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**Original Article**

# Preparation, Optimization and Evaluation of Intravenous Curcumin Nanosuspensions Intended to Treat Liver Fibrosis

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Curcumin, an ayurvedic natural product has many pharmacological properties including liver protection. The objective of this study was to investigate parenteral nanosuspensions of curcumin for enhancing the solubility, Cmax and liver protection and achieve sustained parenteral drug release after intravenous administration. Curcumin nanosuspensions were prepared using solvent-antisolvent precipitation method. The formulations were characterized using techniques such as powder x-ray diffractometry (XRPD), scanning electron microscopy, saturation solubility, in-vitro dissolution, pharmacokinetics and liver protection in rats. Nanosuspensions of curcumin were successfully prepared using solvent-antisolvent precipitation. The size of the particles was below 600 nm. XRPD studies indicated transformation of curcumin from crystalline to amorphous form upon fabrication into nanosuspensions. Saturation solubility and dissolution rate were higher for nanosuspensions when compared to pure drug. The optimum formulation sustained the drug release for 8 days *in vivo*. Cmax and liver protection of curcumin in the form of intravenous nanosuspensions was significantly higher than that of intravenously administered pure drug in the solution form. Therefore, in this study a parenteral sustained release curcumin nanosuspension formulation intended for sustained systemic release of the drug, enhanced Cmax and enhanced liver protection/targeting was successfully developed. The formulation can be successfully used in liver fibrosis/cirrhosis.

**Key words**: Curcumin, Liver protection, Sustained release, Nanosuspension, Intravenous

## Karaciğer Fibrozisi Tedavisine Yönelik Olarak Intravenöz Kurkumin Nanosüspansiyonlarının Hazırlanması, Optimizasyonu ve Değerlendirilmesi

Ayurvedik doğal bir ürün olan kurkumin, karaciğer koruma da dahil pek çok farmakolojik özelliğe sahiptir. Bu çalışmanın amacı; kurkuminin çözünürlüğünü, Cmax'ı, karaciğer korumasını artırmak için parenteral nanosüspansiyonu hazırlayarak incelemek ve intravenöz uygulama sonrası sürekli parenteral ilaç salımı sağlamaktı. Kurkumin nanosüspansiyonları solvan-antisolvan çöktürme metodu ile hazırlandı. Formülasyonlar; X-ışını toz difraktometresi, (XRPD), taramalı elektron mikroskobu, doygunluk çözünürlüğü, in vitro çözünme, farmakokinetik ve ratlarda karaciğer koruma gibi tekniklerle karakterize edildi. Kurkumin nanosüspansiyon formülasyonları solvan-antisolvan presipitasyonu ile başarılı şekilde hazırlandı. Partiküllerin boyutu 600nm'nin altındaydı. XRPD çalışmaları nanosüspansiyon üretimiyle kurkuminin kristal halden amorf forma dönüştüğünü gösterdi. Saf etken madde ile karşılaştırıldığında nanosüpansiyonların doygunluk çözünürlüğü ve çözünme hızı daha yüksekti. Optimum formülasyon, *in vivo* etken madde salımını 8 güne kadar sürdürdü. Intravenöz kurkumin nanosüspansiyonunun karaciğer koruma ve Cmax'ı çözelti halinde verilen saf etken maddeninkinden anlamlı derecede yüksekti. Böylece, bu çalışmada, parenteral sürekli etkili kurkumin nanosüspansiyon formülasyonu etken maddenin sürekli sistemik salımı için tasarlandı, artırılmış Cmax ve artırılmış karaciğer koruma/hedefleme başarılı şekilde geliştirildi. Formülasyon karaciğer fibrosis/siroz'da başarılı şekilde kullanılabilir.

**Anahtar kelimeler:** Kurkumin, Karaciğer koruma, Sürekli etki, Nanosüspansiyon, Intravenöz.

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## INTRODUCTION

The parenteral administration route is the most effective and common form of delivery for active drug substances with metabolic high first pass metabolism, physicochemical limitation and narrow therapeutic index. For this reason, whatever drug delivery technology that can reduce the total number of injections throughout the drug therapy period will be truly advantageous not only in terms of compliance, but also for potential to improve the quality of the therapy. Such reduction in frequency of drug dosing is achieved, in practice, by the use of specific formulation technologies that guarantee that the release of the active drug substance happens in a slow and predictable manner. For several drugs, depending on the dose, it may be possible to reduce the injection frequency from daily to once or twice monthly or even less frequently. In addition to improving patient comfort, less frequent injection of drugs in the form of depot formulation smoothes out the plasma concentration time profiles by eliminating the peaks and valleys. Such smoothing out of the plasma profiles has the potential to not only boost the therapeutic benefit but also to reduce unwanted events and side effects. The objective of this study was to develop a novel parenteral sustained release dosage form of curcumin in the form of nanosuspension so as to develop an improvised sustained release parenteral dosage form for this drug useful in liver fibrosis. Curcumin is a poorly soluble compound which previously proved to have liver protection property and thus can be conveniently used in liver fibrosis/cirrhosis.

The present approaches for parenteral delivery include micellar solutions, salt formation, solubilization using cosolvents, cyclodextrin complexation, and more recently vesicular systems such as liposomes and niosomes. Vesicular systems can also be used for drug targeting (1). These methods have limitations like solubilization capacity, parenteral acceptability, high manufacturing cost, lack of flexibility for sustained release, etc. To solve the above problems, the nanosuspension technology is used. Nanosuspensions are administered through various parenteral routes such as intraarticular, intraperitoneal, intravenous, etc. Nanosuspensions increase the efficacy of parenterally administered drugs. Nanosuspensions can also be used for drug targeting (2). Paclitaxel nanosuspension was reported to have their superiority in reducing the median tumor burden (3). Clofazimine nanosuspension showed an improvement in stability as well as efficacy above the liposomal clofazimine in Mycobacterium avium-infected female mice (4). Rabinow et al. showed that intravenous nanosuspension of itraconazole enhanced efficacy of antifungal activity in rats relative to the solution formulation (5). Nanosuspensions/nanoparticles can enhance the intravenous solubility of the drug thereby enhances Cmax and AUC and also result in enhanced therapeutic efficacy of the poorly soluble drug (6). On contrary, it has been found that nanosuspensions reduce the Cmax by forming a depot in the reticular endothelial cells in the liver and then slowly releasing the drug. This is particularly helpful for drugs which have high toxicity (5). The reduction in Cmax with nanosuspensions in these cases is thus helpful. As there is a sustained parenteral release of the drug, it can result in enhanced activity with prolonged systemic action and enhanced AUC with same dose that has been used with other intravenous formulations. Curcumin nanosuspensions have been previously prepared and demonstrated enhanced efficacy following various mechanisms (7-9). These were administered via oral route to enhance the bioavailability and injected via intravenous route for enhancement in pharmacokinetic properties. However, studies that address enhancement in activity with curcumin nanosuspensions after intravenous administration were not reported. Thus, this study addressed this issue by taking curcumin nanosuspensions to treat liver fibrosis/cirrhosis. The results can be extrapolated to other diseases such as cancers in which curcumin nanosuspensions are useful.

Liver fibrosis/cirrhosis is the scarring process that represents the liver's response to injury. Cirrhosis and liver cancer resulting from cirrhosis are now among the top ten causes of death worldwide, and in many developed countries liver disease is now one of the top 5 causes of death in middle-age (10). There is no approved drug for this disease and thus new and effective therapies are thus urgently needed. Further, as liver fibrosis/cirrhosis is a chronic disease initiated and aggrevated at certain liver cells, a parenteral sustained release dosage form along with drug targeting activity is beneficial. Curcumin, anti-inflammatory and antioxidant drug has been previously proved to be effective in the treatment of liver fibrosis/cirrhosis (11). However, the clinical utility of this molecule can be further enhanced by drug targeting and parenteral sustained release delivery (12,13). In the past decade, several kinds of delivery system targeting to activated hepatic stellate cells (HSC) of the liver, the cells widely known to be involved in liver fibrosis have been designed and explored (14). This is based on the premise that HSC secrete fibrosis depositing material and are the main culprits for the formation of liver fibrosis. This targeting technique has demonstrated some success. But the clinical realization for these technologies is far from achievement with current technology. On the other hand Kupffer cells (KC) can also be targeted to resolve fibrosis/cirrhosis (15). Nanosuspensions, a colloidal formulation for curcumin can lead to higher intracellular drug levels at KC after drug encapsulation and targeting using intravenous route by passive drug targeting. This leads to enhanced therapy, reduced dose and reduced side-effects. This study investigates nanosuspensions of sizes greater than 100 nm so as to achieve passive targeting to KC. The formulation also provides parenteral sustained release.

## MATERIALS AND METHODS

Curcumin was obtained from Yucca Enterprises, Mumbai. Ethanol, Tween 80 and Sodium lauryl sulphate were obtained from Sd fine chemicals limited. All the other ingredients used were of analytical grade.

*Preparation and optimization of curcumin nanosuspensions*

Curcumin nanosuspension was prepared by solvent-antisolvent precipitation method as described previously (16). Different compositions were used as shown in Table 1. Two media were used in the preparation of nanosuspensions. In media 1, curcumin was dissolved in ethanol and used. In media 2, tween 80 was dissolved in distilled water and used and was contained in a Buchner funnel. Ethanol was used as a solvent and Tween 80 as a surfactant to stabilize the nanosuspension formulation. Media 1 was added to media 2 drop wise with a constant stirring on a magnetic stirrer using a butterfly syringe. Vacuum was applied by placing paper on Buchner flask while stirring till the solvent evaporated. Product obtained was filtered and dried at room temperature in a desiccator over night. The product thus obtained was again reconstituted filtered and centrifuged at 7000 rpm for 10 min. The pellet was reconstituted and was subjected to bath sonication for 5 min. The nanosuspension thus obtained was freeze dried to obtain the final form. The two factors: surfactant concentration and solvent-antisolvent ratio were investigated at different levels to study their effect on particle size. Several previous studies indicated that these two factors significantly affect the particle size of the fabricated nanosuspensions. In F1 to F4 and F8 tween 80 was kept constant and the solvent:antisolvent ratio was changed while in F4-F7, solvent-antisolvent was constant and tween 80 was altered. The volume of the solvent and antisolvents were also considered in the preparation method. When F-1 and F-4 are compared, the solvent-antisolvent ratios as well as surfactant concentrations are the same. However, the volumes of solvent and antisolvent were drastically reduced in F4 compared to F1. This resulted in reduction in the particle size. Thus, volumes of similar range were taken in the next batches F5-F8. Of the several formulations prepared, an optimum formulation was selected for further studies.

Optimization was based on particle size, surface morphology, saturation solubility and *in vitro* dissolution. A formulation is considered optimized when its particle size is less, surface morphology is smooth, saturation solubility and dissolution rate are the highest. F-7 formulation was finally considered to be optimized and is thus written in bold.

The mean particle size of nanosuspensions was determined using optical microscope. In this method size of 200 particles was determined by using stage micro meter. The average article size was determined. In order to examine the particle surface morphology and shape, Scanning Electron Microscopy (SEM) was used. A concentrated aqueous suspension was spread over a slab and dried under vacuum. The sample was shadowed in a cathodic evaporator with gold layer 20 nm thick. Photographs were taken using a JSM-5200 Scanning Electron Microscope (Tokyo, Japan) operated at 20 kV. Drug content, saturation solubility and dissolution rate with the nanosuspensions were determined. Drug content in the formulations was determined after dissolving the drug from the nanosuspensions in ethanol. The samples were suitably diluted and the curcumin concentration in the sample was analyzed by UV spectrophotometer at 421nm. The solubility of drug is an important physicochemical property because it effects the bioavailability of the drug, the rate of drug release into dissolution medium and consequently, the therapeutic efficiency of the pharmaceutical product. The solubility of material is usually determined by the equilibrium solubility method, which employs a saturated solution of the material, obtained by stirring an excess of material in the solvent for a prolonged until equilibrium achieved. The saturation solubility of curcumin nanosuspension and pure drug were evaluated in phosphate buffer (7.4). The nanosuspension powder was dispersed in 500mL of phosphate buffer (7.4). The prepared nanosuspension was kept for magnetic stirring for 72hrs. The pure drug was dispersed in 500 ml of phosphate buffer (7.4). The prepared suspension was kept for magnetic stirring for 72hrs. The samples were suitably diluted and the curcumin concentration in the sample was analyzed by UV spectrophotometer at 421nm. In-vitro dissolution studies of samples were carried out using USP apparatus II paddle method by

**Table 1.** Different batches of curcumin nanosuspension preparation with particle size

| Formulation | F-1 | F- 2 | F- 3 | F-4 | F-5 | F- 6 | F-7 | F- 8 |
|---|---|---|---|---|---|---|---|---|
| Curcumin | 100mg | 100mg | 100mg | 100mg | 100mg | 100mg | 100mg | 100mg |
| Ethanol | 25mL | 5mL | 5mL | 5mL | 5mL | 5mL | 5mL | 10mL |
| Distilled water | 50mL | 50mL | 25mL | 10mL | 10mL | 10mL | 10mL | 5mL |
| Tween 80 | 0.5mL | 0.5mL | 0.5mL | 0.5mL | 0.25mL | 0.75mL | 1.0mL | 0.5mL |
| Solvent: antisolvent | 0.5 | 0.1 | 0.2 | 0.5 | 0.5 | 0.5 | 0.5 | 2 |
| Average particle size | 3.74µ | 2.47µ | 2.22µ | 0.68µ | 0.70µ | 0.57µ | **0.47µ** | 0.81µ |

dispersed powder technique. Dissolution was performed for 1 hr. Accurately weighed nanosuspension samples containing 50 mg of drug were added to 900 ml of PBS (pH 7.4) at 37 ± 0.5°C and stirred at 75 rpm. An aliquot of 10ml was withdrawn at different time intervals. An equal volume of fresh dissolution medium was immediately replaced. The samples were assayed spectrophotometrically at 421 nm. The dissolution of nanosuspensions was compared with that of dissolution of equivalent amount of the pure drug.

*In vitro characterization of optimized curcumin nanosuspension*

Zeta potential of the optimized formulation was measured. The zeta potential was used to predict the storage stability of dispersed systems. The zeta potential was measured using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). Samples were diluted with the respective original dispersion medium which provides information regarding the thickened of the diffuse layer. Diluted nanosuspension was added to the sample cell (quartz cuvette) and was put into the sample holder unit and measured. The higher the measured zeta potential better was the physical long-term stability of the dispersion. The drug crystalline state in the optimized nanosuspension sample was evaluated by Powder X-Ray Diffraction (PXRD) analysis. X-ray spectra were recorded with X'Pert-PRO multipurpose X-Ray diffractometer (PAN analytical, Tokyo, Japan) using Ni-filtered, Cu K radiation, a voltage of 45 kV, and a current of 40 mA with a scintillation counter. The instrument was operated in the continuous scanning speed of 4°/min over a 2θ range of 5° to 40°. The samples were grinded using a mortar and pestle, placed into the cavity of an aluminum sample holder and packed smoothly using a glass slide. To determine the sustained release of the drug from optimized nanosuspension, in vitro release studies were performed. In-vitro release studies of samples were carried in a drug release study apparatus developed in our laboratory (17). Accurately weighed nanosuspension samples containing 10 mg of drug dispersed in 10 ml of phosphate buffer (pH 7.4) was taken in the donor compartment. The receptor compartment contained 100 ml of phosphate buffer media (pH 7.4) at 37 ± 0.5°C and stirred at 75 rpm. An aliquot of 10ml was withdrawn at different time intervals. An equal volume of fresh dissolution medium was immediately replaced. The samples were assayed spectrophotometrically at 421 nm. The release of nanosuspensions was compared with that of release of equivalent amount of the pure drug.

*In vivo pharmacokinetic and pharmacodynamic studies in rats with optimized formulation*

All the animal studies were conducted as per the guidelines of CPCSEA, India. The protocol was approved by Institutional Animal Ethics Committee of Geetanjali College of Pharmacy, Hyderabad (IAEC No. 1648/PO/a/12/CPCSEA). Wistar rats (weighted 180-220 g) were used as experimental animals. Twenty eight rats were randomly divided into seven groups with four rats in each group. Prior to the experimentation the rats were fasted for 12 h with free access to water. Group 1 was control rats. To induce hepatotoxicity, Group 2, Group 3, Group 4 and Group 5 were injected with carbon tetrachloride as described earlier. The next day after administering carbon tetrachloride, optimized curcumin nanosuspensions were given to Group 3 and Group 6 intravenously. In Group 4, 5 and 7, curcumin dissolved in ethanol was administered. In Group 4, curcumin was administered in equal daily divided doses for 8 days, while in Group 5 and Group 7, curcumin was administered one time iv bolus. A 50 mg equivalent drug was administered in all the drug treated groups. In Group 6 and 7, about 0.5 ml blood samples were collected via the orbit vein at 0.125, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24 hr and daily once for 10 days after administration. The collected blood samples were placed in heparinized tube and then separated immediately by centrifugation at 3000 rpm for 10 min and stored at -20ºC prior to the analysis. The plasma samples were then extracted and the curcumin levels were analyzed using a HPLC method as described earlier (18). In Group 1, 2,

4 and 5, blood was collected for every 6 hours on day 1 and then daily once for 10 days. Serum was separated and SGOT and SGPT levels were determined to assess liver damage with carbontetrachloride and liver protection with curcumin formulations as described earlier.

*Statistics*

All experiments were done six times and the data were expressed as mean ± STDEV and Tukey's post hoc test was done to analyze significance of difference between different groups using the statistical analysis software package SPSS (Version 16.0, IBM, USA).

Table 1. The influence of various parameters on particle size is shown in Figure 1A and Figure 1B.

Different batches of curcumin were prepared considering the solvent and anti-solvent ratio and concentration of surfactant to obtain nanoparticles with a reduced particle size. In batch 1, 1:2 concentration of solvent to anti-solvent ratio was considered,with 0.5ml surfactant concentration. It was observed that there was significant change in the particle size. The particles appeared to be crystals with the size of 3.74µ. In batch 2, 1:10 concentration of solvent to anti-solvent ratio was considered, with 0.5ml surfactant concentration. It was observed that there was a slight change in the

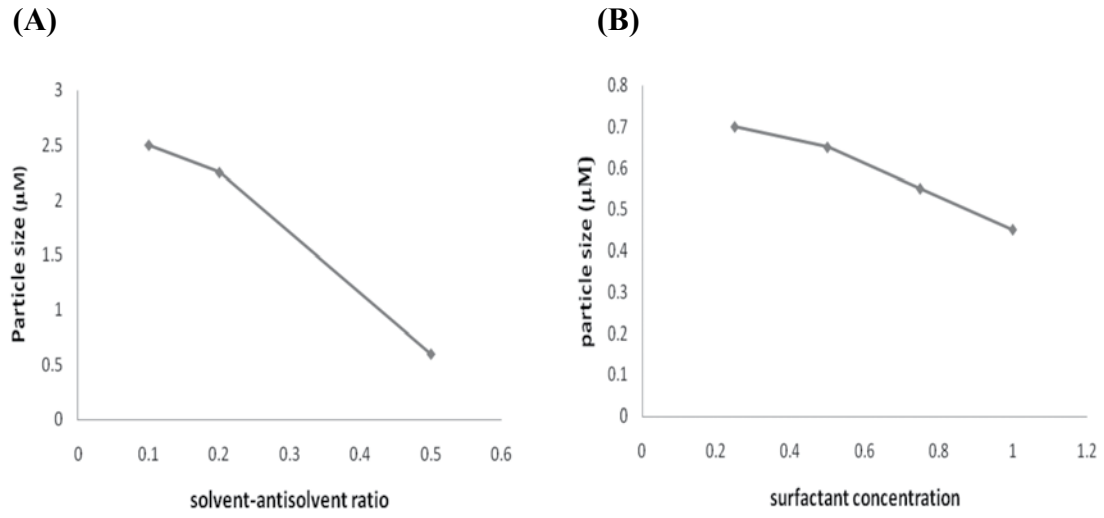



**Figure 1.** (A) Graphical representation showing effect of solvent-antisolvent ratio on particle size; (B) Graphical representation showing effect of surfactant concentration on particle size

## RESULTS

The mean particle size was determined by using optical microscopy. In this method particle size was determined by using stage micrometer and the average particle size was determined. The particle size is shown in

particle size the particles appeared to be crystals with the size of 2.47µ. In batch 3, the concentration of solvent to anti-solvent was reduced to 1:5 and surfactant concentration of 0.5ml. The particle size reduced to 2.22µ and the particles appeared to be spherical in shape. In batch 4 and 5,the concentration of solvent to

anti-solvent were kept constant by changing the surfactant concentration to 0.5mL and 0.25mL respectively. Particle size was found to be 0.68µ and 0.70µ. In batch 6,7 and 8 by changing the surfactant concentrations such as 0.75mL,1.0mL and 0.5ml respectively. The average particle size was found to be 0.578µ, 0.475µ and 0.81µ respectively. From this study it has been concluded that there is a size reduction of particles. It was observed that by increasing the concentration of surfactant reduces the particle size (Figure 1). The particle size was reduced with an increase in solvent-antisolvent ratio.

The drug content in all the formulations was above 99% suggesting less coating with the surfactant. The saturation solubility of the pure drug in phosphate buffer pH 7.4 was found to be 0.39 mg/mL. The saturation solubility with all the batches was higher than that of the pure drug (Table 2). Dissolution rate was also higher with nanosuspensions compared to conventional suspensions.

**Table 2**. Saturation solubility of different nanosuspension formulations

| Formulation | Saturation solubility(µg/mL) |
|---|---|
| F-1 | 1.8 |
| F-2 | 2.2 |
| F-3 | 2.3 |
| F-4 | 5.8 |
| F-5 | 3.7 |
| F-6 | 6.0 |
| **F-7** | **7.3** |
| F-8 | 3.4 |

Considering the yield and reduction in particle size, F- 7 was preferred to be the best among all the batches. Surface morphology and shape were visualized. The particles appeared as smooth, crystal, porous and spherical (Figure 2).

The zeta potential and charge of the optimized nanosuspension was evaluated by measuring the zeta potential of the nanosuspension by the Malvern zeta sizer. The zeta potential of the optimized formulation (F-7) was found to be -27.92 mV. From the Figure 3 XRPD Graph it was observed that the crystallanity of the drug was changed to amorphous form in the nanosuspension. The peaks obtained for pure drug was very clear and sharp the intensity of the peaks was very high when compared to peaks of curcumin nanosuspension. Reduction in the peak intensity indicates the change in crystal structure. From this we can conclude that there was reduction in the crystallanity and change into amorphous structures as: polymorphic transformations; alterations in crystallanity, changes in state. The state of drug has changed from one form to another form so there is change in drug solid state property from crystalline to amorphous.

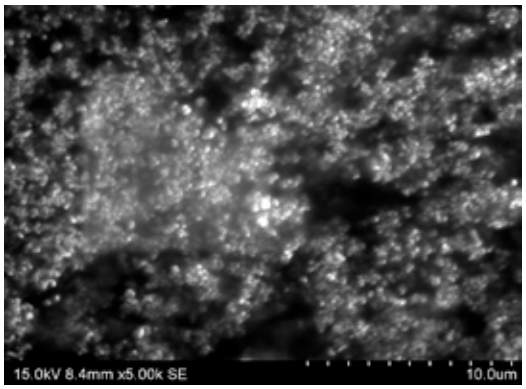
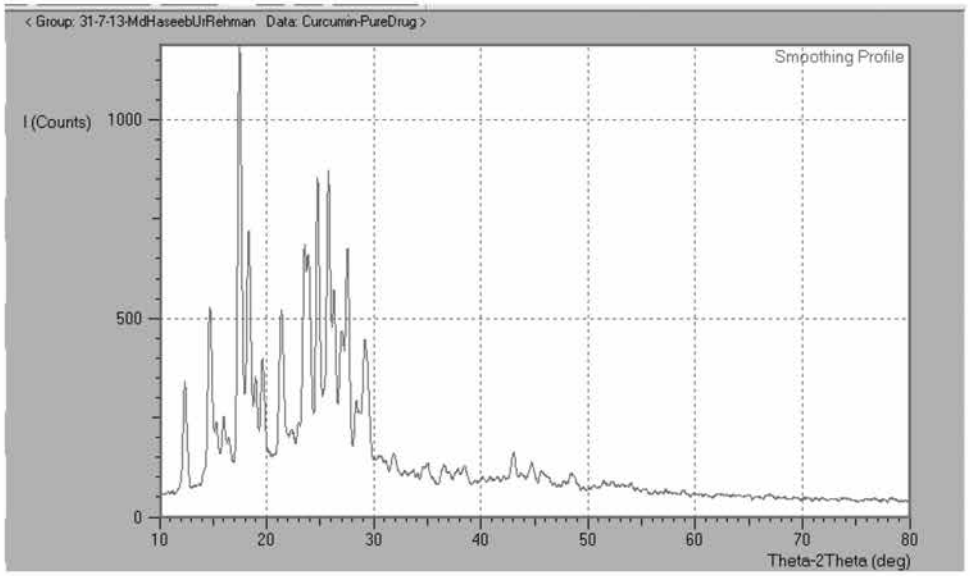



**Figure 2.** SEM picture of curcumin nanosuspension

**(A)**

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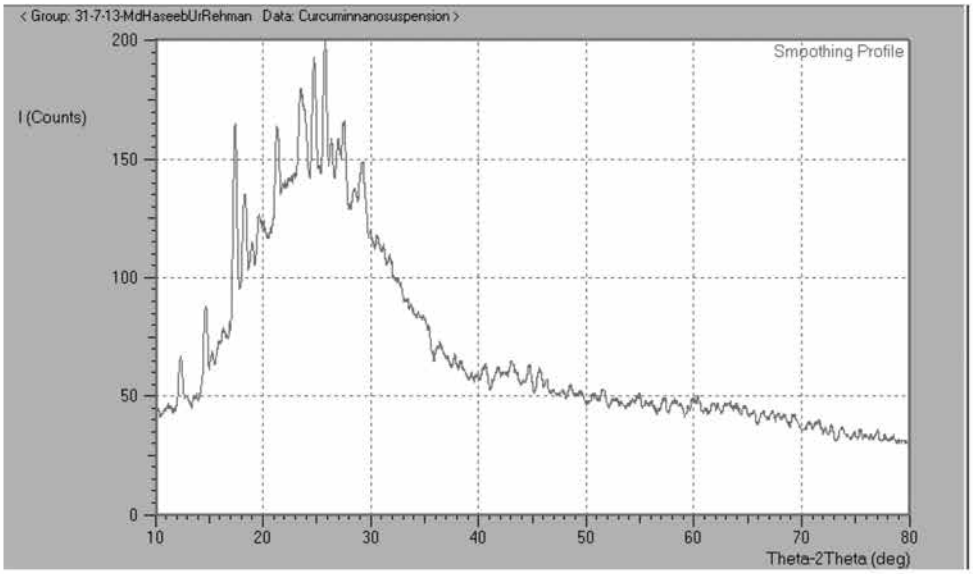
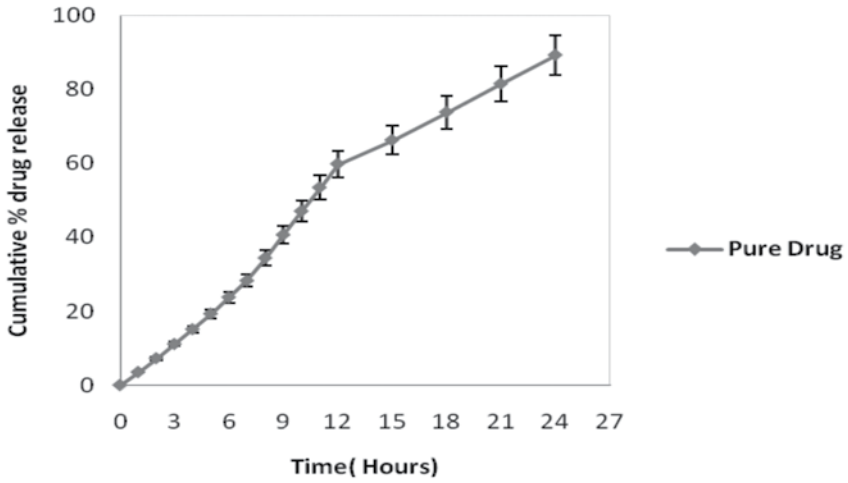



**Figure 3.** (A) XRPD graph of pure drug and (B) XRPD graph of nanosuspension

The results of drug release demonstrated sustained release of the drug from optimized curcumin nanosuspension. The results of drug release were expressed in terms of % drug release as the function of time (Figure 4). The data obtained revealed that the release of pure curcumin was (3.5 % in 1 hr) and (89.3 % in 24 hrs). From the data obtained it was observed

**(A)**

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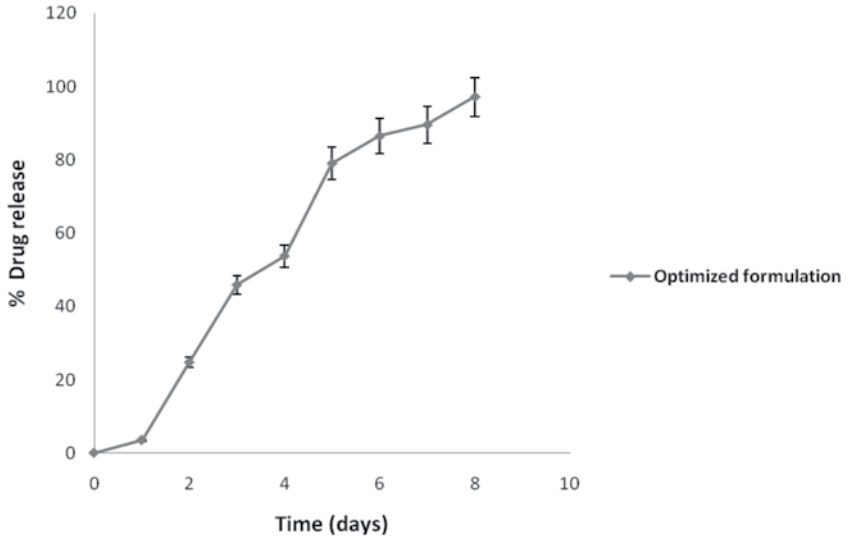


**Figure 4. (**A) Graphical representation of release profile of pure drug; (B) Graphical representation of release profile of nanosuspension (F-7) formulation

that there was sustainment in % drug release (3.52 in 1st day) and (97.12 in 8th day). It clearly indicates that nanosuspension formulation has been a successful technique to sustain the drug release. The drug is released *in vitro* for 8 days. HPLC method has been utilized to calculate the drug levels in plasma and tissues. Extraction efficiency was 76%. The retention time was 10.4 min and the minimum detection level was 20 ng/mL. Drug concentration in plasma following intravenous administration of the curcumin nanosuspension and the drug solution was presented in Figure 5. There was a sustained release of curcumin in

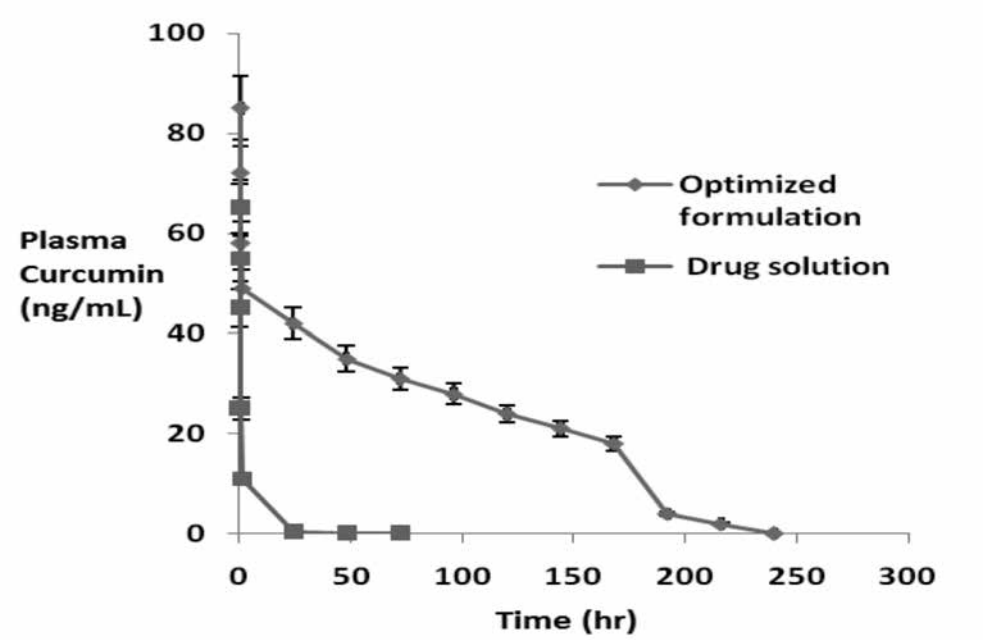


**Figure 5.** Plasma levels of curcumin upon administration of drug solution and optimized

plasma for 8 days with one time administration of nanosuspension while with bolus solution, the drug disappeared within 24 hours. The Cmax was found to be 85 ng/mL with nanosuspension and 65 ng/mL for i.v. solution administration. The tmax in both the cases was 0.75 hr. From the experimental studies it was witnessed that drug was found to be more in liver in comparison to kidney, lungs and brain (data not shown). The drug deposition order in various tissues is as follows: liver > lung > kidney > brain.

Two rats died in $CCl_4$ group, and no rats died in other groups during the whole experimental period. The ability of curcumin nanosuspensions to protect rat livers against damage as well as fibrogenesis activated by means of $CCl_4$ was assessed using SGOT and SGPT levels (Figure 6). Increased serum level of SGPT and SGOT is associated with liver damage. $CCl_4$ administration generated a significant raise in serum SGOT to 59.1 ± 4.48 U/L in comparison with normal value that was 18 ± 3.35 U/L. Administration of curcumin nanosuspension and drug treatment produced a substantial lowering in SGOT and SGPT levels. With nanosuspension administration there was 90% reversal in serum SGOT and SGPT levels while with repeated i.v. solution administration there was 70% reversal in serum levels. With one time bolus administration of curcumin, the liver protection as assessed by SGOT and SGPT levels is very insignificant.

## DISCUSSION

Nanosuspensions are suitable injectable forms for poorly soluble drugs such as curcumin. Solvent-antisolvent precipitation technique a commonly used technique to prepare nanosuspensions. Nanosuspensions of this study were prepared using solvent-antisolvent precipitation technique. The production of nanosuspensions using this technique is quite simple and scalable. Stabilization of nanosuspensions requires a stabilizer. Tween 80

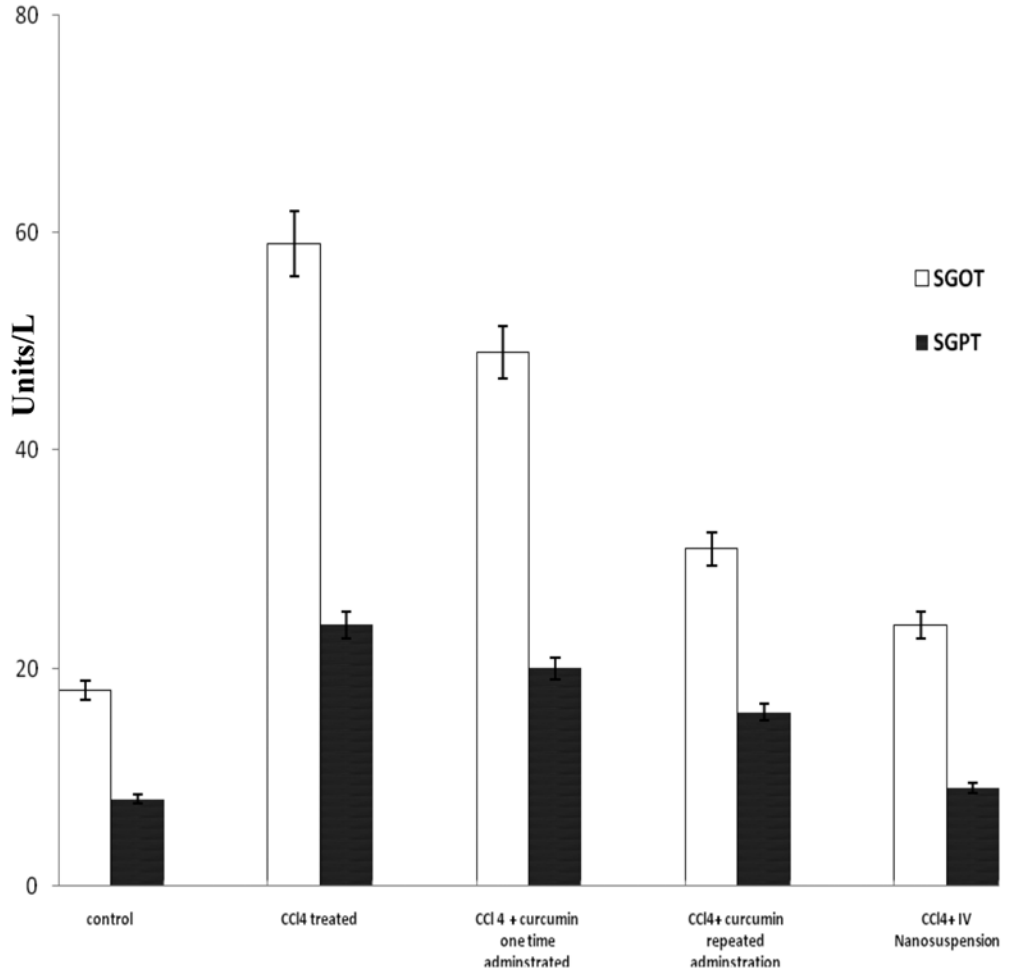


**Figure 6.** SGOT, SGPT levels after various administrations

was successfully used to prepare curcumin nanosuspensions. Tween 80 is a surfactant useful in formulations that are also administered intravenously (19). Thus, the formulations prepared can be conveniently administered via intravenous route. Two factors mainly influenced the particle size of nanosuspensions in solvent-antisolvent precipitation technique. These are solvent-antisolvent ratio and surfactant concentration. Both these factors influence particle size in our study. Thus, suitable formulations of curcumin nanosuspensions can be prepared by altering the solvent-antisolvent ratio or the concentration of the surfactant. Other stabilizers can also be used to prepare curcumin nanosuspensions. The saturation solubility that determines the Cmax after intravenous administration has been increased with the formation of nanosuspensions. The dissolution rate was also increased. Previously such results were obtained with curcumin nanoparticles (6). In this study by Mohanty and Sahoo, nanoparticular curcumin was developed to overcome major

obstacles association with curcumin delivery poor solubility, rapid degradation and poor bioavailability. They concluded that enhancement of water solubility as well as stability will undoubtedly bring curcumin to the forefront of existing anticancer therapeutic agents. It was concluded that the encapsulation of curcumin within nanoparticulate curcumin brought about a new avenue to improve the bioavailability of curcumin and make the drug amenable to intravenous dosing for the treatment of cancers. Similar results were obtained in our study. From the various formulations we prepared, we selected an optimum formulation with most desired properties. This formulation was taken for further studies. SEM, XRPD, sustained in vitro/in vivo drug release and pharmacodynamic activity of liver protection were determined with optimized formulation.

SEM pictures confirmed the formation of nanosuspensions. The particles were spherical in nature. XRPD was used to investigate the physical nature of the encapsulated drug in the optimized formulation. XRPD was used for analysis of a variety of transformations during pharmaceutical processing and storage such and degree of hydration. Upon preparation to nanosuspensions, the crystalline nature of curcumin was transformed to amorphous form. Thus, improvement in saturation solubility may be due to the formulation of nanosize as well as change to amorphous form. Thadkala et al., demonstrated similar results with amorphous ezetimibe nanosuspensions (20). Further, in the sustained release of the drug both in vitro drug release was determined with optimized nanosuspension. The release of the drug from the nanosuspension was sustained for a period of 8 days. This was demonstrated *in vitro*. Thus, improvement in saturation solubility and the sustainment in drug release indicates that a sustained release formulation of curcumin with improved Cmax can be obtained with nanosuspensions. We further demonstrated this speculation with in vivo studies. The Cmax of the curcumin with nanosuspension was increased when compared to that of solution administration. Previously it has been demonstrated with nanosuspensions and nanoparticles that Cmax can be reduced as well as Cmax can be enhanced. With itraconozole nanosuspensions, Cmax of the drug was reduced while with curcumin nanoparticles Cmax was enhanced. In our study, we demonstrated an enhancement in Cmax. This indicates that for some drugs, nanotechnology can result in enhanced Cmax while it can be reduced with other drugs. Several factors including the pharmacokinetics of the drug, the physico-chemical properties of the drugs, the pharmacodynamic activity can have profound effect on this effect. We then aimed to demonstrate the benefits of curcumin nanosuspensions in liver protection. This property is particularly useful in liver fibrosis and cirrhosis where in significant morbidity is seen in recent years. When compared to solution form of curcumin, nanosuspension form demonstrated enhancement in liver protection activity. This could be attributed to enhanced Cmax, sustained in vivo drug release and localization in liver cells. As the particles are of sizes greater than 100 nm, it could be inferred that they can be quickly taken in the KC of the liver. These are also called reticular endothelial cells. Since, studies clearly indicated that KC are the main culprits in the initiation of fibrosis which results from enhanced oxidative stress, the enhanced cellular levels of antioxidant curcumin could conveniently arrest the progression of liver toxicity with carbontetrachloride administration. To test the hepatoprotective activity of nanosuspensions, the formulation was administered to $CCl_4$ induced model. Carbon tetrachloride, hepatotoxin that is a widely used model for hepatoprotective drug screening, and the intensity of the liver damage is assessed by increased levels of cytoplasmic enzymes (SGOT and SGPT) in circulation. Evaluation of the serum enzymes can be a beneficial quantitative marker of the degree and type of hepatocellular damage. Upon administration of curcumin nanosuspensions, it was able to reduce all the elevated enzyme levels. In this study, curcumin nanosuspensions having particle size of 470 ± 5.3 nm, a charge of -27.92

± 2.5 mv, and surface hydrophobicity might be largely taken on through RES and transferred directly into nonparenchymal hepatic cells in liver. The curability involving liver toxicity had been a lot more for nanosuspensions when compared to the solution because of smaller size particles were instantaneously taken up by the RES of liver thus the drug was aggregated in the liver and the drug release was more focused at the cellular level. This results in the hepatoprotection. The reversal of biochemical end points in a $CCl_4$ hepatotoxic model is more preferable with curcumin nanosuspensions in comparison to intravenously administered curcumin solution. The outcomes could be extrapolated to additional drugs recommending the significant advantage of passive targeting of drugs to the liver. Thus, curcumin nanosuspensions can be conveniently used in other diseases where in curcumin has a significant therapeutic potential.

## CONCLUSIONS

Curcumin nanosuspensions can be conveniently prepared using solvent-antisolvent precipitation technique. These suspensions after intravenous administration can result in enhanced Cmax and lead to sustained release of the drug into systemic circulation. In liver fibrosis model, the nanosuspension is more effective when compared to the solution form of its administration intravenously.

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