Adlin et.al IJPPA, 2018; VOL-02(01); 37-44

Original Research Article

ISSN: 2394-1618

IP INDEX Impact Factor is 2.608

INTERNATIONAL JOURNAL OF PHARMACY AND PHARMACEUTICAL ANALYSIS

"PREPARATION, CHARACTERIZATION AND EVALUATION OF CURCUMIN-LOADED CHITOSAN NANOPARTICLES"

J Adlin Jino Nesalin*[a], T Tamizhmani[a]

*[a]Bharathi College of Pharmacy, Bharathinagara, KM Doddi, Maddur Taluk, Mandya District, Karnataka-571422, India. E-Mail ID: adlinjino@gmail.com

ABSTRACT

Curcumin is a highly potent, nontoxic; bioactive agent found in turmeric and has been known for centuries as a household remedy to many ailments. Among the potent anticancer agents, curcumin has been found to be very efficacious against different types of cancer cells. The only disadvantage that it suffers is of low aqueous solubility and poor bioavailability. The curcumin loaded nanoparticles were prepared by ionic gelation of chitosan with tripolyphosphate anions (TPP). Nanoparticles of different core: coat ratio were formulated and evaluated for process yield, loading efficiency, particle size, zeta potential, in vitro drug release, kinetic studies and stability studies. The chitosan nanoparticles have a particle diameter ranging approximately 342-712 nm and a zeta potential -30.35to -15.9 mV. There was a steady increase in the entrapment efficiency on increasing the polymer concentration in the formulations. The in vitro release behavior from all the drug loaded batches were found to follow first order and provided controlled release over a period of 24 h. No appreciable difference was observed in the drug content and in vitro release of product during 6 months in which nanoparticles were stored at 5°C and room temperature. According to the data obtained, this chitosan- based delivery system opens new and interesting perspectives as drug carriers.

Keywords: Nanoparticles; Chitosan; Curcumin; Ionic gelation technique.

1. INTRODUCTION

Curcumin, a major polyphenolic pigment of the turmeric root [Curcuma longa L., Family: Zingeberacea] found widely cultivated in several tropical parts of Asia. Turmeric commonly finds use as a spice in Indian cooking, a cosmetic agent for skin care and a traditional Indian and Chinese medicine. It has low intrinsic toxicity but a wide range of pharmacological activities including antitumor, antioxidant, anti-amyloid and anti-inflammatory properties [1]. Several clinical trials dealing with cancer have stated the pharmacokinetics, safety and efficacy of curcumin in humans. However, clinical applications of Curcumin have been hampered by its extremely poor water solubility i.e., less than 1 μg/ml [2]. Many methods have been developed to improve solubility of poor water-soluble drugs but they induce severe side effects such as neurotoxicity, nephrotoxicity and hypersensitivity etc. To evade this difficulty, considerable

efforts have been devoted to the research and development of alternative methods to improve drug solubility without the use of organic solvents and other relatively harmful excipients. The development of drug nanocarriers for poorly soluble pharmaceuticals seems to be more promising. The therapeutic application of hydrophobic, poorly water-soluble agents is associated with some serious problems, such as poor absorption and low bioavailability. Colloidal drug delivery systems such as liposomes, micelles and nanoparticles have been intensively examined for their use in tumor therapy. The effectiveness of drug delivery systems can be attributed to their physical and chemical properties. The only factor that limits the use of free curcumin for cancer therapy is its poor solubility in water, which in turn limits its systemic bioavailability when administered orally. Polymeric nanoparticle-based drug delivery is being increasingly investigated as this delivery route is known to overcome many obstacles associated with the delivery of free drugs. Nanoparticles may become one of the successful carriers by overcoming problems caused by infections that are refractory to conventional treatment. Chitosan possesses some ideal properties of a polymeric carrier for nanoparticles such as biocompatibility, biodegradability, non-toxicity, 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 [3]. Depending on the desired administration way, the size of the carriers should be optimized. Thus, if the carrier size is under 1 μm, an intravenous injection (the diameter of the smallest blood capillaries is 4 μm) is enabled and this carrier is also desirable for intramuscular and subcutaneous administration, minimizing any possible irritant reactions. Hence, the objective of the work was to formulate chitosan nanoparticles containing Curcumin by Ionic gelation method, evaluate its physicochemical characteristics such as particle size, shape, zeta potential, drug loading capacity and in vitro release characteristics.

2. MATERIALS AND METHODS

2.1. Materials

Curcumin used was purchased from M/s. Sigma Aldrich Chemicals Ltd, Bangalore and chitosan from Central Institute of Fisheries Technology, Cochin, India (deacetylation degree 86%, molecular mass 110 kDa). Glacial acetic acid and sodium tripolyphosphate were obtained from Merck Specialties Private Limited, Mumbai, India. All other chemicals used were of analytical grade.

2.2. Preparation of nanoparticles [4-11]

Chitosan nanoparticles 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 1.0, 2.0, 3.0, 4.0, 5.0 mg/ml. Under magnetic stirring at room temperature, 5 ml of 0.84% (w/v) TPP aqueous solution was added drop wise 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 continued for about 30 min. The resultant nanoparticles suspensions were centrifuged at 12000 × g for 30 min using C24 centrifuge. The formation of the particles was a result of the interaction between the negative groups of the TPP and the positively charged amino groups of chitosan (ionic gelation) (Table 1).

ISSN: 2394-1618
IP INDEX Impact Factor is 2.608

2.3. Characterization of prepared nanoparticle [12,13]

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. Drug entrapment efficiency
Drug content was determined by centrifugation method. The redispersed nanoparticles suspension 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

2.3.3. Surface morphology study
Scanning electron microscopy (SEM) of the chitosan nanoparticle was performed to examine the particle size and surface morphology. The nanoparticles were mounted on 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 7500–20000 ×.

2.3.4. Particle size distribution
The particle size distribution of the nanoparticles was determined by photon correlation spectroscopy (PCS, Coulter Counter model N4 MD, Coulter Counter Co.USA). 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.

2.3.5. Zeta potential
The Zeta-potential of drug loaded nanoparticles was measured by Zeta sizer (Malvern Zetasizer 3000HS, UK). 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.

2.3.6. *In vitro* release studies [14]
In vitro release studies were carried out by using dialysis tubes with an 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 agitated continuously using a magnetic stirrer and the temperature was maintained at 37 ± 1°C. 5ml sample of receptor compartment was taken at various intervals of time over a period of 24 h and each time 5 ml fresh buffer was replaced. The amount of drug released was determined spectrometrically at 425 nm.

2.3.7. Kinetic modeling [15-17]
In order to understand the kinetic and mechanism of drug release, the result of in vitro drug release study of nanoparticles was fitted with various kinetic equation like zero order (cumulative% release vs. time), first order (log% drug remaining vs. time),

ISSN: 2394-1618
IP INDEX Impact Factor is 2.608

Higuchi's model (cumulative% drug release vs. square root of time), Peppas plot (log of cumulative% drug release vs. log time). R2 (coefficient of correlation) and k (release rate constant) values were calculated for the linear curve obtained by regression analysis of the above plots.

2.3.8. Stability studies [18]

The stability study was carried out using the batch FC-4. Formulation FC-4 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 drug content at 0 month, 3months and 6 months. *In vitro* release study of formulation FC-4 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 TPP solution to the chitosan solution under magnetic stirring. Chitosan nanoparticles are 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. Nanoparticles prepared by ionic gelation technique were found to be discrete and through SEM analysis (Fig. 1), their mean size distribution was found to be 342-712 nm. Since the particle size is less than 1 µm, this drug delivery system can be used for parenteral formulations, drugs administered by such routes will achieve direct systemic delivery, thereby avoiding first pass hepatic metabolism and reaching a reduction in the dose delivered. The drug entrapment efficiency of nanoparticles containing drug: polymer in various ratios of 1:1, 1:2, 1:3, 1:4 and 1:5 were found to be 60.5±0.63%, 67.8±0.06%, 71.80±0.28%, 82.5±0.16%, 74.50±0.82% (Table 1). Thus there was a steady increase in the entrapment efficiency on increasing the polymer concentration in the formulation. The formulation FC-4 registered highest entrapment of 82.5±0.16% (Table 1). The high entrapment efficiency is likely due to electrostatic interactions between the drug and the polymer. Zeta potential of all formulated nanoparticles was in the range of -30.35to -15.9 mV, and it shows good stability.

3.2. *In vitro* release of nanoparticles

Cumulative percentage drug released for FC-1, FC-2, FC-3, FC-4 and FC-5 after 24 h were found to be 85.28±2.1, 83.73±3.2, 81.38±2.4, 76.57±2.1, 80.26±2.1 respectively (Fig. 2). It was apparent that in vitro release of curcumin showed a very rapid initial burst, and then followed by a very slow drug release. An initial, fast release suggests that some drug was localized on the surface of the nanoparticles.

3.3. Kinetic studies

In order to describe the release kinetics of all five formulations the corresponding dissolution data were fitted in various kinetic dissolution models like zero order, first order and Higuchi, respectively (Table 2). As indicated by higher R2 (coefficient of correlation) values, the drug release

from all formulations follows first order release and Higuchi model. Since it was confirmed as Higuchi model, the release mechanism was swelling and diffusion controlled. The Peppas model is widely used to confirm whether the release mechanism is Fickian diffusion, non-Fickian diffusion or zero order. 'n'(release exponent of Korsmeyer-Peppas model) value could be used to characterize different release mechanisms. The 'n' values for all formulations were found to be less than 0.50. This indicates that the release approximates Fickian diffusion mechanism.

3.4. Stability studies

The results of drug content of ideal formulation FC-4 after 6months of stability testing at different storage conditions were shown in Fig. 3. In vitro release profiles for the same formulation stored at different storage conditions were also showed in Fig. 4. On comparing this data with the previous data of FC-4, it was observed that there was a slight decrease in drug content when the formulation was stored at $5^0\pm 3^0$C, Room temperature and at 40 ± 2˚C/75% RH.

4. CONCLUSION

Based on drug content, drug entrapment efficiency, particle size morphology, zeta potential and in vitro release, formulation FC-4 was selected as an optimum formulation. Stability studies were carried out for the selected formulation FC-4. The stability studies showed that maximum drug content and closest in vitro release to previous data was found for FC-4 stored at $5^0\pm 3^0$C, Room temperature and at 40 ± 2˚C/75% RH. Thus nanoparticles of curcumin (FC-4) with core: coat ratio 1:4 was found to be spherical, discrete and free flowing and able to control the drug release effectively.

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

This research was fully supported by the grants from the Advance Research Wing, Rajiv Gandhi University of Health Sciences, Bengaluru, Karnataka. The authors acknowledge the financial support received from RGUHS, for their support and encouragement in carrying out her project work.

Table 1 Formulation and physicochemical characterization of Curcumin nanoparticles

Sl.No	Batch Code	Drug: Carrier ratio	Entrapment efficiency[a] (%)	Particle size[a] (nm)
1	FC-1	1:1	60.5±0.63	426±5.04
2	FC-2	1:2	67.8±0.06	642±4.20
3	FC-3	1:3	71.80±0.28	712±1.90
4	FC-4	1:4	82.5±0.16	342±12.4
5	FC-5	1:5	74.50±0.82	517±10.7

[a]Mean ± SD. FC-1, FC-2, FC-3, FC-4 and FC-5 represent formulations 1 to 5, respectively

ISSN: 2394-1618
IP INDEX Impact Factor is 2.608

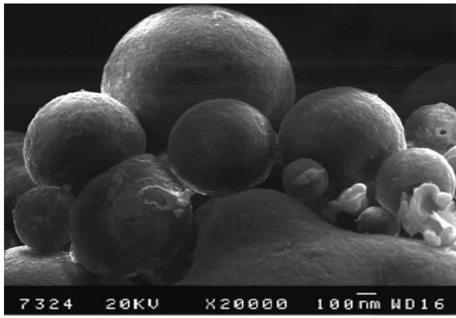

Fig. 1. SEM of formulation FC-4.

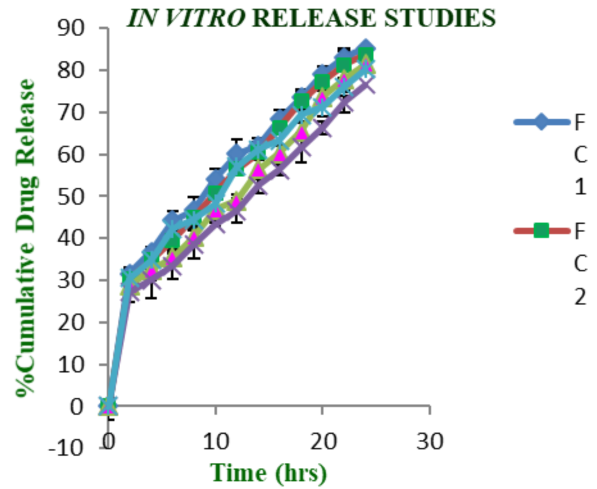

Fig. 2. Comparative *in vitro* release profiles of Curcumin nanoparticles (mean ± SD, *n* = 3).

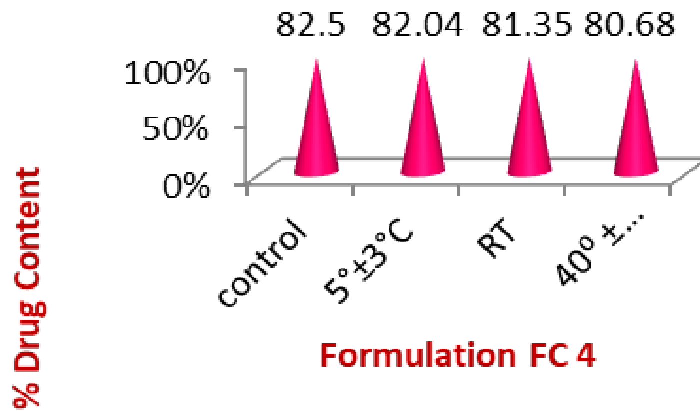

Fig. 3. Stability study: comparison of drug content of formulation FC-4 at 5⁰± 3⁰C, Room temperature and at 40 ± 2˚C/75% RH after 6 months

Table 2 Correlation coefficients according to different kinetic equations.

Formulation	Zero order	First order	Higuchi plot	Peppas plot	'n' values
FC–1	0.9157	0.9714	0.9905	0.9717	0.4252
FC–2	0.9318	0.9712	0.9878	0.9597	0.4428
FC–3	0.943	0.9478	0.9729	0.9282	0.4567
FC–4	0.941	0.9581	0.9754	0.9395	0.441
FC–5	0.905	0.981	0.988	0.9662	0.4091

FC-1, FC-2, FC-3, FC-4 and FC-5 represent formulations 1 to 5, respectively

ISSN: 2394-1618
IP INDEX Impact Factor is 2.608

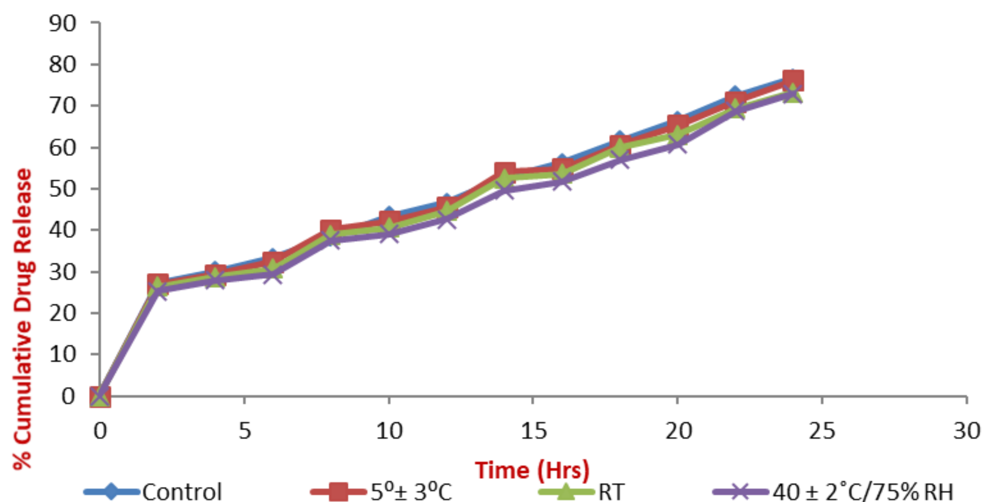

Fig. 4. Stability study: comparison of *in vitro* drug release profile for Formulation FC-4 at 5^0± 3^0C, Room temperature and at 40 ± 2˚C/75% RH after 6 months

7. REFERENCES

[1] Aggarwal BB, Kumar A, Bharti AC. Anticancer potential of curcumin: preclinical and clinical studies. Anticancer Res 2003; 23:363-98.

[2] Maheshwari RK, Singh AK, Gaddipati J, Srimal RC. Multiple biological activities of curcumin: a short review. Life Sci 2006; 78:2081-87.

[3] Tilak R, Bharadwaj, Meenakshi K, *et al.* Natural Gums and Modified Gums as sustained release carriers. Drug Dev Ind Pharm 2000, 26: 1025-38.

[4] Yan W, Wuli Y, Changchun W, *et al.* Chitosan nanoparticles as a novel delivery system for ammonium glycyrrhizinate. Int J Pharm 2005, 295: 235-45.

[5] Yesim A, Karine Andrieux B, Maria Jose Alonso C, et al. Preparation and *in vitro* evaluation of chitosan nanoparticles containing a caspase inhibitor. Int J Pharm 2005;298: 378-83.

[6] 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.

[7] Lopez-Leon T, Carvalho ELS, Seijo B, *et al.* Physicochemical characterization of chitosan nanoparticles: electrokinetic and stability behavior. J Colloid Interface Sci 2005;283: 344-51.

[8] Yan P, Ying-jian L, Hui-ying Z, *et al.* Bioadhesivepolysaccharide in protein delivery system: chitosan nanoparticles improve the intestinal absorption of insulin *in vivo*. Int J Pharm 2002; 249:139-47.

[9] Ana G, Begona S, Carmen R. Microencapsulated chitosan nanoparticles for lung protein delivery. Eur J Pharm Sci 2005;25: 427-37.

[10] Xiong Wei L, Daniel Ken Lem L, Albert Sun Chi C, *et al.* Sustained expression in mammalian cells with DNA complexed with chitosan nanoparticles. Biochimi Biophys Acta 2003; 1630:7-18.

[11] Tang ESK, Huang M, Lim LY. Ultrasonication of chitosan and chitosan nanoparticles. Int J Pharm 2003; 265:103-14.

[12] Peltonen L, Koistinen P, Karjalainen M, *et al.* The effect of cosolvents on the formulation of nanoparticles

from low molecular weight polylactide. AAPS PharmSciTech 2002;3:1-7.

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

[14] Pandey R, Ahmad Z, Sharma S, *et al.* Nanoencapsulation of azole antifungals: Potential applications to improve oral drug delivery. Int J Pharm 2005; 301:268-76.

[15] Saparia B, Murthy RSR, Solanki A. Preparation and evaluation of chloroquine phosphate microspheres using cross-linked gelatin for long term drug delivery. Indian J Pharm Sci 2002; 64: 48-52.

[16] Haznedar S, Dortunc B. Preparation and evaluation of Eudragit microspheres containing acetazolamide. Int J Pharm 2004; 269:131-40.

[17] Higuchi T. Mechanism of sustained action medication: theoretical analysis of rate of release of solid drugs dispersed in solid matrices. J Pharm Sci 1963; 52:1145-49.

[18] Joseph Nisha M, Palani S, Sharma PK, *et al.* Development and evaluation of nanoparticles of mitomycin-c. J Pharm Res 2006; 5:53-6.