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REDEVELOPMENT, OPTIMIZATION AND *IN VITRO* CHARACTERIZATION OF DASATINIB-LOADED CHITOSAN NANOPARTICLES TO ENHANCE THE DISSOLUTION RATE

SHYAM S KUMAR[1*], GOPALAKRISHNAN G[2] AND GOWRISHANKAR N L[1]

1: Research Scholar, Department of Pharmacy, Faculty of Engineering and Technology, Annamalai University, Chidambaram, Tamilnadu, India

2: Department of Pharmacy, Faculty of Engineering and Technology, Annamalai University, Chidambaram, Tamilnadu, India

***Corresponding Author: Shyam S Kumar: E Mail: shyamchennath@gmail.com**

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ABSTRACT

The main objective of the present study is to formulate dasatinib loaded nanoparticles to enhance the solubility and dissolution rate of the drug. The nanoparticles were prepared by simple ionic gelation method using various concentrations of chitosan and TPP. The prepared nanoparticles were evaluated for particle size, shape, charge, encapsulation efficiency and *in vitro* drug release**.** The optimized drug loaded nanoparticles showed the size of 125 ± 4mn with PDI 0.25 ± 0.05, potential of +40 ± 2mV, encapsulation efficiency of 65.5 ± 1.2% and the drug release of 79 % with an initial burst effect of 22% in one hour followed by sustained release up to 24 hrs. These preliminary results demonstrate that the possibility of delivering hydrophobic drugs to target with enhanced encapsulation efficiency and dissolution rate

Keywords: Chitosan, Hydrophobic drugs, Nanoparticles, cancer

INTRODUCTION

Noncommunicable diseases (NCDs) are now responsible for the majority of global deaths and cancer is expected to rank as the leading cause of death and the single most important

barrier to increasing life expectancy in every country of the world in the 21st century. The unsatisfactory outcome of drug treatment is because of not only the drugs' low efficacy to stop the tumor growth and progression, but also the serious side effects, drug resistance and cancer relapse, especially for chemotherapy drugs **[1]**. However, the growing evidence shows that the molecularly targeted therapeutics may face the similar issues as other anticancer drugs, such as poor solubility, low bioavailability, insufficient tumor specificity, and drug resistance **[2]**.

Chronic myeloid leukemia (CML) is a common type of cancer of white blood cells that affect both blood and bone marrow with a rising morbidity **[3]**. Dasatinib (DAS), a small molecule tyrosine kinase inhibitor, can effectively fight against CML and ALL by inhibiting the activity of both Src and BCR-ABL tyrosine kinases in leukemia cells **[4, 5]**. However, DAS treatment has been reported to cause serious hematologic and non-hematologic adverse effects due to its interaction with non-disease-related processes and cells, which often leads to a dose reduction or treatment discontinuation in clinic **[6]**. Peripheral edema and pleural effusion are the common non-hematologic side effects occurred during DAS treatment, which is likely caused by endothelial hyper permeability **[7-9]**. NPs showed various advantages including the increased drug solubility and stability, improved Pharmacokinetics, enhanced permeability and retention (EPR) effect-mediated tumor targeting, and capability of further engineering to impart various functionalities Polymer based NPs are commonly used to improve drug bioavailability and/or reduce drug associated side effects **[10]**. Chitosan is a natural hydrophilic polysaccharide copolymer of glucosamine and N-acetylglycosamine. It is considered as a safe excipient due to its biocompatibility, biodegradability and lack of toxicity **[11]**. Further it is cationic in nature and posses mucoadhesive property it will enhance the cellular uptake by ionic interaction. Recently it has received lots of interest for drug delivery as well as biomedical applications like wound healing ointment and dressings **[12],** artificial membranes **[13]** contact lenses and bandages.

The purpose of this study is to develop, optimize and characterize DAS loaded polymeric nanoparticles with the primary objective to enhance the dissolution rate and to investigate the effect of stabilizers on particle size, zeta potential, drug entrapment, morphology and *in vitro* drug release.

MATERIALS AND METHODS

Materials and Methods

Dasatinib and chitosan (Medium Mol.Wt, Viscosity of 200 cps) were purchased from Sigma Aldrich, U.S.A, Sodium tri poly phosphate was purchased from Loba Cheme, glacial acetic acid was obtained from Fishier Scientific, Dialysis membrane with a molecular weight cut off of 12,000-14,000 daltons was purchased from HiMedia laboratories, Mumbai, Potassium dihydrogen phosphate, tetra butyl methyl ether, ortho phosphoric acid, sodium hydroxide, were of analytical grade and acetonitrile used was HPLC grade

Preparation of chitosan nanoparticles

Dasatinib loaded CS-NPs were prepared using an ionic gelation method **[14]**. Determinate weights of chitosan were dissolved in glacial acetic acid 1% (v/v) 10 mg of dasatinib was added to the above solution under constant magnetic stirring, followed by addition of aqueous TPP solution in a drop wise manner. Then the solution was kept on constant magnetic stirring for 30 min and sonicated using probe sonicator (Vibra Sonics). The nanoparticle suspension was centrifuged at 13,000 rpm and 4ºC for 30 min using Eppendorf Ultracentrifuge to remove excessive amounts of TPP and free dasatinib. The pellets were dispersed in deionized water. Finally, NPs were lyophilized for 24 h using freeze dryer (Lyodel) for storage in powdered form.

Physiochemical characterization of nanoparticles

Particle size and Zeta potential using photon correlation spectroscopy The average hydrodynamic diameter and polydispersity index (PDI) of the formulated nanoparticles were determined by dynamic light scattering (DLS) analysis using Zetasizer Nano ZS90 (Malvern Instruments limited, UK), 1ml of sample of nanoparticles dispersion was placed in disposable cuvettes for particle size measurements. Each experiment was conducted in triplicate. The electrophoretic mobility (zeta potential) measurements were made using the Malvern Zetasizer (Nano ZS90, Malvern Instruments) at 25ºC. Samples were diluted with double distilled water **[15]**.

Atomic Force Microscopy (AFM)

The surface properties of drug loaded nanoparticles were visualized by an atomic force microscope (Nova NTEGRA prima, Russia) Russia) under normal atmospheric conditions. Explorer atomic force microscope was in tapping mode, using high-resonant-frequency (F0 = 4-150 kHz) pyramidal cantilevers with silicon probes having force constants of 0.35-6.06 N/m. Scan speeds were set at 2 Hz. The samples were diluted

10 times with distilled water and then dropped onto glass slides, followed by vacuum drying during 24 hours at 25ºC. Height measurements were obtained using AFM image analysis software (Multimode Scanning probe microscope (NTMDT, NTEGRA prima, Russia)

Encapsulation efficiency

Nanoparticles were separated from aqueous phase by ultracentrifugation (Eppendorf) at 13000 rpm and 4°C for 45 minutes. The supernatants were collected and evaluated for dasatinib residue by UV. The encapsulation efficiency (EE) was determined indirectly by measurement of the amount of free dasatinib in the supernatant after ultracentrifugation and was calculated according to the following equation:

$$\mathbf{EE}= \frac{Amount\ of\ total\ drug - amount\ of\ drug\ in\ supernant}{Amount\ of\ total\ drug} x100$$

In vitro* drug *release

A modified dialysis method was used to evaluate the *in vitro* release of dasatinib loaded chitosan NPs. Two milliliters of nanoparticles suspension (corresponding to 2 mg of dasatinib) was placed in a dialysis bag (cellophane membrane, molecular weight cut off 10,000–12,000, Hi-Media, India), which was tied and placed into the phosphate buffer(0.1 M, pH 7.4) maintained at 37ºC with continuous magnetic stirring. At selected time intervals, aliquots were withdrawn from the release medium and replaced with the same amount of phosphate buffer. The sample was assayed spectrophotometricaly for dasatinib at 220 nm **[16]**.

***In vitro* release kinetics**

The drug release data were computed using DDsolver, which is an Excel plugin module and the resultant data were fitted to the Korsmeyer-Peppas exponential equation (1) to establish the mechanism of drug release where Q is the percentage of drug released at time t and k is a constant incorporating the structural and geometric characteristics of the dosage form. The diffusional exponent n is an important indicator of the mechanism of drug transport from the dosage form **[17]**.

$$Q = ktn$$

RESULT AND DISCUSSION

In the present study we have developed a Nano particulate system which is composed of hydrophobic polymer chitosan possessing the following advantage like 1) obtaining NP spontaneously by mild agitations absence of organic solvents and high temperature 2) obtaining NP with positive charge which could enhance the cellular uptake chitosan produces low to high positive charge which could enhance the cellular uptake and has mucoadhesive property. Conditions for

formation dasatinib loaded chitosan nanoparticles Chitosan NPs were prepared by simple scale up ionotropic gelation method similar to the method developed **[18]**. Chitosan is a cationic polyelectrolyte the nanoparticles were formed by inducing the gelation by controlling its interaction with polyanion TPP which leads to reduce the aqueous solubility of CS this system based on inter and intramolecular linkages created between TPP and positive charge of charged amino groups of CS which are responsible for the successful formation of the nanoparticles. The CS/TPP ratio is rate limiting step and controls the size and size distribution of nanoparticles **[19]**. In order to obtain nanoparticles with less size. We studied the effect of the CS/TPP ratio on the formation of nanoparticles. The maximum concentration of CS used was up to 6 mg/ml while the maximum concentration of TPP was 4 mg/ml .The particle size, PDI, drug encapsulation and zeta potential were analyzed and the result are presented in **(Table 1)** Our results indicated that particle size depend on both CS and TPP concentration that the specific concentration of CS/TPP can only form the nanoparticles with smaller size.

Effect of chitosan concentration

The role of chitosan concentration (0.2, 0.4 & 0.6%) on formation of nanoparticles and its influence on particle size was evaluated. When the amount of TPP was kept constant as 0.2% and an increase in CS concentration from 0.2% to 0.6% showed a decrease in the particle size with favorable PDI value. When the amount of chitosan exceeded 0.6% of CS a highly opalescent suspension is formed and it also leads to aggregation. Recent studies reported20 that when the concentration of CS is low (0.6%) it forms a low viscosity gelation medium resulting in a decrease in liquid phase dispersion, thus promoting formation of smaller particles.

Effect of TPP concentration

The role of TPP (0.2, 0.4 & 0.6%) concentration on particle size formation was studied. The increase in TPP concentration showed an increase in particle size. The TPP concentration with 0.2 and 0.4% with 0.6 and 0.2% chitosan forms particle 200 nm at the same time TPP concentration at 0.4 and 0.6% with 0.4 and 0.6% of CS concentration it showed a huge increase in particle size results in microparticles. When TPP concentration above 0.4% it results in highly opalescent suspension on storage it starts settling of particles.

Effect of sonication on particle size

The sonication time in the formation of CS-NP played a crucial role in the formation of smaller size nanoparticles. The smallest nanoparticles (125 ± 4 nm) were obtained with the sonication time of two minutes. While employing ultra-sonication formation of acoustic cavitation is the main cause for decreasing particle size. Acoustic cavitation by creating a large shear force on the chitosan molecules breaks the particles in to smaller ones. The increase in the sonication time from 30, 60 and 120 seconds showed the decreased particle size presented in **(Figure 1)**. The sonication time beyond two minutes showed no further decrease in particle size.

Particle size and Zeta potential

The nine formulations were prepared with various concentrations of chitosan and TPP. The particle size distribution of prepared CS nanoparticles was ranged from 125 ± 4 to 2852 ± 8 nm. With increasing the concentration of CS we observed decrease in particle size and increase in zeta value. At 0.2% concentration of TPP the cross linking with chitosan is high (0.6%) this result in more compact particle structure and the neutralization degree of charged amino acid is improved leading the good net charge of the particles. Due to the compact structure and net charge the particles prepared at this concentration have a smaller size. The zeta potential of the prepared CS nanoparticles was ranged from +5 to +40 mV. When increase in the concentration of CS the zeta value increases due to the higher degree of protonation of amino group in the CS molecule with the strong positive charge which leads to the higher zeta potential. The optimum concentration of CS/TPP was identified as 0.6% of CS with 0.2% TPP (CT3) with size of (125 ± 4) nm showed in (**Figure 2)**. the zeta potential for the prepared dasatinib loaded CS-NP (CT3) was 40 ± 2 mV which indicates the good colloidal stability of the prepared CS NP. Further the morphology of the nanoparticles was also analyzed using AFM showed in (**Figure 3)**. The physical state of dasatinib loaded CS-NP (CT3) was analyzed using selected area electron diffraction pattern (SAED) **(Figure 4)** showed that entrapped drug was in amorphous or in molecular dispersed state.

The encapsulation efficiency of dasatinib loaded CS-NP were ranged from 42.3 ± 1.6 to 70.6 ± 1.5%. The increase in chitosan concentration from 0.2 to 0.6% increases in encapsulation was observed at constant TPP concentration of 0.2%. Out of these formulations CT3 was selected as the best formulation based on particle size, zeta

potential (>+30 mV) and encapsulation of 65.5 ± 1.2% . The optimized formulation was selected for further studies.

***In vitro* release study**

The cumulative percentage release of optimized dasatinib loaded CS-NP (CT3) and free drug solution (D-sol) was studied in phosphate buffer pH 7.4 and showed in **(Figure 5)**. The percentage release was found to be 79 % up to 24 hrs and percentage release of drug in solution was about 85% in 6 hrs. The release profile of dasatinib loaded CS–NP exhibits an initial release burst release of 22% in one hour followed by the sustained release of 57% for following 24 hrs. The observed burst effect was due to the dissociation of drug molecules that were loosely bound to the surface of the chitosan nanoparticles. The second part of the release was slow and sustained release of encapsulated dasatinib at an approximately constant rate from the nanoparticles. In order to investigate the mode of drug release from chitosan nanoparticles. The release data of the optimized formulation were fitted to korsmeyer peppas model it showed the regression coefficient (r2 value) of 0.9916. And the 'n' were in the range of 0.307 it indicates the drug release follows fickian diffusion controlled release pattern.

Table 1: Optimization of DAS loaded chitosan nanoparticles

Formulation Code	CS (%)	TPP (%)	Size (nm)	PDI	Zeta potential (mV)	EE (%)	Physical appearance and opacity
CD1	0.2	0.2	414 ±4	0.49±0.03	+6±1	46.9±1.8	opalescent suspension
CD2	0.4	0.2	290±6	0.31±0.31	+12±1.2	55.5±1.1	opalescent suspension
CD3	0.6	0.2	125±4	0.25±0.05	+40±2	75.5±1.2	opalescent suspension
CD4	0.2	0.4	155±10	0.34±0.07	+31±2.5	70.6±1.5	opalescent suspension
CD5	0.4	0.4	1574±2	0.31±0.04	+5.7±1.5	52.6±2.05	Highly opalescent suspension
CD6	0.6	0.4	1953±3	0.28±0.07	+4.6± 2	42.3±1.6	Highly opalescent Suspension
CD7	0.2	0.6	1731±2	0.42±0.05	+5±1.6	47.8±1.4	Highly opalescent suspension
CD8	0.4	0.6	2852±8	0.56±0.07	+4± 2.5	55.06±1.9	Highly opalescent suspension
CD9	0.6	0.6	2764±6	0.84±0.08	+3±3	43.2±1.7	Highly opalescent suspension

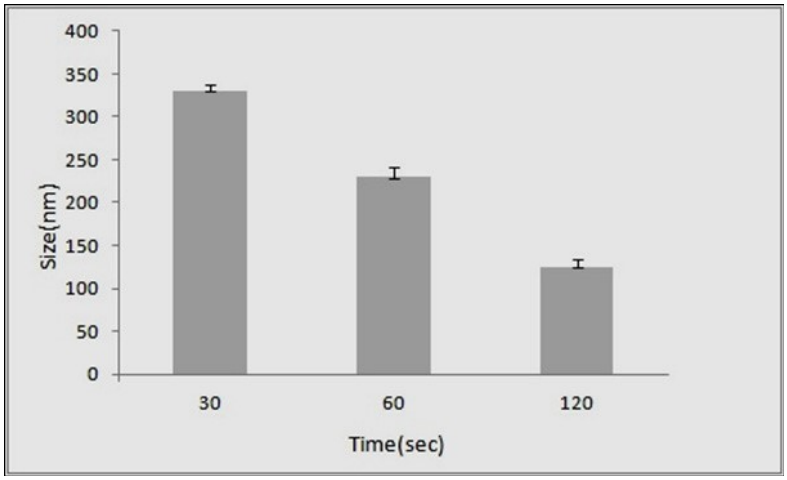

Figure 1: Influence of sonication time on particle size

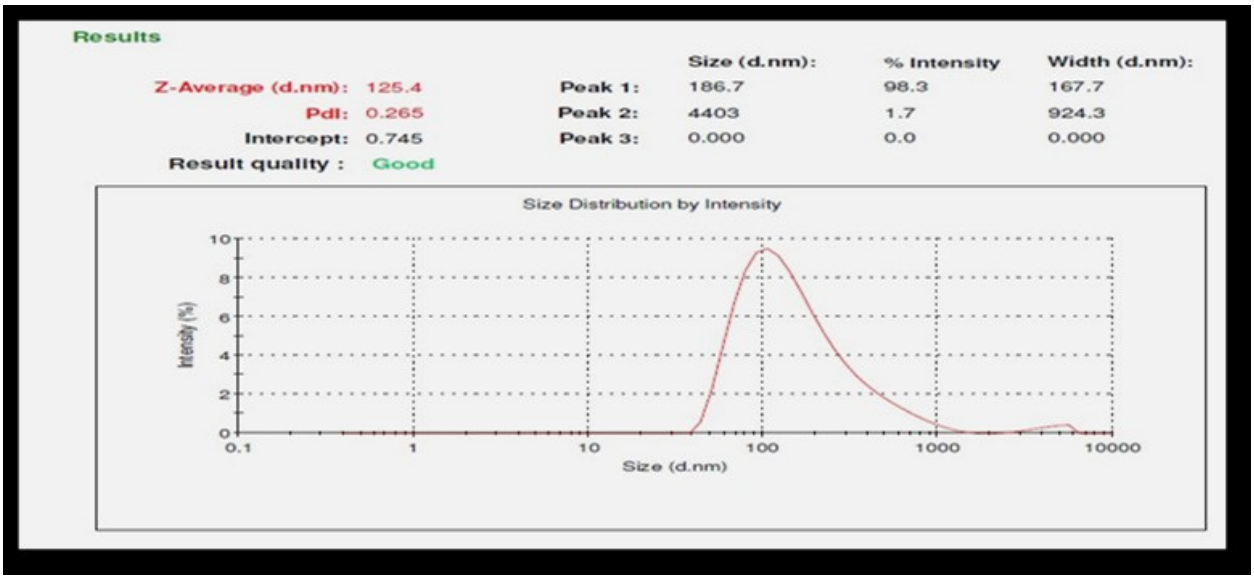

Figure 2: Particle size distribution of dasatinib loaded CS-NP containing CS of 0.6%and TPP 0.2%

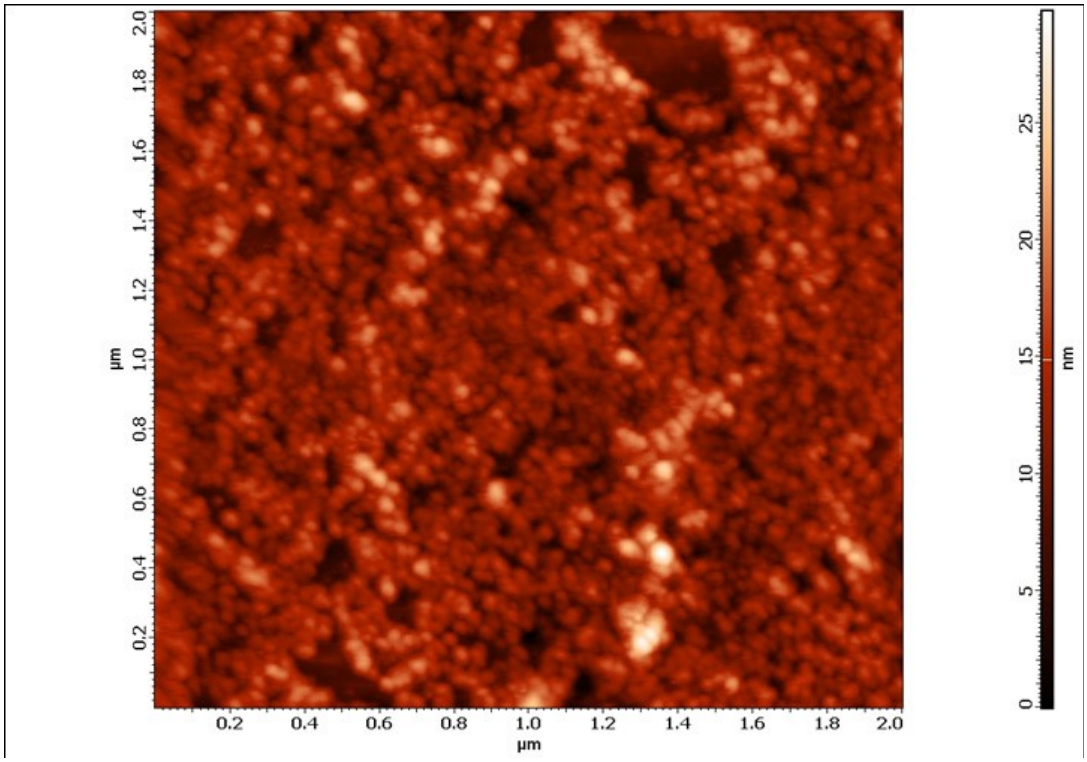

Figure 3: AFM images of dasatinib loaded CS-NP (2D)

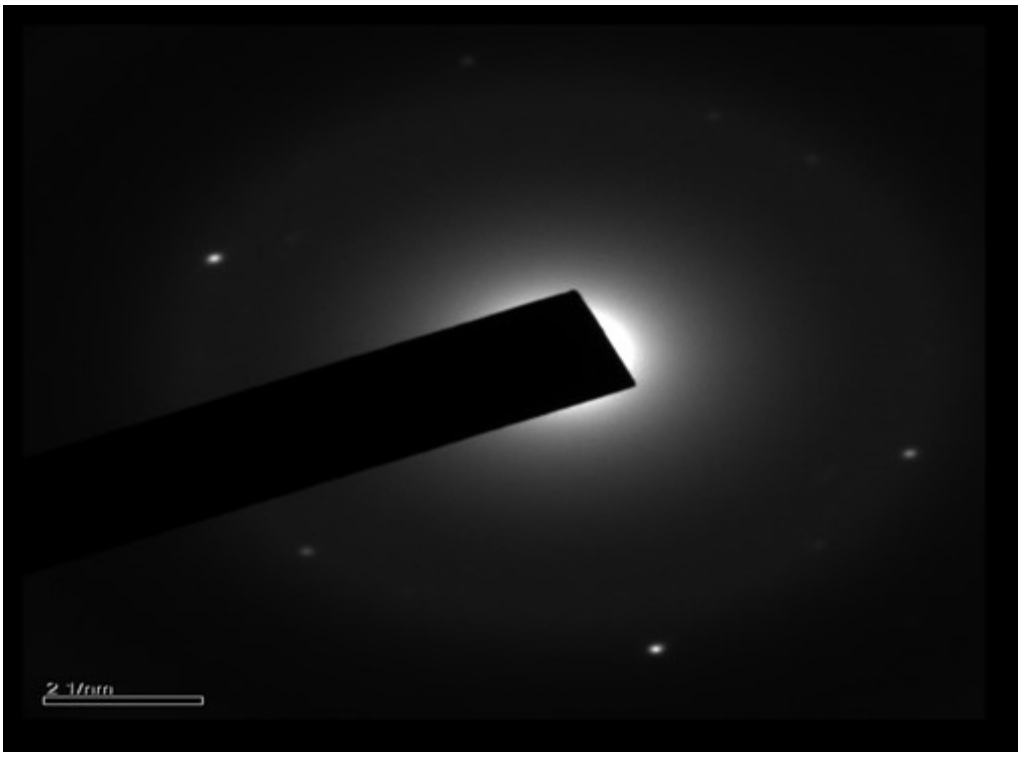

Figure 4: selected area electron diffraction pattern (SAED)

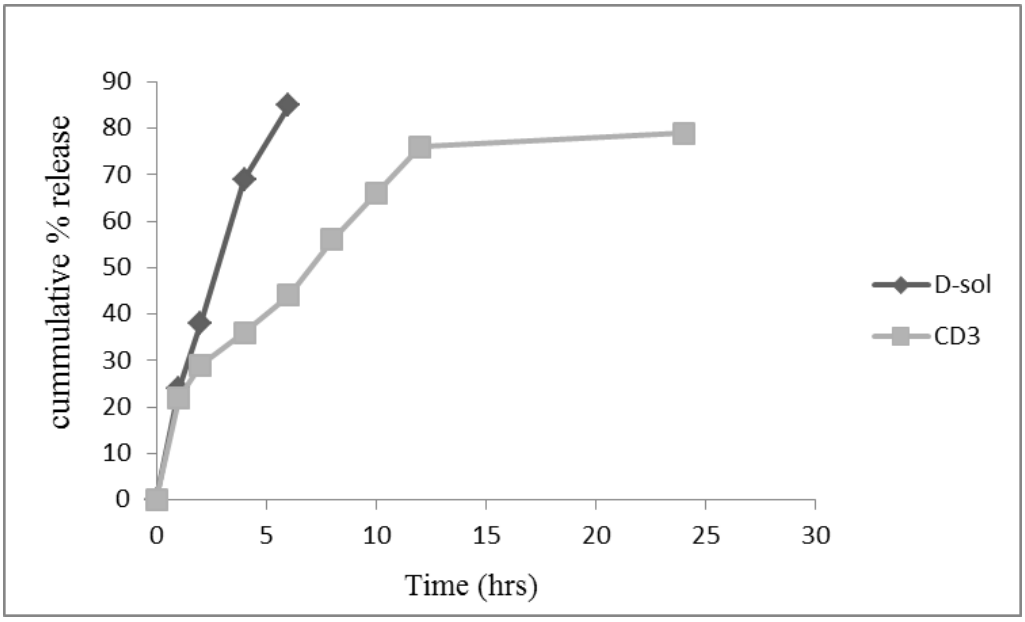

Figure 5: *In vitro* drug release profile of the optimized DAS CS-NP in pH 7.4 phosphate buffer

CONCLUSION

This study demonstrates the ionic gelation method can be used to enhance the solubility and dissolution rate of hydrophobic drugs and produce the size of less than 200 nm. The concentration of CS, TPP and sonication time strongly effect the particle size formation of the DAS CS-NP. The DAS CS-NP composed of 0.6% CS and 0.2% TPP was selected as the optimized formulation which produced smaller particle with better encapsulation. Based on this preliminary data the optimized formula will be extended for *In vivo* pharmacokinetic study to confirm the drug uptake in the target site.

CONFLICT OF INTEREST

Authors declare no conflict of interest

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