INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

FORMULATION DEVELOPMENT OF CHITOSAN GELS ENRICHED WITH OFLOXACIN SOLID LIPID NANOPARTICLES

Samyuktha Metta* and Sravya Maddukuri

Department of Pharmaceutics, M L R Institute of Pharmacy,
Hyderabad, Telangana, India.

ABSTRACT

The purpose of this research was to investigate novel particulate carrier system such as SLN for ocular application of Ofloxacin and to evaluate its beneficial effects on eye. The SLN dispersions were prepared using High pressure homogenization technique which involved hot homogenization and were incorporated into polymeric gels of chitosan. The SLN were prepared by using the lipid phase consisting of Dynosan 118 (4.0gms) as the solid lipid, Soyabean lecithin (3.0gms) as a lipid phase surfactant and the aqueous phase containing Poloxamer 188 (Pluronic F68) as an aqueous phase surfactant. The nanoparticles dispersion were evaluated by various parameters like particles size, OSLNF1 (250.1 ±30.2) and OSLN F6 (98.5 ± 12.8), zeta potential OSLN F1 (+29.3±3.1) and OSLN F6 (+31.2±1.2), Drug Loading OSLN F1(99.1 ± 0.32) and OSLN F6(96.1 ± 0.30), Entrapment efficiency OSLN F1(64.2 ±0.19) and OSLN F6 (62.4 ± 2)and polydispersity index (OSLN,0.310 ± 0.01 and OSLN, (0.318 ± 0.02). Differential scanning calorimetry and FTIR were conducted for the optimized formulae and was found to have no incompatibility between the drug and exipients. Transmission electron microscopy (TEM) was done to know the surface morphology.

The in vitro release profile of Ofloxacin from SLN dispersion prolonged drug release for 10hrs. It concluded that SLN represented a potential particulate carrier with increase drug release and improved characteristics.

Keywords: Ofloxacin, Chitosan Gel, Solid lipid Nanoparticles, UV spectrophotometer methods.

INTRODUCTION

Ofloxacin (OFC) is chemically 1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxilic acid which has broader spectrum of antibacterial activity than the older fluoroquinolones[1]. Solid lipid Nanoparticles (SLN), introduced in 1991 has attracted increasing attention as an alternative colloidal carrier system for controlled drug delivery because of their good tolerability, biodegrability and physical stability. SLN are composed of physiologically compatible lipids with a high melting point as the solid core, which is coated by non toxic amphilic surfactant as the outer shell. SLN dispersions have been proposed as a new type of colloidal drug carrier system suitable for intravenous administration. The system consists of spherical SLN in the nanometer range which are dispersed in water or aqueous surfactant solution[2]. Generally they are made of solid hydrophobic core having a mono layer of phospholipids coating. The solid core contains the drug dissolved or dispersed in the solid high melting fat matrix. The hydrophobic chains of phospholipids are embedded in the fat matrix. They have potential to carry liphophilic or hydrophilic drugs or diagnostic.

There are two well established methods reported in literature for the production of SLN. Homogenization of melted lipids at elevated temperature is termed as hot homogenization technique and homogenization of a suspension of solid lipids at room temperature or below is referred to as cold homogenization technique[3].

To be successful, an ocular drug delivery system should have small particle size (less than 50-100 µm) with a narrow size range, should be non-irritant, adequately bioavailabile, be compatible with ocular tissue and cause no blurred vision. Systemic administration of drugs to treat eye conditions such as ocular disease can only be optimized if the medicament is lipophilic, because the hydrophilic agents remain behind the blood–retina barrier unless it is interrupted.

The SLN are comfortable than insoluble or soluble insertion, less blurred vision as compared to ointment and gels. Increased bioavailability due to increased precorneal residence time, decreased nasolacrimal drainage of the drug. Chances of undesirable side effects arising due to systemic absorption of the drug through naso-lacrimal duct are reduced[4]. Drug effect is prolonged and hence frequent instillation of drug is not required. The chitosan polymeric gel base itself has been used successfully to treat moderate to severe cases of dry eye such as keratoconjuctivitis.

Chitosan molecules are aminoglucopyrans composed of N-acetylglucosamine and glucosamine residues. These polysaccharides are renewable resources which are currently being explored intensively for their applications in pharmaceutical, cosmetics, biomedical, agricultural, food, and non-food industries as well[5]. These unique polymers have emerged as a new class of physiological materials of highly sophisticated functions due to their versatile biological activity, excellent biocompatibility, and complete biodegradability in combination with low toxicity.

MATERIALS AND METHODS

Ofloxacin was kindly gifted from Wockhardt Pharmaceuticals, Aurangabad, India., Dynasan 118 (Rohm Gmbh & CoKG, Germany), Soyabean lecithin (Ruchi Global limited, Madhya pradesh), Poloxamer 188 (Pharmaceutical Pvt Ltd, Navi, Mumbai), Chitosan (India sea foods, cochin, india), All chemicals and reagents used were of analytical grade.

Preparation of SLN

Ofloxacin , Dynasan 118 and Soybean lecithin were dissolved in 10ml of chloroform. The organic solvent was completely removed using rotaevaporator at 75^{o}C and then the melted lipid phase was dispersed in a 191.7 ml hot water solution containing 1.0g of poloxamer 188. The pre emulsion was stirred for 15 min at 1800 rpm using a high speed homogenizer at 75^{o}C for five cycles[6]. After the homogenized samples was cooled at 5^{o}C, the OFC-loaded SLN dispersion containing 0.15% OFC stabilized by poloxamer 188 and soy bean lecithin (P-SLN) was obtained. Formulae of Ofloxacin SLN is given in Table 1.

Preparation of SLN Loaded Gels

Then the Ofloxacin SLNs were incorporated in the Chitosan gels at the concentration of (0.5%, 1%, 1.5% and 2.0 w/w).

Particle size analysis and ζ-potential measurements

The particle size of the SLN was determined by Photon Correlation Spectroscopy using Zetasizer 3000 (Malvern Instruments Ltd., UK). Samples were appropriately diluted with double-distilled 0.45 µm filtered water before measurement. The ζ-potentials of the formulated SLN were also determined using the same instrument[7]. For the ζ-potential measurements, each sample was diluted with bi distilled water and the electrophoretic mobility determined at 25°C and keeping the dispersant dielectric constant as 78.5 for water. Each experiment was repeated thrice.

$$A_{spec} = \frac{\text{Surface area}}{\text{density X volume}} = \frac{4\pi r^2}{p^4/3\pi r^3} = \frac{3}{p \text{ X } r}$$

Fourier Transform Infra Red (FTIR) studies

FTIR studies were carried out on pure Ofloxacin, Soya bean lecithin, Poloxamer, Dynasan 118 and SLN loaded with Ofloxacin[8]. (Perkin Elmer Jasco FTIR- 401, Japan)

Differential scanning calorimetry (DSC) STUDIES

Differential scanning calorimetry can be described as a thermal analysis technique used in the investigation of melting, crystallization, solid-to-solid transition temperatures of lipids, and determination of the solid fat content of the excipient. It enables the measurement of temperature change and heat flow which occurs when a material undergoes phase transition. Basically it involves the comparison of the

heat required to raise the temperature of a sample and a reference, where in both the sample and the reference are maintained at the same temperature throughout a given experiment[8]. Such data allows the quantitative and qualitative assessment of physical and chemical properties of the material by measuring changes in either enthalpy or heat capacity of a sample. The thermal events that can be detected by this method may be endothermic phenomena such as melting or exothermic phenomena such as crystallization.
The crystallinity index (CI) was calculated from the heat of fusion according to equation to determine the degree of crystallinity of SLN.

$$CI\ [\%] = \frac{\text{Enthalpy}_{SLN}\ (ml/mg)}{\text{Enthalpy}_{Bulk}\ \text{lipid (ml/mg)} \times \text{Concentration}_{\text{lipid phase}}\ [\%]} \times 100$$

Scanning electron microscopy (SEM)
The developed nanosuspensions of OSLN F1 to OSLN F6 were freeze-dried both in the presence and in the absence of a cryoprotectant (5%, w/w mannitol), formed a solid residue which could be easily redispersed in NaCl (0.9%, w/v) solution simply by gentle hand agitation without evident size and *ζ*-potential changes of the resulting nanosuspensions. This method offers excellent resolution and an easier sample preparation procedure for morphological examination of SLNs. SEM has high resolution and the sample preparation is relatively easy. SEM measures electrons transmitted from the particle surfaces to evaluate their morphology.

Drug entrapment efficiency and loading efficiency
The entrapment efficiency (EE) was determined as per the method discussed by Friedrich I et al.[9] The amount of free OFC in the supernatant was analyzed by UV-spectrophotometry at 294 nm and calculated by the calibration curve, $y = 0.016x + 0.006$, $R^2 = 0.9999$. The entrapment efficiency was determined in triplicate and calculated by the following equation

$$\%\ EE = \frac{M_{\text{Initial drug}} - M_{\text{Free drug}}}{M_{\text{Initial drug}}} X100$$

Where
"$M_{\text{Initial drug}}$" is the mass of initial drug used for the assay.
"$M_{\text{Free drug}}$" is the mass of free drug detected in the supernatant after centrifugation of the aqueous dispersion.

Freeze-drying and redispersibility of SLN-suspensions
All the prepared batches were lyophilized to verify the physical stability and redispersibility by using the method followed by Friedrich I et al [10]

Determination of pH
The pH of the Chitosan gel was determined using a calibrated pH meter. The readings were taken for average of 3 samples[11].

Measurement of Gel Strength
A sample of 50 gm of chitosan gel was placed in a 100 ml graduated cylinder and gelled in a thermostat at 37^0C. The apparatus for measuring gel strength was allowed to penetrate in Chitosan gel. The gels strength, which means the viscosity of the gels at physiological temperature, was determined by the time (seconds), the apparatus took to sink 5cm down through the prepared gel[12].

Viscosity Studies
Rheological studies of the prepared formulations were carried out by Brookfield synchroelectric viscometer (LVDV Pro II) using spindle S18 (small sample adaptor)[13].

Determination of mucoadhesive Force
The mucoadhesive force of all the optimized batches was determined as follows; A section of mucosa was cut from the chicken cheek portion and instantly fixed in between two glass vials using rubber band. The vial with chicken cheek mucosa was connected to the balance at an inverted position, while first vial was placed on a height adjustable pan. Oral gel was added into the nasal mucosa of first vial. The tear solution have the pH 5.5 was evenly spread on the surface of the test memberane. Then the height of second vial was so adjusted that the mucosal surfaces of both vials come in intimate contact.

RESULTS AND DISCUSSION
Particle size and ζ-potential
Particle size distribution of nanosuspension particles are in nanometric range suitable for ophthalmic use. For SLN

prepared with mixed lipid matrix and drug, the particle size distribution of OSLNF1 (250.1 ±30.2nm) and OSLN F6 (98.5 ± 12.8nm) as shown in table 2. In both formulations the z-average diameter and PI remained almost same after three months of storage at 4 ± 2°C but there was a significant variation when stored at room temperature[15]. The nanosuspension exhibied ζ-potential of OSLN F1 (+29.3±3.1mV) and OSLN F6 (+31.2±1.2mV). Such a positive charged of nanoparticles are supposed to be important for ophthalmic use as mucin layer of corneal surface is negatively charged, so it can facilitate an effective adhesion to the corneal surface which in turn would enhance ocular bioavailability.

Drug entrapment efficiency (%EE) and loading efficiency (%IE)

Percentage entrapment efficiency was found to be 76.2 ± 0.19 (OSLN F1) and 75.4 ± 2.50 (OSLN F6).The high drug incorporation may be attributed to the fact that rapid quenching of drug occurred in lipid phase due to presence of poloxamer-188 and Soya bean lecithin as surfactant phase and the drug incorporation followed core-shell model with drug-enriched core[16]. Drug loading efficiency of 75.1 ± 0.32 (OSLN F1) and 73.1 ± 0.30 (OSLN F6) was observed. There was an increase in the entrapment (82.6% ± 2.10) and loading efficiency (80.5% ± 0.50) in case of OSLNF5 was observed which may be attributed to the higher percentage of lipid mixture which was used in SLN preparation.

Infrared spectroscopy analysis

From FTIR study, the characteristic peaks of drug such as of N-H Stretch (3383-3412 cm–1), aldehyde C-H stretch (2629 – 2812 cm^{-1}), aldehydlic C-H bending (1392 cm^{-1}) and aromatic ketone C=O (1110-1178 cm^{-1}) appeared for the pure drug Ofloxacin. For SLN all peaks which have been obtained for the pure drug were avaiable at same wave length for N-H stretching, C-H bending, C-H Stretching and aromatic ketone (1141-1178 cm^{-1})[17]. Remaining peaks also either shifted or replaced in the IR spectrum of pure Ofloxacin(a), optimized formulation OSLN F5(b) and for physical mixture Dynasan 118, Soyabean lecithin and Poloxamer-188(c) are shown in Fig.1. The Pure Ofloxacin contain aromatic C-H stretching (3010.98 cm^{-1}), symmetric C-0-C stretching, N-H & C-H stretching (2478.61 cm^{-1}), methoxy group (2843.17 cm^{-1}). The above mention values of pure Ofloxacin is not there in Physical mixture dynasan-118, soyabean lecithin and poloxamer-188. This established drug entrapment in lipid matrix which was compared with standard functional group frequencies of Ofloxacin as shown in Table 3.

DSC Spectra

The pure drug Ofloxacin showed an endothermic peak at 170.97°C. The peak is not shifted in the case of DSC of the SLN formulation containing Ofloxacin + Dynasan 118 + Soyabean lecithin and Poloxamer188. The DSC of physical mixture of the dynasan 118, soya bean lecithin and Poloxamer showed an endothermic peak at 100°C. The DSC of Dynasan 118 showed an endothermic peak at 75°C[18]. The DSC of Poloxamer showed a peak at 90°C which emphasized that there was no incompatability exist between the drug and other excipients which were used in the formulation. The DSC interpretations and DSC graphs are shown in Fig.2 respectively.

SEM (Scanning eletron microscopy)[18]

The photographs of the optimized OSLN F5 formulation taken by Scanning eletron microscopy are shown in the fig.3.

In vitro release studies were performed in a modified Franz diffusion cell over a period of 12 hours[19]. At specific time intervals, aliquots of samples containing the released drug are taken from the acceptor compartment and are quantified using a suitable method of determination such as UV spectroscopy at a 294nm.

Release kinetics parameters from optimized formula of OSLN F5 of zero order R^2 is 0.985, m(5.565), First order R^2 (0.799), m (0.985), Higuchi matrix R^2 (0.988), m (8.565), Korsmeyer Peppar R^2 (0.982), m (8.488), are shown in table 5. The release data were best fitted for zero order kinetic models which reveal that the drug release from SLN enriched chitosan gel of Ofloxacin followed zero order kinetics and non fickian diffusion mechanism.

Viscosity study

All SLN enriched gels formulations exhibited a viscosity range of 530±1.05 to 700±1.50cps. OSLNF1(530±1.05), OSLN F2 (520±1.10), OSLN F3 (598±0.53), OSLN F4 (612±1.25), OSLN F5 (650±1.25), OSLN F6 (700±1.50) and were existing as a liquid form at ambient

temperature and also exhibited a broad range of shear rates[20] as shown table 6.

Gel Strength[20]

At 37^0 C, the gel strength of formulation OSLNF1 (82±1), OSLN F2 (91±3), OSLN F3 (101±2), OSLN F4 (109±3), OSLN F5 (115±2), OSLN F6 (120±2) as shown table 6.

Spreadability[20]

The gel strength of formulation OSLNF1 (10), OSLN F2 (11), OSLN F3 (13), OSLN F4 (14), OSLN F5 (17), OSLN F6 (18) as shown table 6.

Determination of mucoadhesive force[20]

Bioadhesive strength for fonrmulations contain Chitosan in concentration of 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 3.5%(% w/w) respectively increased with respect to bioadhesive strength of different proportion of chitosan gels. The best formulation of OSLN F1(16.11±0.7), OSLN F2 (17.01±1.4), OSLN F3 (18.11±1.9), OSLN F4 (19.13±2.2), OSLN F5 (20.12±2.9), OSLN F6 (15.98±1.9), as shown table 6.

CONCLUSION

It found that hydrophilic drugs like Ofloxacin can be successfully incorporated in the solid lipids nanoparticles. In the presence Dynasan 118 there was reduction in the crystallinity of the formulated SLN, this is propitious in lipid nanoparticulate drug delivery systems where drug entrapment is troublesome because of highly crystalline nature of pure solid lipids. Low crystallinity of mixed lipid nanoparticles (Dynasan 118, Soyabean lecithin) can be considered as better drug delivery systems as they could hold more drugs as compared to single lipid. The prepared SLNs were found in the colloidal size range with good particle size distribution, PI, high entrapment efficiency, and showed good stability on storage. The optimized SLN loaded chitosan gel of Ofloxacin would enhance corneal retention of the lipid nanoparticles which in turn would enhance ocular bioavailability of the drug.

ACKNOWLEDGEMENTS

The authors wish to thank: Ofloxacin was kindly gifted from Wockhardt Pharmaceuticals, Aurangabad, India. and also thankful to management of MLR Institute of Pharmacy, Hyderabad for providing the all facilities for carried out this research work.

Table 1: Formulae of Ofloxacin loaded SLN

Ingredients (%, w/w)	F1	F2	F3	F4	F5	F6
Ofloxacin (mg)	150	150	150	150	150	150
Dynasan118 (gm)	2.0	1.5	1.0	1.5	1.5	1.5
Soyabean lecithin(gm)	0.5	0.5	0.5	1.0	1.5	2.0
Poloxamer188 (gm)	0.2	0.2	0.2	0.4	0.4	0.5

Table 2: Characterization of SLN

Formulation Code	Particle Size(nm)	Polydispersity index	Ent.Efficiency (%)	Drug loading (%)
OSLN F1	250.1 ±30.2	0.310 ± 0.01	76.2 ± 0.19	75.1 ± 0.32
OSLN F2	216.2 ±15.7	0.312 ± 0.01	78.8 ± 0.25	74.4 ± 0.75
OSLN F3	155.2 ±13.7	0.314 ± 0.01	77.3 ± 1.78	76.6 ± 0.65
OSLN F4	96.2 ± 12.8	0.315 ± 0.02	75.8 ± 1.90	78.6 ± 0.65
OSLN F5	96.9 ± 12.2	0.317 ± 0.02	82.6 ± 2.10	80.5 ± 0.50
OSLN F6	98.5 ± 12.8	0.318 ± 0.02	75.4 ± 2.50	73.1 ± 0.30

Table 3: IR Interpretations for Pure drug and SLN

Funtional groups	Puredrug Ofloxacin	Dynosan 118 + Soyabean lecithin + Poloxamer	For SLN (Ofloxacin + Dynasan118 +Soyabean lecithin + Poloxamer)
F group	1037cm^{-1}	--	1038 cm^{-1}
N-H stretch	3383 – 3412 cm^{-1}	3250-3500 cm^{-1}	3385 – 3443 cm^{-1}
Aldehyde C-H stretch	2629 – 2812 cm^{-1}	2740 – 2965 cm^{-1}	2742 cm^{-1}
Aldehydic C-H bending	1392 cm^{-1}	1346- 1371 cm^{-1}	1392 cm^{-1}
Aliphatic Ketone C=O	1110-1178 cm^{-1}	1112 – 1193 cm^{-1}	1114- 1180 cm^{-1}
Aromatic C-H stretch	3010.98 cm^{-1}	--	--
Methoxy group	2843.17 cm^{-1}	--	--
Symmetric C-0-C stretch	1041.60 cm^{-1}	--	--
N-H & C-H stretch	2478.61 cm^{-1}	2468 - 2712 cm^{-1}	--

Table 4: Melting peaks, enthalpies and crystallinity of bulk dynasan-118, Soyabean lecithin, Ofloxacin, physical mixture of dynasan 118 & Soyabean lecithin (PM1), and lyophilized Ofloxacin-SLN (OSLN-F5)

Parameters	Dynasan 118 (Bulk)	Soyabean (Bulk)	PM1	Ofloxacin bulk	Lyophilized-SLN(OSLN-F5)
Melting peak (^{o}C)	60.54	79.21	59.2	189.9	120.41
Enthalpies (J/g)	250.21	545.87	530.2	105.3	29.321
Crystallinity index (%)	--------	---------	98	---------	34.23

Table 5: Release kinetics SLN loaded chitosan gels

Model	Equation	OSLN F1		OSLN F2		OSLN F3		OSLN F4		OSLN F5		OSLN F6	
		R^2	M	R^2	m	R^2	m	R^2	m	R^2	m	R^2	m
Zero order	Mo-Mt=kt	0.981	8.651	0.812	8.151	0.915	7.257	0.926	7.127	0.985	8.565	0.930	8.152
First order	InM=InMo	0.985	0.172	0.830	0.162	0.916	0.190	0.930	0.190	0.799	0.985	0.925	0.135
Higuchi's Matrix	$M_0-M_t = kt1/2$	0.850	7.132	0.835	7.156	0.930	7.160	0.946	7.150	0.988	8.565	0.932	8.565
Korsmeyer -Peppar	log (M_0-M_t)= log k + n log$t$	0.890	8.516	0.840	8.416	0.940	7.142	0.950	6.152	0.982	8.488	0.980	8.490
Hixon crowell	$M_0^{1/3}-M_t^{1/3}$	0.905	0.172	0.845	0.232	0.918	0.185	0.970	0.182	0.938	0.125	0.915	0.312

Table 6: Charaterization of SLN enriched chitosan gels (n=3)

SLN Gels	Chitosan gels Concentation (%)	Viscocity (cps)	Gelling strength (sec)	Spreadability coefficient gms\sec	Mucoadhesive force (dynes\cm^2)
OSLN F1	0.5	530±1.05	82±1	10	16.11±0.7
OSLN F2	1	520±1.10	91±3	11	17.01±1.4
OSLN F3	1.5	598±0.53	101±2	13	18.11±1.9
OSLN F4	2.0	612±1.15	109±3	14	19.13±2.2
OSLN F5	2.5	650±1.25	115±2	17	20.12±2.9
OSLN F6	3	700±1.50	120±2	18	15.98±1.9

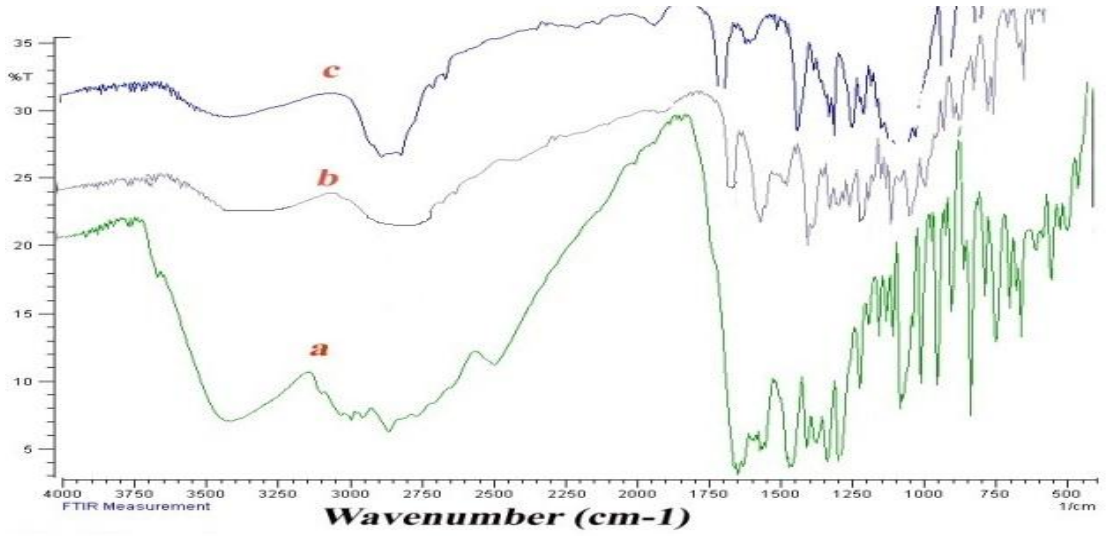


Fig. 1: IR Spectra

a. Olfloxacin

b. Ofloxacin, Dynosan 118, soya bean lecithin, Poloxamer 188

c. Dynosan 118, soya bean lecithin, Poloxamer 188

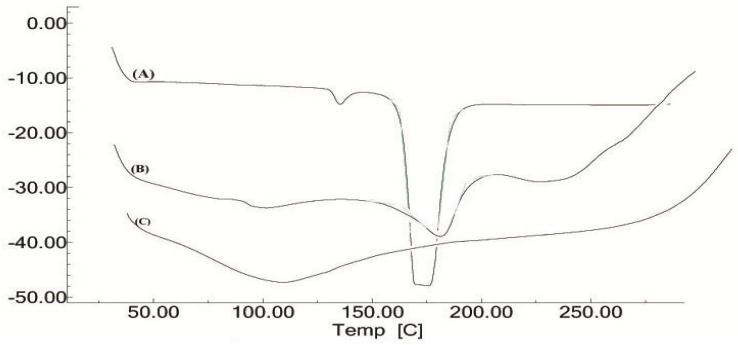


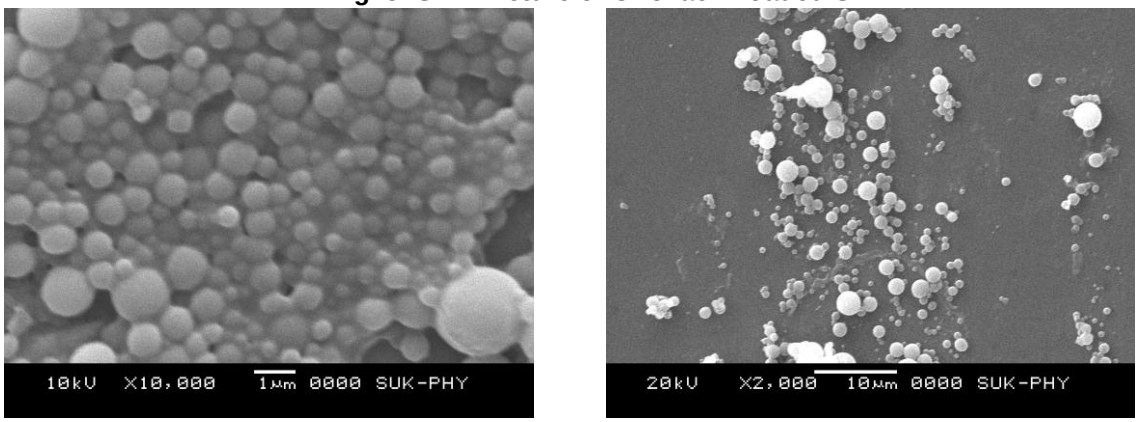
Fig. 2: DSC Spectra

A. Olfloxacin

B. Ofloxacin, Dynosan 118, soya bean lecithin, Poloxamer 188

C. Dynosan 118, soya bean lecithin, Poloxamer 188

Fig. 3: SEM Picture of Ofloxacin loaded SLN



A. In vitro drug Permeation studies

B. In vitro release drug diffusion studies were performed using modified Franz diffusion cell

REFERENCES

1. Attama AA, Reichl S and Muller-Goymann CC. Diclofenac sodium delivery to the eye: in vitro evaluation of novel solid lipid nanoparticle formulation using human cornea construct. Int J Pharm. 2008;355:307–313.
2. Attama AA, Schicke BC and Müller-Goymann CC. Further characterization of theobroma oil-beeswax admixtures as lipid matrices for improved drug delivery systems. Eur J Pharm Biopharm. 2006;64:294–306
3. Attama AA, Schicke BC, Paepenmüller T and Müller-Goymann CC. Solid lipid nanodispersions containing mixed lipid core and a polar heterolipid: characterization. Eur J Pharm Biopharm. 2007; 67: 48–57.
4. Bristol Myers Squibb Company. New Drug Application for Tequin (Ofloxacin Tablets and Injections). Environmental Assessment. Center for Drug Evaluation and Research, Website. 2001, Accessed May 31, 2006.
5. Bunjes H, Westesen K and Koch MHJCrystallization tendency and polymorphic transitions in triglyceride nanoparticles. Int J Pharm. 1996;129:159–173.
6. Cavalli R, Caputo O, Carlotti ME, Trotta M, Scarnecchia C and Gasco MR. Sterilization and freeze-drying of drug-free and drug-loaded solid lipid nanoparticles. Int J Pharm. 1997;148:47– 54.
7. Cavalli R, Gasco MR, Chetoni P, Burgalassi S and Saettone MF. Solid lipid nanoparticles (SLN) as ocular delivery system for tobramycin. Int J Pharm. 2002;238: 241–245.
8. Dingler A and Gohla S. Production of solid lipid nanoparticles (SLN): scaling up feasibilities. J Microencapsul. 2002;19: 11–16.
9. Freitas C and Müller RH. (Correlation between long-term stability of solid lipid nanoparticles (SLN) and crystallinity of the lipid phase. Eur J Pharm Biopharm, 1999;47:125–132.
10. Friedrich I, Reichl S and Müller-Goymann CC. Drug release and permeation studies of nanosuspensions based on solidified reverse micellar solutions (SRMS). Int J Pharm. 2005;305:167–175.
11. Gan L, Gan Y, Zhu C, Zhang X and Zhu J. Novel microemulsion in situ electrolyte-triggered gelling system for ophthalmic delivery of lipophilic cyclosporine A: in vitro and in vivo results. Int J Pharm. 2009;365:143–149.
12. Hu FQ, Jiang SP, Du YZ, Yuan H, Ye YQ and Zeng S. Preparation and characterization of stearic acid nanostructured lipid carriers by solvent diffusion method in an aqueous system. Colloids Surf B Biointerfaces. 2005;45:167–173.
13. Kalam MA, Sultana Y, Samad A, Ali A, Aqil M, Sharma M and Mishra AK. Gelrite-based in-vitro gelation ophthalmic drug delivery system of Ofloxacin. J Disp Sci Tech. 2008;29:89–96.
14. Liu J, Gong T, Wang C, Zhong Z and Zhang Z. Solid lipid nanoparticles loaded with insulin by sodium cholate-phosphatidylcholine- based mixed micelles: preparation and characterization. Int J Pharm. 2007; 340:153–162.
15. Solid Lipid Nanoparticles (SLN)—ein neuartiger Wirkstoff-Carrier fur Kosmetika und Pharmazeutika. II. Wirkstoff-Inkorporation, Freisetzung und Sterilizierbarkeit. Pharm Ind, (59): 511–514.
16. Motwani SK, Chopra S, Talegaonkar S, Kohli K, Ahmad FJ and Khar RK. Chitosan-sodium alginate nanoparticles as submicroscopic reservoirs for ocular delivery: formulation, optimisation and in vitro characterisation. Eur J Pharm Biopharm. 2008;68:513–525.
17. Max Farlane TW in L.P.Samarayake and T.W. Maxfarlane,(Eds.), Buttertworth and Co. London,1st Edn.,Britan. 1990;.20-21
18. BASF Wyandotte. Pluronic surfactants, product Brochure BASF Corporation, Mount olive New jersey. 1997.
19. Collectt JH and Davies MC.Effect of batch variability on molecular properties and in-vivo release characteristics of pluronic PF-127

gels. J pharm Pharmacology. 1984;36:52-57.
20. Verger ML, Fluckiger L and Kim Y. Preparation and characterization of nanoparticles containing an anti-hypertensive agent. Eur J Pharm Biopharm. 1999;46:137-143.