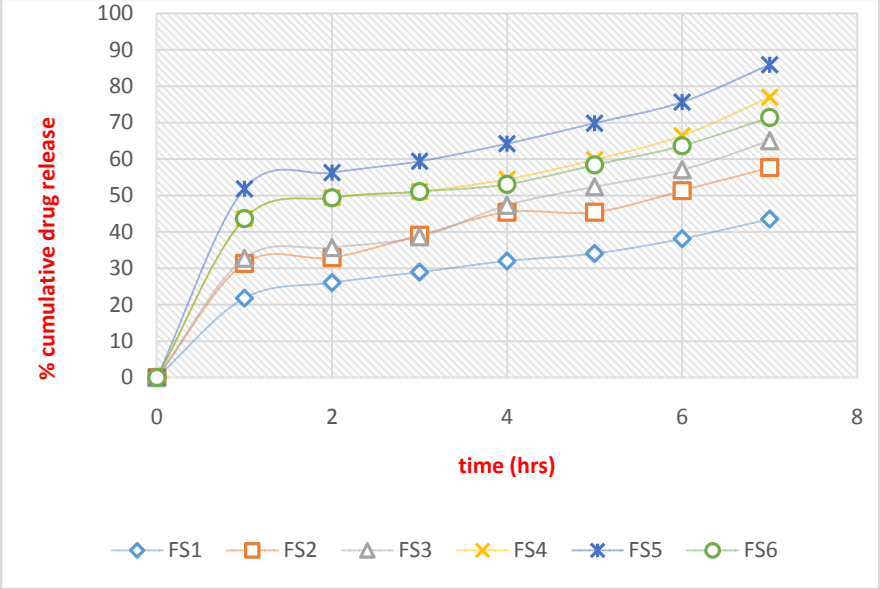

Figure 8: *In vitro* release profiles of Curcumin nanoparticles

Table 3: 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

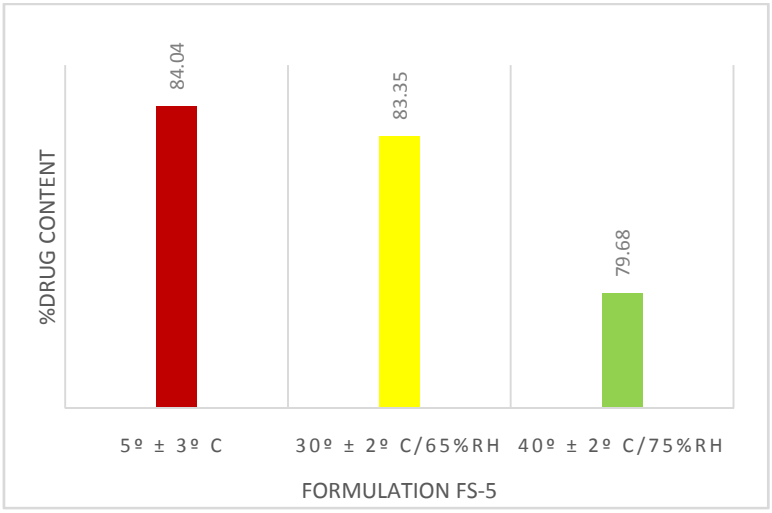

Figure 9: Stability study: comparison of drug content of formulation FS-5 at $5^0 \pm 3^0$C, Room temperature and at 40 ± 2˚C/75% RH after 6 months

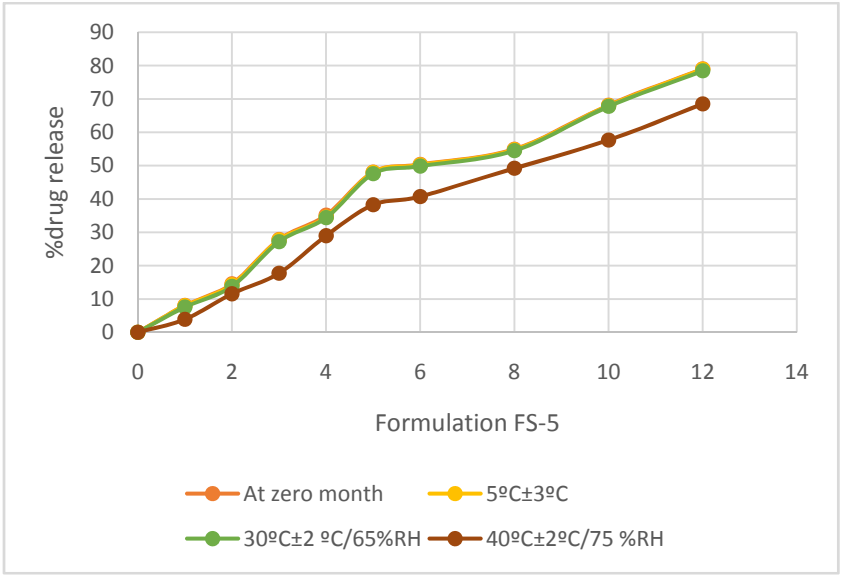

Figure 10: Stability study - *in vitro* Drug Release of Formulation FS-5 after six month of storage at 5º± 3ºC, room temperature 30º ± 2ºC/65% ± 5% RH and 40 ± 2ºC/75% ± 5% RH.

7. REFERENCES

[1] *Duse L, Baghdan E, Pinnapireddy SR, Engelhardt KH, Jedelská J, Schaefer J, Quendt P, Bakowsky U. Preparation and characterization of curcumin loaded chitosan nanoparticles for photodynamic therapy. physica status solidi (a). 2018;215(15):1700709.*

[2] *Mehanny M, Hathout RM, Geneidi AS, Mansour S. Exploring the use of nanocarrier systems to deliver the magical molecule; curcumin and its derivatives. J cont rel 10;225:1-30.*

[3] *Trapani A, Denora N, Iacobellis G, Sitterberg J, Bakowsky U, Kissel T. Methotrexate-loaded chitosan-and glycolchitosan-based nanoparticles: a promising strategy for the administration of the anticancer drug to brain tumors. Aaps Pharmscitech 2011;12(4):1302-11.*

[4] *Agel MR, Baghdan E, Pinnapireddy SR, Lehmann J, Schäfer J, Bakowsky U. Curcumin loaded nanoparticles as efficient photoactive formulations against gram-positive and gram-negative bacteria. Colloids and Surfaces B: Biointerfaces 2019;178:460-8.*

[5] *Sommerwerk A, Brüßler J, Schäfer J, Baginski L, Bandulik M, Bakowsky U. Lipid coated chitosan microparticles as protein carriers. physica status solidi c 2011;8(6):1978-84.*

[6] *Kumar MR, Muzzarelli R, Muzzarelli C, Sashiwa H, Domb AJ. Chitosan chemistry and pharmaceutical perspectives. Chemical reviews 2004;104(12):6017-84.*

[7] *Wu Y, Yang W, Wang C, Hu J, Fu S. Chitosan nanoparticles as a novel delivery system for ammonium glycyrrhizinate. Int j pharm 13;295(1-2):235-45.*

[8] *Aktaş Y, Andrieux K, Alonso MJ, Calvo P, Gürsoy RN, Couvreur P, Çapan Y. Preparation and in vitro evaluation of chitosan nanoparticles containing a caspase inhibitor. Int J pharm 2005;298(2):378-83.*

[9] *Zengshuan M, Tit Meng L, Lee-Yong L, et al. Pharmacological activity of peroral chitosan insulin nanoparticles in diabetic rats. Int J Pharm 2005; 293:271-80.*

[10] *Peltonen L, Koistinen P, Karjalainen M, Häkkinen A, Hirvonen J. The effect of cosolvents on the formulation of nanoparticles from low-molecular-weight poly (I) lactide. Aaps Pharmscitech 2002;3(4):52-8.*

[11] *Cui F, Oian F, Yin C. Preparation and characterization of mucoadhesive polymercoated nanoparticles. Int J Pharm 2006; 316:154-61.*

[12] *Pandey R, Ahmad Z, Sharma S, Khuller GK. Nano-encapsulation of azole antifungals: potential applications to improve oral drug delivery. International Journal of Pharmaceutics 2005;301(1-2):268-76.*

[13] *Chandrudu J. Preparation and evaluation of repaglinide nanosuspensions. International Journal of Novel Trends in Pharmaceutical Sciences 2019;9(1):6-11.*