Compritrol ATO 888 based solid lipid nanoparticles of cefixime: Formulation and evaluation

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ABSTRACT

Solid lipid nanoparticles of cefixime were prepared by solvent- evaporation method using compritrol ATO 888 as the lipid core. The mean particle size of solid lipid nanoparticles, determined by Photon Correlation Spectroscopy (PCS), was 586.3 nm. The entrapment efficacy (EE) was measured using double beam UV/Vis spectrophotometry, reported 72.43. In vitro release was determined using USP apparatus type II (paddle type) in phosphate buffer saline pH 7.4. The drug release in the first 10 h was about 95.37 % of the total drug. In-vivo study was conducted on male rats after oral administration of cefixime solution and cefixime solid lipid nanoparticles in different formulations, the relative bioavailability of cefixime-SLNs was found to be 1.87. These results showed that cefixime absorption is enhanced by using solid lipid nanoparticles formulations.

Keywords: SLNs, Photon Correlation Spectroscopy, spectrophotometry.
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INTRODUCTION

Solid lipid nanoparticles are one of the most popular approaches to improve the oral bioavailability of the poorly water soluble drugs. solid lipid nanoparticles are having size (50-1000 nm) and are composed of lipid components which are in solid state at room temperature[1,2]. Due to their inherent biodegradability and biocompatibility, lipids are now beingextensively investigated as carriers (3) for drugs and proteins. Nanoparticles can protect the drug from degradation, enhance its transport and prolong its release; therefore they may improve the plasma –half life of the drug[4]. Several polymer-based nanotechnologies are being intended in order to optimize the technological (e.g., solubility, stability, bioavailability, etc.) aspects of drugs[5].

They have many advantages such as low toxicity, good biocompatibility and lipophillic drugs are better delivered by solid lipid nanoparticles and the system is much physically stable [6]. A variety of lipids have been shown to change the physical barrier function of the gut wall and hence, enhance the permeability [7]. The Solid lipid nanoparticles mainly those in the range of 120-200 nm are not taken up readily by the cells of the Reticuloendothelial System and thus bypass liver and spleen filtration[5]. Compounds from BCS class II and class IV are most likely the suitable candidates of choice for preparing solid lipid nanoparticles [8,9]. Siekmann prepared solid lipid nanoparticles of 30 to 100 nm by dissolving tripalmitin in chloroform. Then the solution was emulsified in an aqueous phase by high pressure homogenization. This study was to investigate the feasibility of preparing solid lipid nanoparticles using compritrol ATO 888 as carrier using solvent evaporation method [11].

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Cefixime is a third generation cephalosporin antibiotic used in the management of various infections caused by *Gram+ve* as well as *Gram-ve* bacterias. Cefixime oral bioavailability is about 40%-50% whether administered with or without food; however, absorption is increased approximately 0.8 hours when it is administered with food [12,13]. It is used to treat or prevent infections that are proven or strongly suspected to be caused by bacteria. One of the major problems with this drug is its very poor solubility in biological fluids that results into poor bioavailability after oral administration. It shows erratic dissolution problem in gastric and intestinal fluid due to its poor water solubility. Rate of absorption and/or extent of bioavailability for such insoluble drugs are controlled by rate of dissolution in gastrointestinal fluids [14].

MATERIALS AND METHODS

Materials
Cefixime from Cosmos pharmaceutical ltd., comprotrol ATO 888 from Gattefossee as a gift sample, tween 20 and span 80 availed by research lab. from rankem. All chemicals are of analytical grade.

Preparation of cefixime-SLNs
Solid lipid nanoparticles were prepared using solvent evaporation method. Cefixime 100 mg was weighed accurately and dissolved in 10 ml of dichloromethane. Compritrol ATO 888 and lipophilic surfactant Span 80 in different proportions dissolved to previous solution. Tween 20 was added to aqueous phase upto 100 ml of water. Aqueous phase was stirred at 3000 rpm for 20 minutes. Then organic phase solution was added slowly to aqueous phase and then stirred at 8000 rpm for 3 hours. The formulations used were shown in table 1.

Table 1: Compositions of different batches of Solid lipid nanoparticles

Ingredients	Formulation B4	Formulation B5	Formulation C3	Formulation C4	Formulation C5
Cefixime (%)	1	1	1	1	1
Compritrol ATO 888 (%)	8	10	6	8	10
Span 80 (%)	1	1	2	2	2
Tween 20 (%)	1	1	1	1	1
Aqueous solvent	Water	Water	Water	Water	Water
Organic solvent	DCM	DCM	DCM	DCM	DCM

Characterization of cefixime-SLNs
Entrapment efficiency
Percentage entrapment efficiency was studied by rupturing of particles by methanol. 100 mg of formulation was taken in volumetric flask with 10 ml of methanol. The formulation was mixed and sonicated till complete rupturing the particles. The solution was filtered and the liberated drug was estimated by spectrophotometrically with UV-double beam spectrophotometer.

Particle size
The mean diameter of solid lipid nanoparticle in the dispersion was determined by a NanoSizer 3000, (Malvern Instruments, Malvern) at a fixed angle of 90 degrees. The particle size analysis data were reported by using the volume distribution as calculated automatically by the device. Samples were diluted by 50-fold with dust-free water to give the recommended scattering intensity of 100,000 counts per second. Each value is the mean of five measurements of 120 seconds each, divided into 10 subruns. The diameter is calculated from the autocorrelation function of the intensity of light scattered from particles, assuming a spherical form for the particles. For mean size calculation, the cumulant algorithm, which takes into account only one population of particles, was used. The polydispersity index (PI) is a measure of dispersion homogeneity and ranges from 0 to 1. Values close to zero indicate a homogeneous dispersion, while those greater than 0.3 indicate high heterogeneity.

Drug release
In-vitro dissolution studies were carried out in 900 ml of PBS 7.4 as a medium using USP apparatus type II (paddle type) with three replicates. The paddle rotation speed was 75 rpm and a temperature of 37 +/- 0.5° C was maintained. In all experiments, 5 ml of dissolution sample was withdrawn at 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 90, 120, 180, and 240, 300 minutes up to12 hours, filtered using a whatman filter and replaced with an equal volume of fresh medium to maintained sink conditions. The samples were analysed by UV double beam spectrophotometer at 286 nm. Cumulative percentages of drug dissolved from solid lipid nanoparticles were calculated and graphs were plotted.

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Oral administration

In-vivo studies approval was obtained from Institutional Animal Ethics Committee, Institute of pharmaceutical sciences and their guidelines were followed for the studies. For the in-vivo studies the optimum solid lipid nanoparticles formulation was taken. *In – vivo* studies were done on albino rats of weight 200-250gm. Six rabbits were kept in two groups. Plasma was withdrawn after oral administration of cefixime solution and cefixime-SLN formulation and was analyzed using HPLC method.

Data analysis

After administration of Cefixime dispersion and Cefixime-SLN formulation, plasma was withdrawn at fixed intervals and analyzed by HPLC method. Pharmacokinetics parameter t_{max}, C_{max} were analyzed. AUC value was calculated using trapezoidal method. AUC value calculated using formula $(C_1 + C_2)(T_2 - T_1)/2$. Scanning at wavelength 286 nm was done and the concentration of Cefixime in each sample was calculated using the areas obtained by densitometric scanning. The concentration of the Cefixime was calculated using the calibration curve prepared in plasma. Noncompartmental analysis was employed for calculating pharmacokinetic parameters like Cmax, Tmax, and AUC_{0-t}.

RESULTS AND DISCUSSION

Characterization of SLNs

The mean particle size of VIN-SLN prepared with different formulations, ranged from 586.3-855.7 nm (Table 2). The SLN formulations size was decreased on increasing the span 80 concentration in batch C (fig. 1).
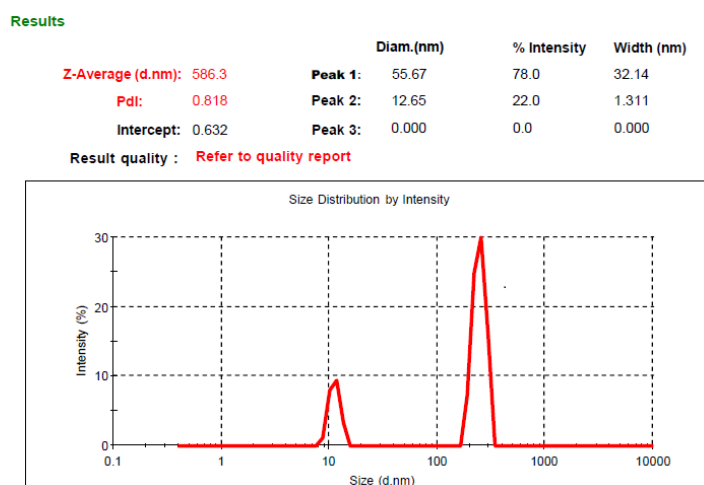

Results			Diam.(nm)	% Intensity	Width (nm)
Z-Average (d.nm):	586.3	Peak 1:	55.67	78.0	32.14
Pdl:	0.818	Peak 2:	12.65	22.0	1.311
Intercept:	0.632	Peak 3:	0.000	0.0	0.000
Result quality :	Refer to quality report				

Fig.1: Size distribution of CIP-SLNs measured using Malvern Zetasizer.

Drug:lipid ratio (1:8) was shows result having better particle characteristics like entrapment efficiency and particle size.

Table 2: The mean diameter and entrapment efficiency of different SLN Formulations

Formulation	Mean diameter	Entrapment Efficiency (%)
Formulation B4	716.6 nm	52.08
Formulation B5	855.7 nm	40.23
Formulation C3	649.4 nm	68.20
Formulation C4	586.3 nm	72.43
Formulation C5	729.2 nm	50.01

Entrapment efficiency decreases with increases in concentration of compritrol ATO 888. This decrease in entrapment efficiency might be due to high diffusion in greater particle size. The entrapment efficiency of formulations containing Tween 20 was a little lower due to the higher solubilization of formulation.

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Drug release of cefixime-SLNs

In vitro release profile of formulations was as follows in fig.2. In-vitro dissolution studies were carried out in 900 ml of PBS 7.4 as a medium using USP apparatus type II (paddle type) with three replicates. The data was processed using Microsoft Excel computer program and various statistical parameters were determined. All the selected batches showed approximately 50 % release of drug in just two hours. But batch C4 and C5 showed controlled release up to 12 hours. C4 batch showed 95.37 % release after 10 hours where as C5 batch showed 94.29 % release in 12 hours. From the results of data fitting to various models, C4 formulation shows controlled release.
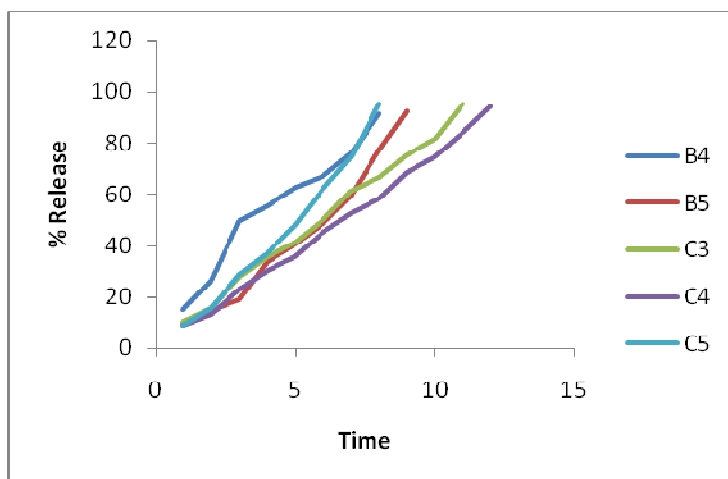

Fig. 2: In-vitro release of cefixime from formulation B4, B5, C3, C4 and C5 in PBS 7.4

Scaning electron microscopy Study

The shape and morphology of the solid lipid nanoparticles was determined by scanning electron microscopy (SEM). The SEM photograph also revealed the spherical shape and smooth surface of the solid lipid nanoparticles in Fig.3. The shape of the particle was found to be identical irrespective of the lipid composition used in the formulations.
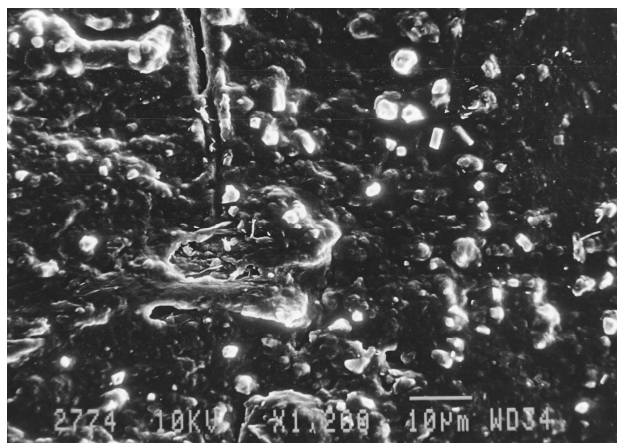

**Fig. 3: Scanning microscopy of cefixime-SLNs.**

In-vivo pharmacokinetic study

In vivo Studies were carried using albino rats. *In vivo* studies were performed to quantify cefixime after oral administration of suspension of solid lipid nanoparticles and pure cefixime. Table 3 shows the data of the plasma concentrations of cefixime after noted time analyzed by HPLC.

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Table 3: plasma concentration profile data for cefixime in rat from cefixime-SLN and pure drug.

Sr. No.	Time (in hrs.)	Pure drug conc.	Cefixime-SLN formulation conc.
1.	0	0	0
2.	0.5	3.14	3.11
3.	1	3.83	4.23
4.	2	4.05	5.73
5.	3	4.32	6.02
6.	4	3.06	6.32
7.	6	0.005	5.88
8.	8	0	3.06

The plasma concentration time profile of cefixime and cefixime-SLN formulation in rats is represented in Figure 4.
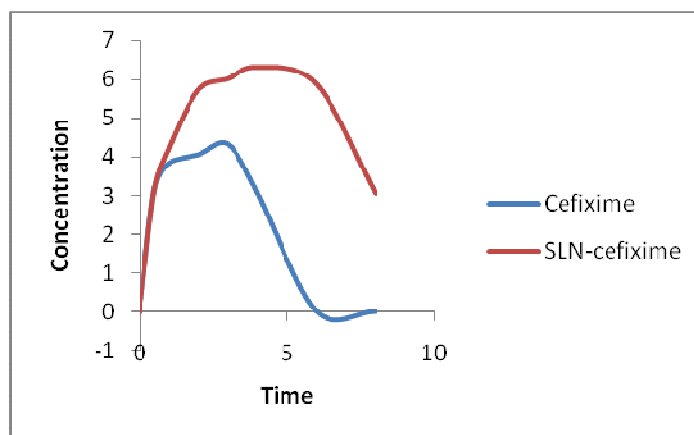
Fig 4: Plasma conc. of Cefixime and Cefixime-SLN

In Vivo study shows inc. in C_{max} values for pure drug and cefixime-SLNs. C_{max} value for pure drug and cefixime-SLNs were found to be 4.32 µg/ml and 6.32 µg/ml respectively. Increase in values of C_{max} and AUC Shows increase in bioavailability of drug in cefixime-SLNs formulation. The surfactants may have contributed to an increase in the permeability of the intestinal membrane or improved the affinity between lipid particles and the intestinal membrane[15,16]. Some particles might be taken up into the lymphatic organs and can be enter the systemic circulation[17,18].

Relative Bioavalability = AUC of Test/ AUC of Std
= 42.15/ 22.44
= 1.87.

CONCLUSION

In our study, poorly bioavailable drug cefixime was successfully incorporated into solid lipid nanoparticles by solvent evaporation method. The entrapment efficiency and size of SLNs were investigated. The in-vitro release tests showed the controled release of the SLNs. An oral pharmacokinetic study was performed on albino rats and the results showed that SLNs produced a significant improvement in the bioavailability of cefixime compared with cefixime solution. The surfactant Compritrol ATO 888 also had significant influence on the oral absorption of cefixime . It finds that SLNs offer a promising delivery system for the enhancement of the bioavailability of poorly soluble drug cefixime.

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