Int J Pharm Bio Sci 2015 Jan; 6(1): (P) 465 - 478

Research Article **Nanotechnology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

PREPARATION, CHARACTERIZATION AND IN-VITRO CELL VIABILITY ASSAY OF CHRYSIN LOADED SOLID LIPID NANOPARTICLES AS DRUG DELIVERY SYSTEM

VEDAGIRI AISHWARYA, RAMACHANDRAN SUREKHA AND THANGARAJAN SUMATHI*

Department of Medical Biochemistry, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai – 600 113, Tamil Nadu, India.

ABSTRACT

The aim of the present study is to prepare and characterize Solid Lipid Nanoparticles (SLNs) encapsulated Chrysin (CN) to improve the bioavailability of the drug. Chrysin-loaded solid lipid nanoparticles (CN-SLNs) with an average particle size of 240.0± 4.79nmand a total drug content of 71.10 ± 3.12% was produced using a hot homogenization followed by micro emulsion technique. The prepared CN-SLNs were also characterized using zeta potential, entrapment efficiency, X-Ray Diffraction (XRD), Transmission Electron Microscope (TEM) and cytotoxicity assay. The particles were found to be spherical in shape, with high drug entrapment of 86.29 ± 3.42%. Zeta potential studies confirmed the stability of the CN-SLNs preparation as indicated by the negative charge (-40.4 ± 2.54 mV). Whereas the XRD studies showed reduced crystallinity and the particles showed less cytotoxic effect when compared with other polymeric nanoparticles (IC_{50} value = 25.0 ± 2.16 µg/ml).The in-vitro release kinetics showed a maximum drug release of 88.80 ± 3.05% for CN-SLNs and 39.28 ± 3.19% of Chrysin suspension(CN–S) in 72 hrs, which ensures the controlled drug release property of CN-SLNs.

KEYWORDS: **Solid lipid nanoparticles, Chrysin, Dynamic light scattering, Entrapment efficiency, Transmission Electron Microscope**.

*Corresponding author

THANGARAJAN SUMATHI

Department of Medical Biochemistry, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai – 600 113, Tamil

INTRODUCTION

Targeted delivery of a drug molecule to specific organ sites is one of the most challenging research areas in pharmaceutical sciences. Nanoparticles are solid colloidal particles, in which the active principles (drug or biologically active material) are dissolved, entrapped, and/or to which the active principle is adsorbed or attached[1]. Solid lipid nanoparticles (SLNs) are considered to be the most effective lipid based colloidal carriers, introduced in early nineties[2,3]. This is the one of the most popular approaches to improve the oralbioavailability of the poorly water-soluble drugs. SLNs arein the range of submicron size (50-1000 nm) and are composed of physiologically tolerated lipid components which are in solid state at room temperature[4 - 7]. SLNs can be employed for various purposes for which nanoparticles have distinct advantages[8]. These advantages include the possibility of incorporating drugs for controlled drug release, the low cytotoxicity due to its composition of physiological compounds, the possibility of loading both lipophilic and hydrophilic drugs into the solid matrix and for its likely large scale production. Also, the solid matrix has been shown to protect the incorporated active ingredients, such as lysozyme, tamoxifen and cyclosporine, against chemical degradation[9 - 11]. Chrysin (5,7-dihydroxyflavone) is a natural flavonoid extracted from many plants, honey and propolis[12,13]. Several studies in recent years have shown that chrysin(CN) has multiple biological activities, such as anti-inflammation, anti-oxidation and vasorelaxation effects[14,15].Several studies also suggested that chrysin could be as a neuroprotective agent in different models. Chrysin markedly decreased the level of MDA as a marker of lipid peroxidation in cortex and hippocampus following chronic cerebral hypoperfusion in rats, while elevated antioxidant enzymes activity[16] and consequently ameliorated brain damage. Another study exhibited that chrysin does-dependently inhibited tunicamycin-induced neuronal cell death in SH-SY5Y cells via inhibition of mitochondrial apoptosis pathway[17]. The present research aims in formulating and characterizingCN-SLNs to improve the pharmacological activity of CN. The characteristics of the CN-SLNs were studied for the purpose of making the effective nanoparticle system for protection of incorporated labile drugs from degradation, controlled release and enhancing the bioavailability.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

Chrysin was obtained from Sigma Aldrich. Stearic acid, Lecithin and Sodium taurocholate were purchased from Hi-Media chemicals. All the other chemicals used in the present study were of analytical grade and purchased from Merck (India) and Sisco Research Laboratories Pvt. Ltd.

VERO CELL LINE

The vero cell culture were obtained from National centre for cell sciences Pune (NCCS). The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100µg/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37 °C.

PREPARATION OF CN-SLNs

The SLNs were prepared as described by Frautschy SA and Cole GM[18], with slight modifications. Briefly, stearic acid mole fraction 0.710, lecithin mole fraction 0.210, taurocholate mole fraction 0.069 and CN mole fraction 0.011 were used to produce the Solid lipid nanoparticles encapsulated CN. Stearic acid was maintained at ~75°C to melt completely, simultaneously distilled water was heated up to ~75°C in a separate beaker. Typically, surfactants were added to distilled water on a magnetic stirrer and allowed to equilibrate at ~75 °C. The water–surfactant solution containing CN was then added to the melted lipid and again allowed to equilibrate at ~75°C. The mixture was then homogenized at 24,000rpm for 150s to form the emulsion. Then the aliquot was continuously stirred near ice

cold water (~2°C), at a ratio of 1:20 (warm microemulsion/cold water) resulting in the formation of solidified solid lipid nanoparticles. The final product was centrifuged at 20,000×g for 15 min, and nanoparticle pellet was resuspended in distilled water. The preparation was stored sterile at 4°C, until delivery by gavage

FORMULATION CHARACTERIZATION PARTICLE SIZE ANALYSIS AND ZETA POTENTIAL

Dynamic light scattering (DLS) was employed to measure particle size distributions and zeta potentials. In this technique, fluctuation in light scattering due to Brownian motion of the particles was analyzed.

PARTICLE SHAPE AND SURFACE MORPHOLOGY

Microscopic analysis of the prepared CN-SLNs was carried out to study the morphology like sphericity and aggregation using a Transmission Electron Microscope (TEM).

TOTAL DRUG CONTENT AND ENTRAPMENT EFFICIENCY

Total amount of drug per unit volume present in CN-SLNs formulation was determined by suitably disrupting SLN dispersion using a mixture of chloroform: methanol (1:1) volumetrically and centrifuged. The pellet containing drug was analyzed spectrophotometrically at 348nm, using a respective blank described by Kakkar[19]. Each experiment was performed in triplicate. TDC was determined by a following equation:

$$TDC = \frac{\text{Amount of drug/ml of SLN dispersion}}{\text{Total amount of drug/ml of SLN dispersion}} \times 100$$

Entrapment efficiency was calculated by the formula mentioned by Rohit B and Pal KI[20].

$$EE = \frac{TDC - D_f}{TDC} \times 100$$

Where, D_f = amount of drug in clear supernatant fluid

IN-VITRO DRUG RELEASE

The drug release from CN loaded SLNs and CN suspension (CN-S) were carried out using the dialysis bag method[21]. The dialysis bags (MWCO 12KD, Hi-Media) were soaked in deionized water for 12h before use. 1 ml of each samples were added into the dialysis bags individually and the bags were placed in a beaker containing a mixture of distilled water and ethanol (50:50 v/v) as the dissolution medium and placed at 37°C at a stirring rate of 100rpm for 72hrs. Aliquots of the dissolution medium were withdrawn at different time intervals and were replaced with the same volume of fresh medium to maintain the sink conditions. CN in the sample solution were analyzed spectrophotometrically at 348nm. All the operations were carried out in triplicate and precautions were taken to protect the drug from heat and light.

X-RAY DIFFRACTION (XRD)

Powder X-ray diffraction patterns were traced for drug, SLNs and CN-SLNs employed by RIGAKU, RV200 using monochromatic Ni-filtered Cu–K radiation, a voltage of 40 kV, a

current of 30 mA radiation scattered in the crystalline regions of the sample, which was measured with a vertical goniometer. Patterns were obtained by using a step width of 0.04° with a detector resolution in 2θ (diffraction angle) between 10°and 70°at ambient temperature.

IN VITRO ASSAY FOR CYTOTOXICITY ACTIVITY

The cytotoxicity activity of sample on Vero cells was determined by the MTT assay[22] (*Mosmann et al*.,1983). Cells (1 × 10^5/well) were plated in 0.2ml of medium/well in 96-well plates. Incubate at 5% CO_2 incubator for 72 hours. Then, added various concentrations of CN-SLNs in 0.1% DMSO for 24hrs at 5% CO_2 incubator. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 20µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) in phosphate-buffered saline solution was added. After 4hrs incubation, 1ml of DMSO was added. Viable cells were determined by the absorbance at 540nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC_{50}) was determined graphically. The effect of the samples on the proliferation of Vero cells was expressed as the % cell viability, using the following formula

$$\% \text{ cell viability} = A540 \text{ of treated cells} / A540 \text{ of control cells} \times 100\%$$

STATISTICAL ANALYSIS

Data from the individual experiments are expressed as the mean ± SD. All statistical analysis were performed by one way analysis of variance (ANOVA) followed by student 't' test using SPSS 20 version. Differences were considered to be statistically significant at $P<0.05$.

Table 1
Characterizations of formulated CN-SLNs

Characterizations	Formulated CN-SLNs
Particle size (nm)	240.0± 4.79
Poly Dispersity Index	0.285
Zeta potential (mV)	- 40.4 ± 2.54
Total drug content (%)	71.10 ± 3.12
Entrapment Efficiency (%)	86.29 ± 3.42
Maximum drug release in 72h (%)	88.80 ± 3.05

Fig. 1A

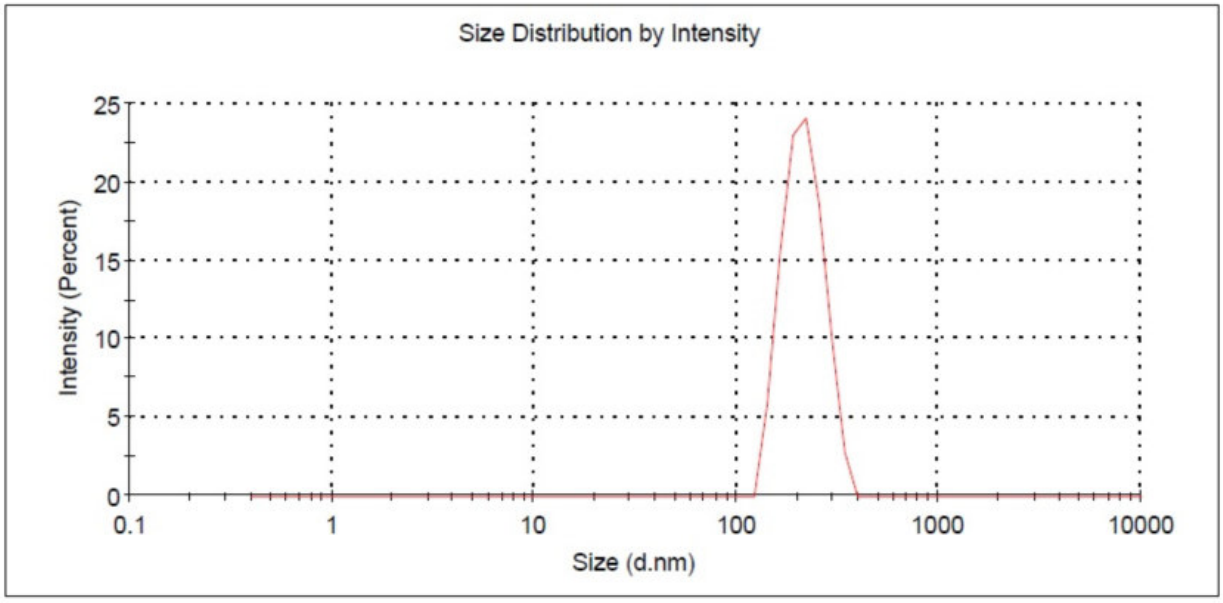

Size Distribution by Intensity
Intensity (Percent)
25
20
15
10
5
0
0.1
1
10
100
1000
10000
Size (d.nm)

Fig. 1B

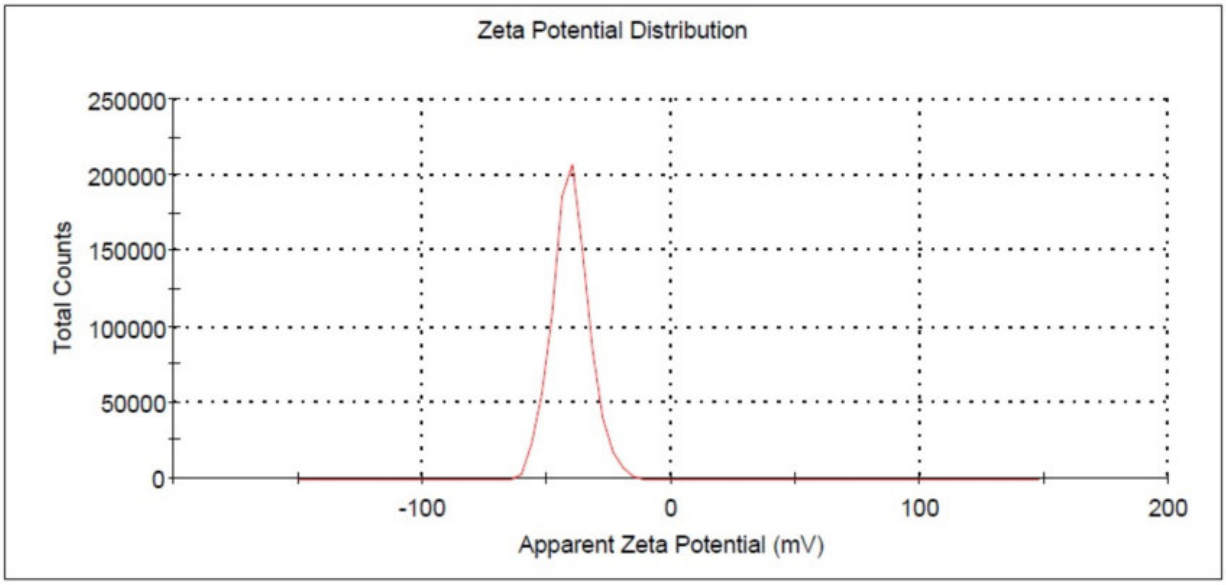

Zeta Potential Distribution
Total Counts
250000
200000
150000
100000
50000
0
-100
0
100
200
Apparent Zeta Potential (mV)

Fig. 1C

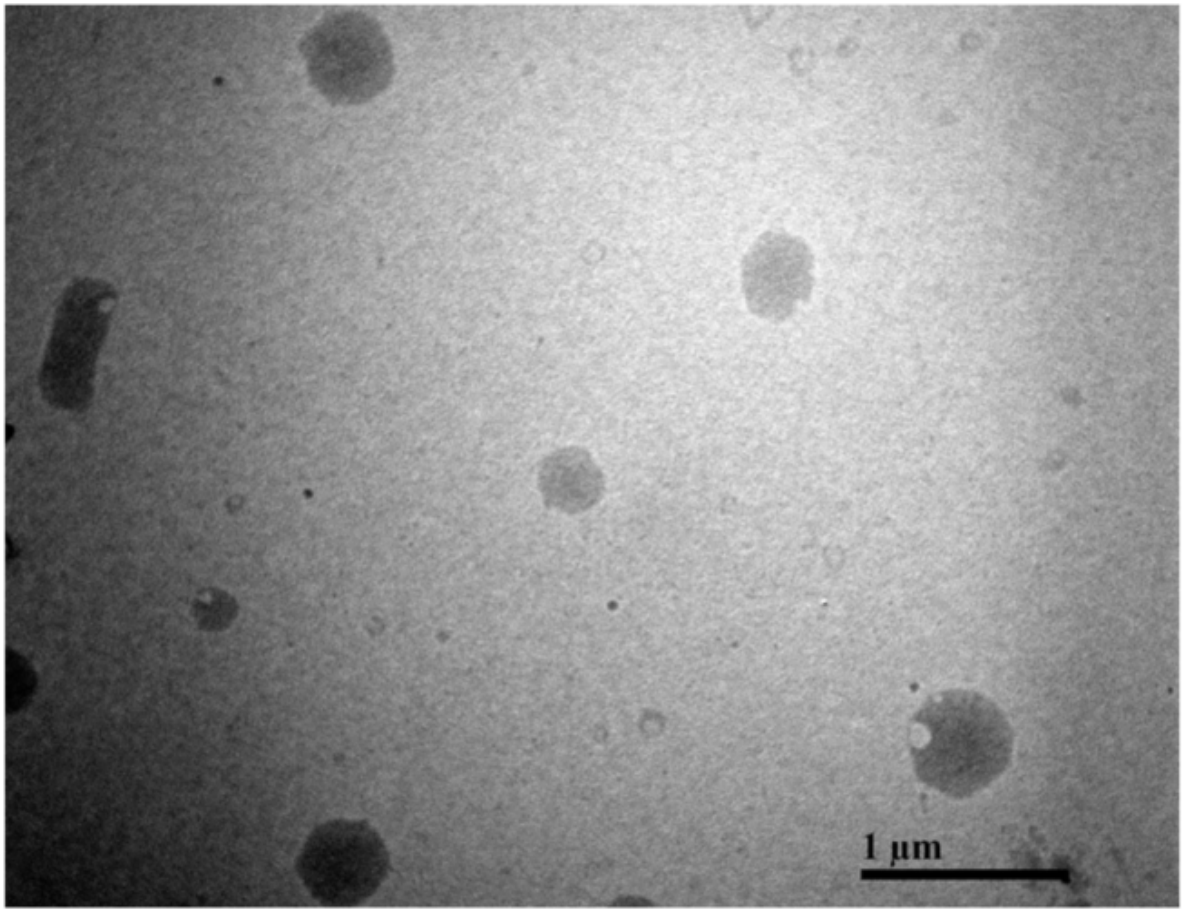

1 µm

Fig. 2

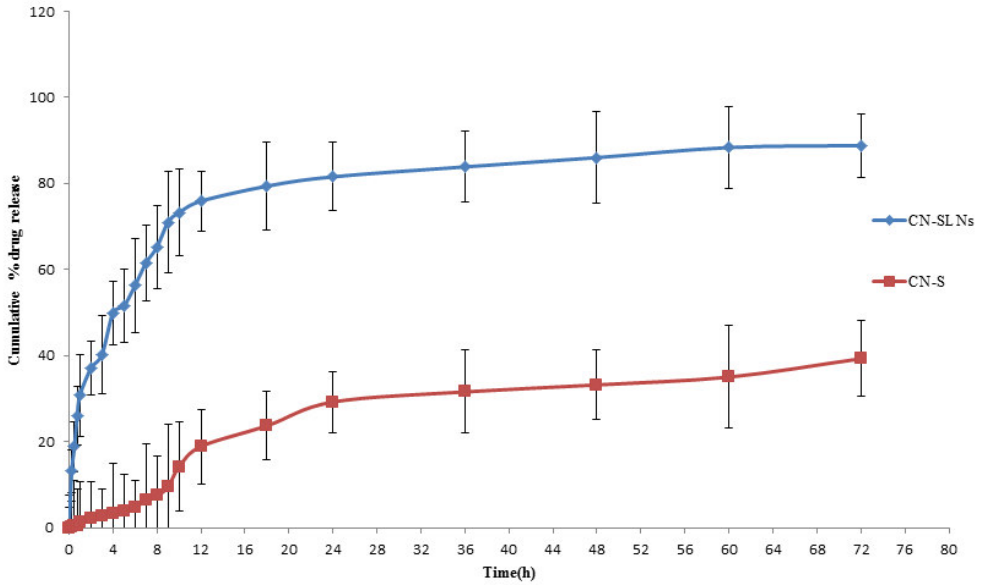

Cumulative % drug release
Time(h)
CN-SLNs
CN-S
0
20
40
60
80
100
120
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
64
68
72
76
80

Fig. 2B

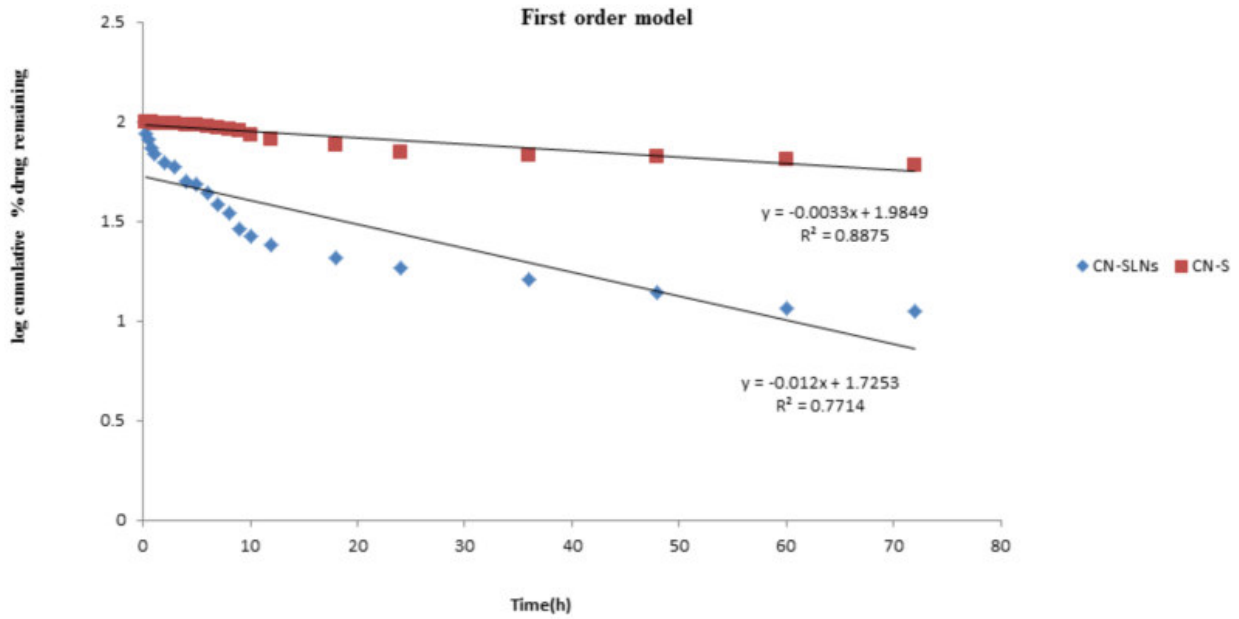

First order model
log cumulative % drug remaining
y = -0.0033x + 1.9849
R² = 0.8875
y = -0.012x + 1.7253
R² = 0.7714
CN-SLNs
CN-S
Time(h)

Fig. 2C

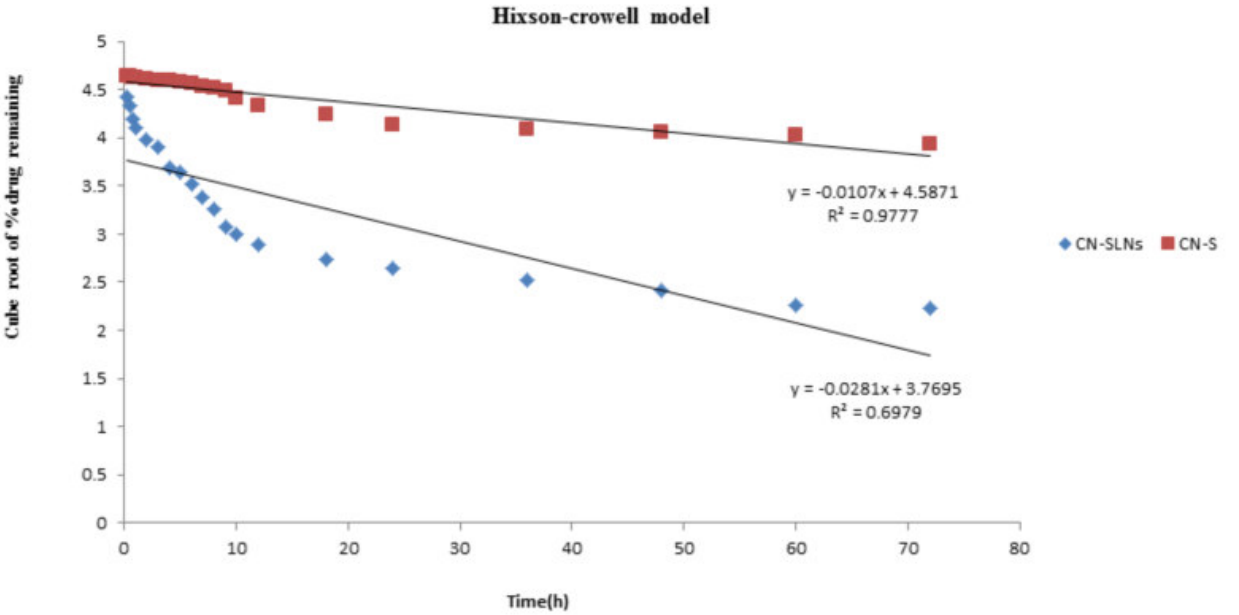

Hixson-crowell model
Cube root of % drug remaining
y = -0.0107x + 4.5871
R² = 0.9777
y = -0.0281x + 3.7695
R² = 0.6979
CN-SLNs
CN-S
Time(h)

Fig. 2D

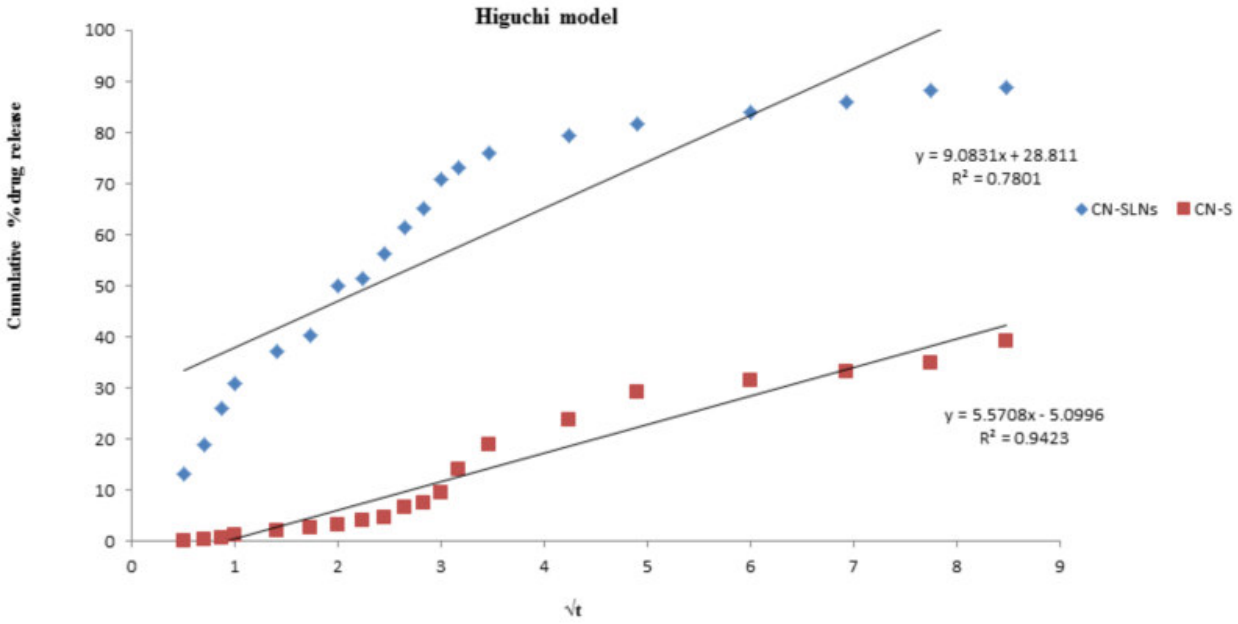

Higuchi model
Cumulative % drug release
√t
y = 9.0831x + 28.811
R² = 0.7801
y = 5.5708x - 5.0996
R² = 0.9423
CN-SLNs
CN-S

Fig. 2E

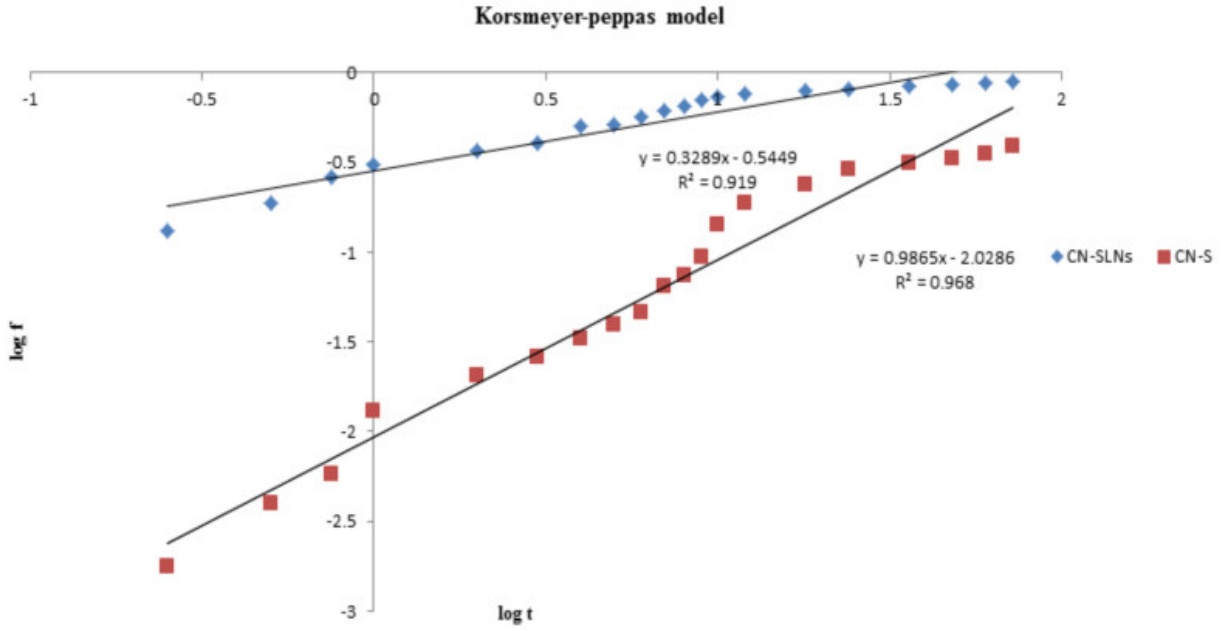

Korsmeyer-peppas model
log F
log t
y = 0.3289x - 0.5449
R² = 0.919
y = 0.9865x - 2.0286
R² = 0.968
CN-SLNs
CN-S

Fig. 2F

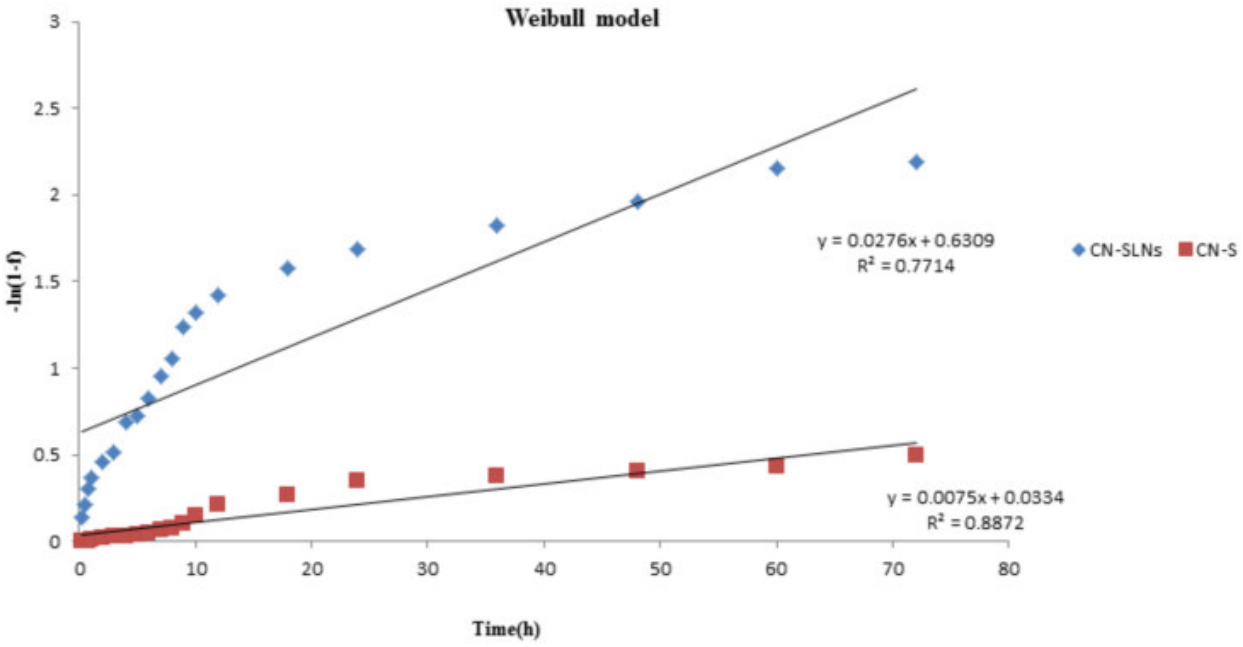

Weibull model
-ln(1-f)
Time(h)
y = 0.0276x + 0.6309
R² = 0.7714
y = 0.0075x + 0.0334
R² = 0.8872
CN-SLNs
CN-S

Fig. 3A

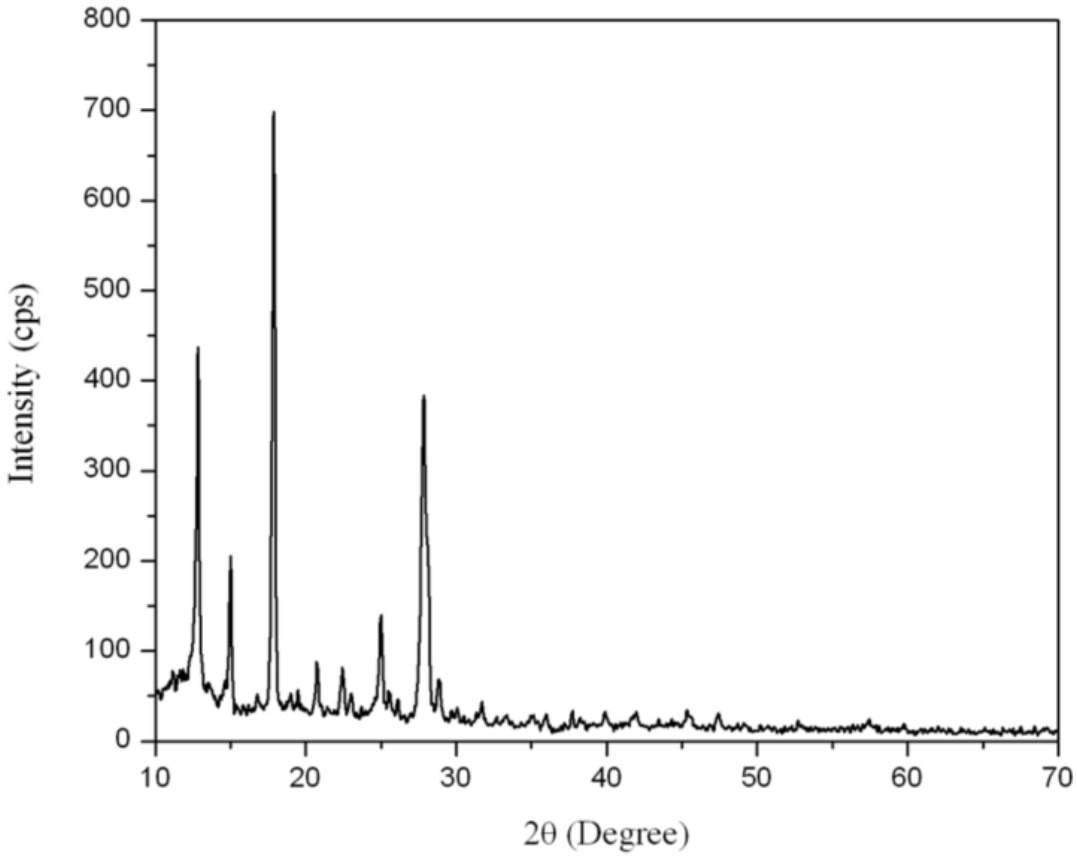

Intensity (cps)
2θ (Degree)

Fig. 3B

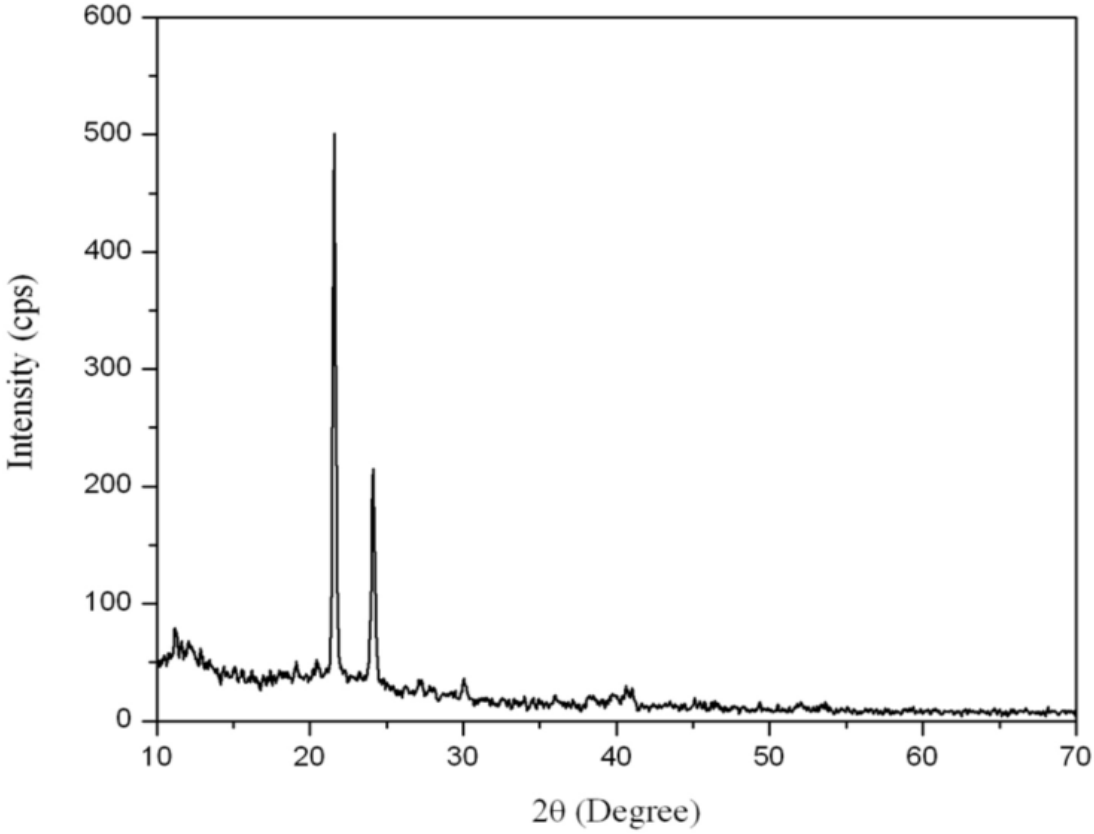

Intensity (cps)
2θ (Degree)
0
100
200
300
400
500
600
10
20
30
40
50
60
70

Fig. 3C

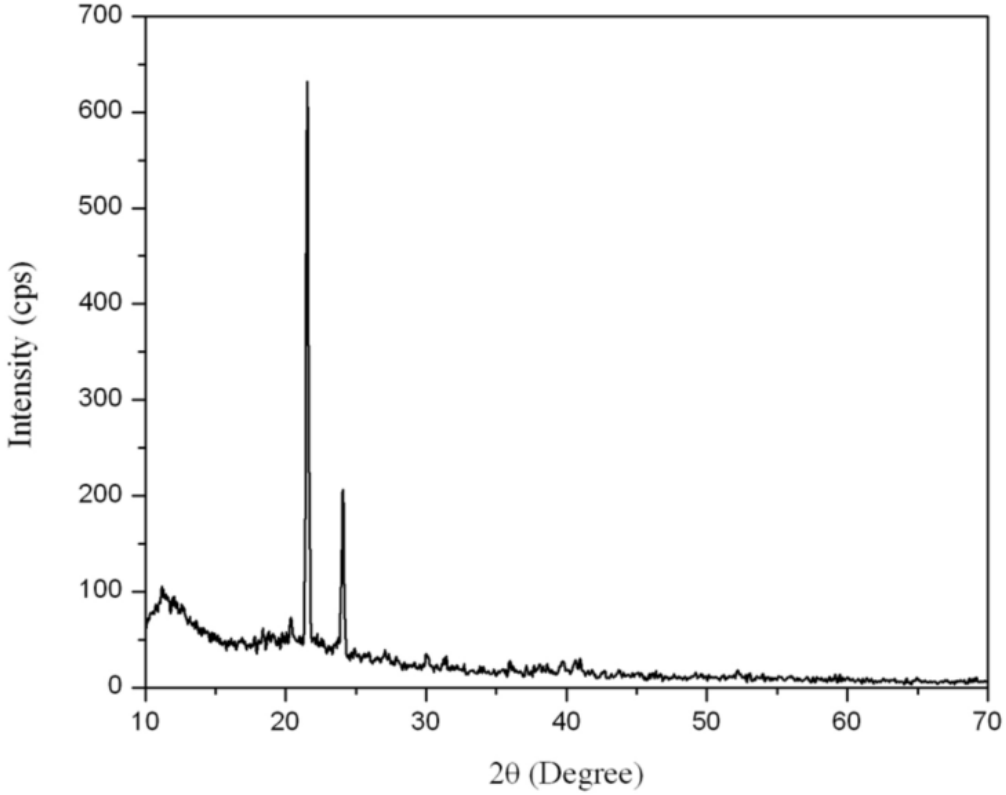
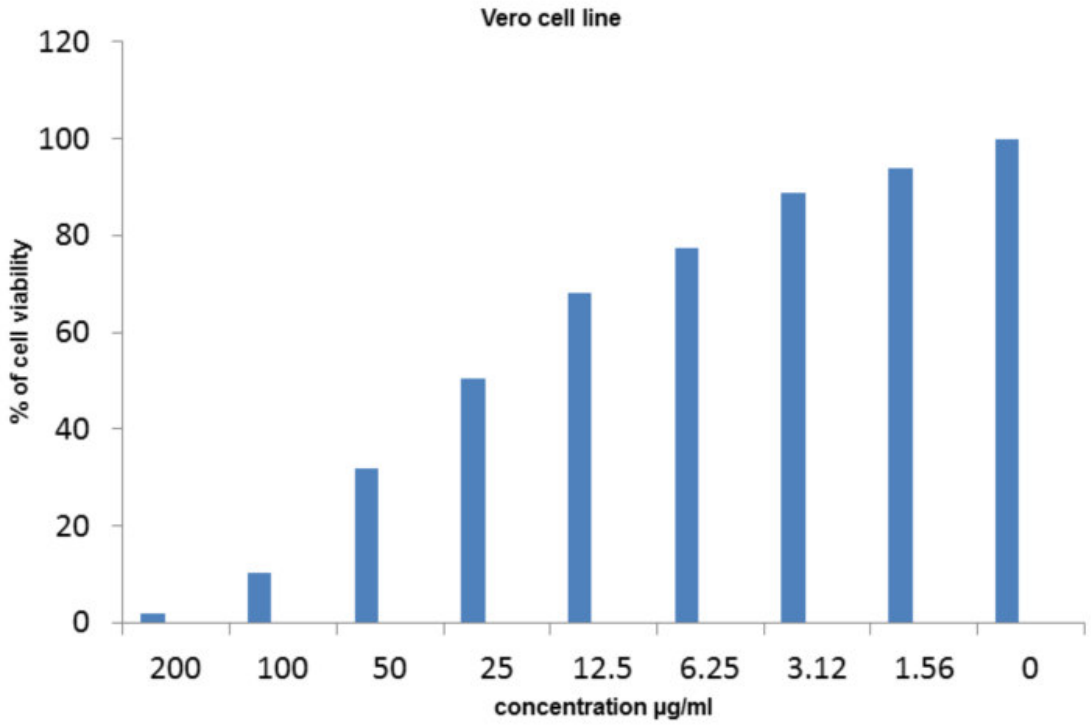

Intensity (cps)
2θ (Degree)
0
100
200
300
400
500
600
700
10
20
30
40
50
60
70

Fig. 4



RESULTS

MEASUREMENT OF PARTICLE SIZE, ZETA POTENTIAL AND ENTRAPMENT EFFICIENCY (EE)

Particle size of optimized formulation CN-SLNs wasfound to be 240.0± 4.79nm (Fig. 1A)with a poly dispersity index of0.285.Zeta potential of CN-SLNs was found to be -40.4 ± 2.54 mV(Fig. 1B). CN-SLNs showed an average drug content of 71.10 ± 3.12% and the EE of the formulation CN-SLNs were found to be 86.29 ± 3.42%. (Table 1)

PARTICLE SHAPE AND SURFACE MORPHOLOGY

The TEM image of CN-SLNs shows relatively spherical particles. (Fig. 1C)

IN VITRO RELEASE

Fig. 2shows the cumulative percentage of drug released with respect to the amount of CN entrapped in SLNs. A maximum drug release was found to be 88.80 ± 3.05% for CN-SLNs and 39.28 ± 3.19% for CN–S in 72 hrs. The release characteristic was incorporated into various release kinetics models and linear regression analysis was carried out (Fig. 2A-F).

XRD STUDIES

Sharp peaks were observed for CN, which indicates the crystalline nature of the drug (Fig. 3A). SLNs showed diffused peaks and undefined sharp peaks (Fig. 3B). CN-SLNs showed diffused peaks where the intensity of lipid peaks and drug peaks was found to be decreased which suggests the deformity of crystal lattice of CN. (Fig. 3C)

IN VITRO CYTOTOXICITY PROFILE

In vitro cytotoxicity of CN-SLNs wasevaluated in Vero cells using MTT assay was shown in Fig. 4. With increase in the concentration of drug present in the formulations, the cell viability was decreased. IC_{50} value of CN-SLNs were calculated after 24 h incubation and found to be 25.0 ± 2.16 µg/ml.

DISCUSSION

Chrysin-loaded Solid lipid nanoparticles were prepared by hot homogenization followed by micro emulsion method.SLNs consist of physiological and biocompatible lipids, which are suitable for the incorporation of lipophilic, hydrophilic and poorly water-soluble active ingredients[23].SLNs are used for many applications including parenteral, peroral and dermal administration. Drug-free SLNs are utilized to improve occlusive properties, whereas drug loaded SLN offer controlled release and stabilization of the encapsulated drugs[24]. Particle size is an important parameter in quality assurance, because the physical stability of nanoparticle dispersion depends on the particle size and its distribution. TEM analysis was used to evaluate the uniformity of size, shape and physical stability characteristics, i.e., aggregation and/or irregularity of the prepared CN-SLNs[25]. In our study, the particle size of CN-SLNs nanoformulation was found to be 240.0 ± 4.79 nm and resulted in particles with a spherical shape as observed by TEM. The zeta potential is known to be predictive of the stability of nanoparticles[26]. The zeta potential of CN-SLNs obtained was -40.4 ± 2.54 mV, which are considered to be highly stable. The entrapment efficiency and loading capacity of SLN were enhanced with increasing the carbon chain length of fatty acid, since the higher hydrophobicity of the longer chain fatty acids resulted in increased accommodation of lipophilic drugs[27,28]. The longer carbon chain length of the fatty acid SLN had slower release rates in-vitro; this was attributed to that the enhanced lipophilicity of longer chain fatty acids had better drug retainingcapacity[29]. Our results showed relatively good EE of the formulation CN-SLNs which were found to be 86.29 ± 3.42%.Prominent ($p < 0.05$) difference in the in-vitro release kinetics was observed between theCN-SLNs and CN-S. A maximum release was obtained for CN-SLNs as compared to CN-S. This shows a typical nanodispersion characteristic of the designed SLNs and form the root cause of their enhanced bioavailability in vivo[30]. In our study of in-vitro release kinetics on different models revealed that CN-SLNs and CN-S were found to fit best in Korsmeyer–Peppas model and Hixson-Crowell model respectively. XRD patterns of CN indicated the crystalline nature of drug .The intensity of crystalline peaks of CN decreased significantly in the formulations submitted after lyophilization, and most of the peaks were absent in the formulation. These results indicate that the drug was not in crystalline form after lyophilization of SLNs. The absence of drug peaks indicated that the drug was encapsulated in amorphous form. The intensity of pure lipid peaks also decreased in SLNs formulation. This reduced intensity indicates the decreased crystallinity of lipid[31,27].The change in crystallinity of lipid and drug can influence the release of CN from SLNs[32].The results of cytotoxicity studies (MTT test) indicated that SLNs are less toxic than polymeric nanoparticles[33,34]. Similarly, low cytotoxicity of SLNs has been observed in a comparable study on poloxamine 908 and poloxamer 407 stabilized SLNs[35]. In conclusion, this highly bioavailable and stable solid lipid nanoparticulate formulation of Chrysin would be a great success in improving the efficacy of Chrysin, hence renovating its reflection from just a preventative dietary supplement to a therapeutic agent. Further studies are being carried out for evaluating the pharmacodynamics and pharmacokinetic properties of CN-SLNs on suitable in-vitro and in-vivo models against Alzheimer's disease

ACKNOWLEDGEMENT

The first author is grateful to UGC for the financial support in the form of UGC – Non Net Fellowship. The authors also thank National center for Nanoscience and Nanotechnology Department, University of Madras, Chennai, Tamilnadu, India for providing the Transmission Electron Microscopy facility.

REFERENCES

1. Chowdary KPR, Rao AS. Nanoparticles as drug carriers. Indian Drugs, 34(10): 549-556, (1997).
2. R.L. Dunn. The atrigel drug delivery system. In: M.J. Rathbone, J.Hadgraft and M. Roberts (eds.),*Modified-Release Drug Delivery Technology*, NY: Marcel Dekker. Inc, NewYork, 2002, pp.647-655.
3. Castelli F, Puglia C, Sarpietro MG, Rizza L and Bonina F. Characterization of indomethacin-loadednanoparticles by differential scanning calorimetry. Int J Pharm, 304: 231-238, (2005).
4. Radtke M and Muller RH. Nanostructured lipid drugcarriers. New Drugs, 2: 48-52, (2001).
5. K. Jores, W.Mehnert, H.Bunjes, M.Drechsler and K.Mader. Solid lipid nanoparticles (SLN) to nanospoons. Visions and reality of colloidal lipid dispersions. International Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Nuremberg, Germany, 2008, pp.15-18.
6. Saupe A, Gordon KV and Rades T. Structural investigations on nanoemulsions, solid lipid nanoparticles and nanostructured lipid carriers by cryo-field emission scanning electron microscopy and raman spectroscopy. Int J Pharm, 314(1): 56-62, (2006).
7. Venkateshwarlu V and Manjunath K. Preparation and in vitro release kinetics of clozapine solid lipid nanoparticles. J Controlled Release, 95: 627- 638, (2004).
8. Wissing SA, Kayser O,& Muller RH. Solid lipid nanoparticles for parenteral drug delivery. Advanced Drug Delivery Reviews, 56: 1257-1272, (2004).
9. Almeida AJ, Runge S, & Muller RH. Peptide-loaded solid lipid nanoparticles (SLN): influence of production parameters. International Journal of Pharmaceutics, 149: 255-265, (1997).
10. Muller HR, Runge SA, Ravelli V, Thunemann AF, Mehnert W, &Souto EB. Cyclosporine-loaded solid lipid nanoparticles (SLN) drug-lipid physicochemical interaction and characterization of drug incorporation. European Journal of Pharmaceutics and Biopharmaceutics, 68: 535-544, (2008).
11. Reddy LH, Vivek K, Bakshi N, & Murthy RS. Tamoxifen citrate loaded solid lipid nanoparticles (SLN): preparation, characterization, in vitro drug release, and pharmacokinetic evaluation. Pharmaceutical Development and Technology, 11: 167-177, (2006).
12. Rapta P, Misik V, Stasko A, Vrabel I. Redox intermediates of flavonoids andcaffeic acid esters from propolis: an EPR spectroscopy and cyclic voltammetry study. Free RadicBiol Med, 18: 901–908, (1995).
13. Williams CA, Harborne JB, Newman M, Greenham J, Eagles J.Chrysin andother leaf exudate flavonoids in the genus Pelargonium. Phytochemistry, 46: 1349–1353, (1997).
14. Duarte J, Jimenez R, Villar IC, Perez-Vizcaino F, Jimenez J, Tamargo J. Vasorelaxanteffects of the bioflavonoid chrysin in isolated rat aorta. Planta Med, 67: 567–569, (2001).
15. LapidotT,Walker MD, Kanner J. Antioxidant and prooxidant effects of phenolicson pancreatic beta-cells in vitro. J Agric Food Chem, 50: 7220–7225, (2002).
16. He XL, Wang YH, Bi MG, Du GH. Chrysin improvescognitive deficits and brain damage induced by chroniccerebral hypoperfusion in rats. Eur J Pharmacol, 680(1-3):41-8, (2012).
17. Izuta H, Shimazawa M, Tazawa S, Araki Y, Mishima S,Hara H. Protective effects of Chinese propolis and itscomponent, chrysin, against neuronal cell death viainhibition of mitochondrial apoptosis pathway in SHSY5Ycells. J Agric Food Chem, 56(19):8944-53, (2008).
18. S.A. Frautschy, G.M. Cole. Bioavailable curcuminoid formulations for treating Alzheimer's disease and other age-related

disorders, United States, US: 2009/0324703 A1, 2009.
19. Kakkar V, Singh S, Singla D, Kaur IP. Exploring solid lipid nanoparticles to enhance the oral bioavailability of curcumin, Molecular Nutrition and Food Research, 55(3): 495–503, (2011).
20. Rohit B, Pal KI, A method to prepare solid lipid nanoparticles with improved entrapment efficiency of hydrophilic drugs, Current Nanoscience, 9(2): 1-9, (2013).
21. Yang KY, Lin LC, Tseng TY, Wang SC, Tsai TH, Oral bioavailability of curcumin in rat and the herbal analysis from *Curcuma longa* by LC-MS/MS. Journal of Chromatography B, Analytical Technologies in the Biomedical and Life Sciences, 853(2): 183–189, (2007).
22. Mosmann T, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods, 65(1-2): 55-63, (1983).
23. Lukowski G, Kasbohm J, Pflegel P, Illing A, Wulff H. Crystallographic investigation of cetylpalmitate solid lipid nanoparticles. Int J Pharm, 196: 201-205, (2000).
24. Jenning V, Gohla S. Encapsulation of retinoids in solid lipid nanoparticles. J Microencap, 18(2): 149-158, (2001).
25. RajatSandhir,AartiYadav,ArpitMehrotra,A dityaSunkaria,Amandeep Singh,Sadhna Sharma.Curcumin Nanoparticles Attenuate Neurochemical and Neurobehavioral Deficits in Experimental Model of Huntington's Disease. Neuromol Med, 16:106–118, (2014).
26. Poullain-Termeau S, Crauste-Manciet S, Brossard D, Muhamed S, Nicolaos G,Farinotti R, Barthélémy C, Robert H, Odou P. Effect of oil-in-water submicronemulsion surface charge on oral absorption of a poorly water-soluble drug in rats. Drug Deliv, 15: 503–514, (2008).
27. Kumar VV, Chandrasekar D, Ramakrishna S, Kishan V, Rao YM, Diwan PV. Development and evaluation of nitrendipine loaded solid lipid nanoparticles: influence of wax and glyceride lipids on plasma pharmacokinetics. Int J Pharm, 335:167-175, (2007).
28. Jenning V, Gohla S. Comparison of wax and glyceride solid lipid nanoparticles (SLN). Int J Pharm, 196: 219–222, (2000).
29. Reddy LH, Murthy RSR. Etoposide-Loaded Nanoparticles Made from Glyceride Lipids: Formulation, Characterization, in Vitro Drug Release, and Stability Evaluation. AAPS PharmSciTech, 6: E158–E166, (2005).
30. Anjali Singh, Iqbal Ahmad, SohailAkhter, Gaurav K Jain, ZeenatIqbal, SushamaTalegaonkar, Farhan J Ahmad.Nanocarrier based formulation of Thymoquinone improves oral delivery: Stability assessment, in vitro and in vivo studies. Colloids and Surfaces B: Biointerfaces,102: 822– 832, (2013).
31. Venkateswarlu V, Manjunath K. Preparation, characterization and in vitro release kinetics of clozapine solid lipid nanoparticles. J Control Release, 95:627-38, (2004).
32. Manjunath K, Reddy JS, Venkateswarlu V. Solid lipid nanoparticles as drug delivery systems. Methods Find ExpClinPharmacol, 27:127-144, (2005).
33. Mosmann T. Rapid colorimetric assay of cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Meth, 65: 55–63, (1983).
34. Muller RH, Maaben S, Weyhers H, Specht F, Lucks JS. Cytotoxicity of magnetite-loaded polylactide, polylactide / glycolide particles and solid lipid nanoparticles. Int JPharm, 138: 85–94, (1996).
35. Muller RH, Maaben S, Weyhers H, Mehnert W.Phagozytic uptake and cytotoxicity of solid lipid nanoparticles (SLN) sterically stabilized with poloxamine 908 and poloxamer 407. J Drug Target, 4: 161–170, (1996).