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Formulation Development of Natamycin Loaded Nanoemulsion for Ocular Drug Delivery

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FORMULATION DEVELOPMENT OF NATAMYCIN LOADED NANOEMULSION FOR OCULAR DRUG DELIVERY

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ABSTRACT

Natamycin (NT), also known as Pimaricin, is predominantly used as an anti-fungal medication for the treatment of fungal infections around the eye. The goal of the present study was to formulate and optimize NT loaded nanoemulsion (NT-NE) and examine its potential application in ophthalmic drug delivery. Placebos were evaluated with respect to particle size, PDI and ZP to identify the aqueous phase and lipid phase concentrations to be carried forward with NT-NE development. NT-NE were prepared by hot homogenization and ultra-probe sonication method, using Castor oil (CO) and Miglyol 812 as liquid lipids, Tween®80 and Poloxomer 188 as surfactants. NT-NE were characterized for physicochemical properties such as particle size, polydispersity index (PDI), zeta potential (ZP) and assay. The *in-vitro* release studies were planned using 10kDa Slide-A-Lyzer™ Dialysis cassettes and compared with the NT suspension used as control (NT-C). Physical stability of NT-NE was measured at room temperature and refrigerated storage conditions. Particle size, PDI, ZP of NT-NE were in the range of 150-350 nm, 0.1-0.45 and -32.0 to -64.73 mV, respectively. The assay of the NT-NE formulations was in the range of 95-101%. From the release studies, sustained and dose-dependent release of the NT was observed from NE compared with NT-C. The approximate %drug release from the castor oil based NT-NE (C1) was 15% and 5% from Miglyol 812 NT-NE (M3) in a 24 hour period. The results, therefore, suggest that the NT-NE system can be a favorable drug delivery platform for the treatment of oculat fungal infections.

DEDICATION

This thesis is dedicated to everyone who helped me and guided me through my own times of stress and anxiety. With greatest thanks to my advisor, Dr. Soumyajit Majumdar and all other lab members who kept wonderful patience, warm humor and continual support. I would like to thank Corrine Sweeney for the practical help that she provided so cheerfully and my post-doctorate Dr. Narendra Reddy for the support and faith in me.

LIST OF ABBREVIATIONS AND SYMBOLS

NT	Natamycin
NE	Nanoemulsion
CO	Castor oil
PDI	Polydispersity Index
ZP	Zeta Potential
NT-C	Natamycin Control
DME	Diabetic macular edema
DR	Diabetic retinopathy
AMD	Macular degeneration
O/W	Oil in water
W/O	Water in oil
NaCMC	Sodium carboxymethyl cellulose
IPBS	Isotonic phosphate buffer solution
HPLC	High performance liquid chromatography

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In addition, I thank them who have introduced me to Linguistics, and whose enthusiasm for the "underlying structures" had lasting effect. I thank the University of Mississippi for permission to include copyrighted pictures as a part of my thesis.

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CHAPTER 1: INTRODUCTION

1.1 Advancements in ocular drug delivery systems

The field of ocular drug delivery is one of the most interesting, but also most challenging, research area for pharmaceutical scientists. The development of new therapeutic compounds and novel drug delivery techniques have been constantly evolving for the treatment of ocular diseases [1]. Ophthalmic formulations in the current market; like solutions, suspensions, implants, ointments and emulsions, have been contributing to the treatment of the ocular diseases. The most important diseases which affect the eye globe include diabetic macular edema (DME), diabetic retinopathy (DR), macular degeneration (AMD), uveitis, fungal/bacterial infections, and glaucoma [2]. These complications could even lead to vision loss and other proliferative issues.

The need for new ocular drug delivery systems has generated many formulations such as bilosomes, nanosuspensions, nanoemulsions, liposomes, microspheres, niosomes, ocular films, in-situ gels and many others [1-2]. Many of them are prepared through non-conventional technologies and sometimes also include nanotechnology systems. These have not just have improved the bioavailability of the drug into the eye but also helped in overcoming drawbacks like increased precorneal elimination, blurred vision and efficiency.

1.2 Barriers

There are certain ocular barriers which need to be outplayed by the formulator without causing any ocular tissue injury. Upon topical administration, only 5-10% of the drug reaches the site of action and rest of about 90-95% is eliminated by several anatomical factors. These

factors are as follows- (i) tear film, (ii) reflexive blinking, (iii) solution overflow, (iv) tear turn over, (v) induced lacrimation, (vi) cornea-corneal epithelium controlling the entry of the drug molecule into the eye [3]. Also, the mucin present in the tear films, responsible for eliminating microorganisms, affects the drug's ocular penetration [4]. Another factor is the anatomical volume of the cul-de-sac (≈30 μl) and the human tear volume (≈7 μl) added to which is the rapid elimination (half-life ≈2-3 min), being washed away over the ocular mucosa [2]. Non-anatomical factor includes patient compliance.

1.3 Anatomy of the eye

The anatomy & physiology of the eye is very much invulnerable to foreign particles. The eye comprises of two segment - anterior and posterior. The anterior segment of the eye is the front third portion of the eye that consists of cornea, iris, ciliary body and lens. The anterior cavity further has anterior chamber (the corneal endothelium) and the posterior chamber (between iris and vitreous humor). The spaces within the anterior segment is filled by aqueous humor; that provides several nutrients to the contiguous structures.

1.4 Fungal Keratitis

Fungal keratitis is an infection causing inflammation around the cornea. The current study of the thesis offers an idea of developing an ophthalmic formulation to treat fungal infections (fungal keratitis) around the eye. Cornea is the outermost, transparent structure present at the front of the eye. It covers iris, pupil and anterior chamber which is the eye's primary light-focusing structure. The presence of immature resident immune cells, immunologic privilege and avascularity property of the cornea makes it a special tissue. The microanatomy of the human cornea includes five layers: - (i) corneal epithelium, (ii) Bowman's layer, (iii) corneal stroma, (iv) Descemet's membrane, (v) Corneal endothelium [5]. Fungal keratitis can develop due to eye injury or because of the usage of contact lens. If not treated at an early stage, it can

cause permanent vision impairment. There are mostly three fungi that can affect the cornea- *Fusarium, Aspergillus,* or *Candida*. There can be two types of fungal keratitis intricate- superficial keratitis & deep keratitis. As the name suggests, superficial keratitis implicates the infection on the outer layers of the corneal tissue whereas deep keratitis affects deeper layers of the cornea. It may not avoid the visibility of the scar after getting healed, and mostly cause vision loss.

1.5 Nanoemulsion system

The anatomical features of cornea which limits the penetration of many lipophilic drugs into deeper layers, has created the need to develop better formulations to decrease the problems associated with corneal permeation, low bioavailability and low precorneal residence time. Solid-lipid nanoparticles (SLN), nanolipid carriers (NLC), polymeric nanoparticles (PN), nanocarriers are some of the emerging lipid/polymeric nano-formulations which have shown success in augmenting the transcorneal drug permeability [9, 10]. Currently, to treat fungal keratitis, the only available ophthalmic product available commercially is Natacyn^{VR} (NT 5% w/v suspension). This suspension contains Natamycin drug, which is a macrolide polyene antifungal agent. US-FDA has approved this NT based suspension commercially for the treatment of fungal keratitis and several other fungal infections [7, 8]. The available therapy needs multiple dosing (a drop every 1-2 h) for several weeks to attain the ideal concentrations at the corneal site [9, 10]. This is because of the low aqueous solubility at the corneal site. Therefore, there is a need to develop a formulation to improve corneal permeability and bioavailability of NT so as to reduce the multiple dosing rate and increase the efficacy, patient compliance through the nano-technique based formulation.

Nanoemulsions are defined as colloidal dispersions wherein two immiscible liquids are mixed with the effect of external forces or energies. There are certain advantages of utilizing

nanoemulsions over conventional emulsions in the current pharmaceutical industry. The two immiscible liquids involve oil and water. Thus, it can be classified into two types- (O/W) and (W/O). The structural characteristics of nanoemulsion was suitably chosen with the idea that the small size of the droplets often leads to a higher bioavailability of the bioactive components. The encapsulated bioactive compound creates an increased equilibrium solubility with the decreased size of the droplets further leading to an enhanced concentration gradient for absorption [11]. Previously, PEGylated nanostructured lipid carriers, in situ gels of bilosomes and transfersomes of NT were reported but no NE formulations have been reported so far [6, 12].

The current investigation mainly focused on NT-loaded nanoemulsion (NT-NE) for improved ocular pharmacotherapy based on the (O/W) nanoemulsion formulations since NT is a highly lipophilic drug. Accordingly, NT-NE were prepared using homogenization followed by probe sonication method and evaluated for particle size, PDI, ZP and drug content. Further, in vitro release studies of an optimized NT-NE were performed, compared with NT control suspension (0.3% NT w/v).

CHAPTER 2. MATERIALS AND METHODS

2.1 Materials

Castor oil (CO) (ricinoleic acid) and egg lecithin (phospholipid) were purchased from Fischer Scientific. NT was purchased from Cayman Chemicals (Ann Arbor, MI, USA). Poloxamer 188, Tween® 80, centrifugal tubes, high-performance liquid chromatography (HPLC)-grade solvents, and other analytical-grade chemicals were purchased from Fisher Scientific (Hampton, NH, USA). Dialysis membrane cassettes (molecular weight cut off 10K) were purchased from Fischer Scientific.

2.2 Methods

2.2.1 Preparation of NT-NE placebos

Different trials were used to develop nanoemulsions with the change in surfactants and their concentrations, different oils and change in homogenization parameters.

2.2.1.1 Assessment of surfactants

Surfactants are the compounds which are used at variable concentrations as emulsifiers. For the preparation of nanoemulsion in the current research, use of nonionic surfactants were chosen due to its high effectiveness, efficiency, biodegradability and low toxicity concerns. The formulations trials were carried with Poloxomer 188, tyloxopol, chremophor el, and Tween® 80. Variable compositions made are listed in Table 1 below.

2.2.1.2 Assessment of oil

Natamycin is from the polyene family of medication which is highly lipophilic in nature. The trials were carried out with oil and liquid lipid fatty acid. Screening of different oils were

carried in previous literature reports [12]. The reported results were in favor of castor oil. Furthermore, Miglyol 812 (fatty acid) were also tried as per many reported stable concerns in previous literature. Reference to Table 1 & Table 2.

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Natamycin	--	--	--	--	--	--				
Castor oil	1%	2%	3%	1%	1%	1%	1%	1%	2%	2%
Miglyol 812	--	--	--	--	--	--				
Egg Lecithin	--	--	--	--	--	--				
Poloxomer ® 188	0.25%	0.25%	0.25%	--	--	0.25%	0.25%	0.25%	0.25%	0.25%
Tween ® 80	0.75%	0.75%	0.75%	0.50%	0.75%	0.75%	1%	2%	1%	2%
Tyloxopol	--	--	--	--	--	0.20%				
TPGS vit. E	--	--	--	--	--					
Chremophor EL	--	--	--	0.25%	0.50%	--				
Glycerin	2.25%	2.25%	2.25%	2.25%	2.25%	2.25%	2.25%	2.25%	2.25%	2.25%
Water	upto 10ml	upto 10ml	upto 10ml	upto 10ml	upto 10ml	upto 10ml	upto 10ml	upto 10ml	upto 10ml	upto 10ml

Table 1: Composition of nanoemulsion placebos

2.2.2 Preparation of NT-NE

After several placebos trials, NT-NE were prepared by using hot homogenization followed by ultra-probe sonication method as per the composition given in Table 2. The oil phase with a combination of oil and egg lecithin (0.5% w/v) was heated to 80 ± 2°C and NT was added with continuous stirring. Simultaneously, the aqueous phase consisting of Tween® 80 (0.75% w/v) and Poloxomer 188 (0.25% w/v) in de-ionized water (upto 10 ml) was heated at same temperature. Aqueous phase then transferred to the molten oil mixture under constant stirring at 2000 rpm. A pre-mixture was then obtained by emulsification process, using a T25 digital

Ultra-Turrax (IKA, Wilmington, NC, USA) for 5 min set at 16000 rpm. Pre-emulsion was then subjected to probe sonication at 40% amplitude for 10 mins with 10 sec pulse on and 15 sec pulse off, using Sonics Vibra Cell Sonicator (Newtown, Connecticut, USA). Compositions are presented in Table 2.

Table 2. Composition of natamycin loaded nanoemulsion with different compositions selected.

	M1	M2	M3	M4	C1
Natamycin	**1%**	**2%**	**1%**	**2%**	**0.3%**
MIGLYOL 812	**3%**	**3%**	**5%**	**5%**	**--**
CASTOR OIL	**--**	**--**	**--**	**--**	**1%**
Egg Lecithin	**0.5%**	**0.5%**	**0.5%**	**0.5%**	**0.5%**
Poloxomer ® 188	**0.25%**	**0.25%**	**0.25%**	**0.25%**	**0.25%**
Tween ® 80	**0.75%**	**0.75%**	**0.75%**	**0.75%**	**0.75%**
Water	**Upto 10 ml**	**Upto 10 ml**	**Upto 10 ml**	**Upto 10 ml**	**Upto 10 ml**

2.2.3. Measurement of Particle size, Zeta Potential and Polydispersity Index

The particle size, zeta potential (ZP) and polydispersity index (PDI) of the placebos and NT-NE prepared were determined by using a Zetasizer Nano ZS Zen3600 (Malvern

Instruments, Westborough, MA, USA); a photon correlation spectroscopy device; at 25 °C in clear, disposable folded capillary cells. The measurements were attained using a helium-neon laser based on which the data was analyzed as per the volume distribution. Ten microliters of sample were diluted 100 times with filtered bi-distilled, 0.2 micron filtered water and measured for particle size and zeta potential in triplicate.

2.2.4. Chromatographic Conditions for Sample Analysis

Drug content (Assay) and in-vitro release study samples were analyzed using high performance liquid chromatography (HPLC) method [12]. The HPLC which was used for the analysis constitutes Waters 717 plus auto sampler, Waters 2487 dual absorbance UV detector, 600 Waters controller pump, and an Agilent 3395 Integrator. A C18 Phenomenex Luna® (5µ, 250 x 4.6 mm) column was used. The mobile phase contained a mixture of phosphate buffer (0.2M, pH 5.5) and acetonitrile (70:30) at a flow rate of 1 ml/min. The analysis was done under 25 °C, injection volume was 20 µL, and the UV detection wavelength (λ_{max}) was set to 304 nm at AUFS 1.00.

2.2.5. Drug content (Assay) of NT-NE

An accurately measured amount of NT-NE (100 µL) was extracted in methanol (filled upto meniscus) in a 10 mL volumetric flask. The mixture was sonicated for 15 min at 15 °C. It was then centrifuged at high speed (13,000 rpm; 10 min; 25 °C) and the supernatant was analyzed for NT content using HPLC.

2.2.6. Physio-chemical stability assessment

The NT-NE formulations were assessed by analyzing physical appearance, change in particle size, PDI, ZP and assay upon storage at 4 °C and 25 °C for a period of one month.

2.2.6. *In vitro* release studies

In vitro release of NT-NE were carried out for 24 hours using 10K dialysis membrane. Prior to the study, cassettes were soaked in de-ionized water overnight at room temperature. The membrane cassettes were carefully placed upon scintillation vials containing isotonic phosphate buffer (IPBS) (20 mL; pH=7.4) which were kept on multi-stationed magnetic stirrer (IKA, USA) at 1000 rpm at static conditions. The temperature was maintained at 34 °C throughout the study. To keep a check, the probe was positioned in another scintillation vial containing same 20 mL (IPBS) without the membrane cassette placed on the device. Freshly prepared NT-NE were prepared for the *in vitro* studies. NT-C was prepared by adding 0.05% sodium carboxymethyl cellulose (NaCMC) to 0.3% NT in a 20 mL scintillation vial and triturated with addition of half quantity of 0.2 micron Millipore water (upto 10 mL). Two hundred microliter of NT-NE and NT-C formulations were put into the membrane cassettes in triplicate. Further at proper time interval, 1 mL of release medium was taken and fresh IPBS was added to maintain the persistent volume of the system. The drug release of NT was analyzed using HPLC as per the above described method.

CHAPTER 3: RESULTS & DISCUSSION

3.1 Assessment of placebos

The placebos made with variant concentrations of non-ionic surfactants and oil differed in either size, PDI or ZP. Also, settling of particles at the bottom of the vial was visually evaluated. P3, P4, P5, P6 & P8 exhibited settling of particles at the bottom mostly within 2-3 days.

Table 3: Particle size, PDI, ZP of NE (mean ± SD, n = 3). Refer to Table 1 for NT-NE compositions.

Formulation	Size (nm)	PDI	ZP (mV)
P1	104.1 ± 2.15	0.27 ± 0.004	-27.2 ± 2.79
P2	339.73 ± 36.8	0.72 ± 0.06	-30.4 ± 0.15
P3	322 ± 5.23	0.46 ± 0.05	-40.1 ± 0.05
P4	5.02E	0.3 ± 0.19	-63.2 ± 0.12
P5	925 ± 194.0	0.96 ± 0.05	-61.5 ± 0.18
P6	1.01E	0.3 ± 0.19	-60.1 ± 0.81
P7	290.4 ± 56.1	0.45 ± 0.01	-41.3 ± 1.9
P8	841.8 ± 151.0	0.75 ± 0.06	-37.1 ± 0.67
P9	370 ± 146.0	0.70 ± 0.17	-42.2 ± 0.28
P10	336 ± 78.40	0.58 ± 0.21	-33.2 ± 0.43

3.2 Assessment of NT-NE

3.2.1. Choice of lipid phase

For further development, the lipid phase was chosen to be formulated in oil (CO) and a fatty acid (Miglyol 812) for NE to compare the results based on size, PDI, ZP and drug content. As per the literature, it was reported that on screening solubility of NT with different oils and liquid lipids; castor oil (CO) had showed best solubility when used in oil phase [12]. Miglyol 812 also showed better solubility of NT. Therefore, NT-NE formulations were prepared with varied concentrations of CO (oil) and Miglyol 812 (fatty acid) with NT in the oil phase. Aqueous phase constituting of Tween® 80 and Poloxomer 188 were optimized based on placebos and were kept same in all the formulations. The percent content for all the excipient were as per the limit mentioned in the inactive ingredients (IIG) database [13].

NT-NE formulations were prepared using homogenization followed by probe sonication method. To develop the NE, varied concentrations of liquid lipids v/s NT ratios were studied on the basis of particle size and PDI. The compositions are listed in the Table 2. It was observed that, when the oil ratio (Miglyol 812) was kept constant in M1 and M2 at 3%, and NT concentration was increased from 1% (M1) to 2% (M2) showed particle size and PDI of 210.2 ± 2.93 and 0.41 ± 0.07 in M1, which is less than the particle size and PDI of 292.6 ± 1.9 and 0.58 ± 0.069 in M2 (Table 2). The increase in particle size is due to the increase in drug load and the increase in PDI could be due to the amount of surfactants ratio are not been sufficient to stabilize the nanoemulsion system.

Table 4: Particle size, PDI, ZP and drug content of NT-NE (mean ± SD, n = 3)

Formulation	Size (nm)	PDI	ZP (mV)	Assay (%)
M1	210.2 ± 2.93	0.41 ± 0.07	-41.06 ± 6.61	79.6 ± 0.55
M2	292.6± 1.9	0.58± 0.069	-43.3±3.21	55.8± 0.07
M3	179.2 ± 0.6	0.29 ± 0.03	-46.1 ± 2.4	97.5 ± 1.9
M4	256.8± 68.8	0.37 ± 0.01	-57.2 ± 1.5	38.7 ± 0.08
C1	212.7 ± 6.7	0.46 ± 0.04	-66.1±0.3	101.2±0.06

On increasing the oil content of Miglyol 812 in M3 & M4 at 5% concentrations and varied NT content of 1% and 2% drug load, there was a higher particle size in M4 (256.86 ± 68.82) with higher PDI (0.37 ± 0.011) as compared to particle size in M3 (179.2 ± 0.66) and PDI (0.29 ± 0.035). The increase in particle size of M4 could be because of the higher drug load amount (NT) not been encapsulated appropriately in the lipid concentration of 5%.

In C1, where the castor oil is being replaced by the Miglyol 812 at 1% concentration with 0.3% NT, showed particle size of 212.7 ± 6.7 and PDI of 0.46 ± 0.04. It has been suggested that PDI values differing in the range of 0.01 to 0.5 represents narrow distribution range [14]. From the results, M1, M3, M4 and C1 falls under the reasonable PDI range. But, M4 constitutes of higher particle size value, this was not considered as a desired NE system.

ZP of NT-NE ranged from -57.2 ± 1.5 to -41.0 ± 6.6 mV for Miglyol 812 based nanoemulsions and -66.1 ± 0.3 that of castor oil based NE. The stability of the NE system also depends upon

the steric stabilization of the formulations. The non-ionic surfactant (Poloxomer 188) used in the nanoparticulate system helps in reducing the electrostatic repulsion between the particles following stabilization when introduced to the corneal site. A lower zeta potential system between (-30 mV) to (+30 mV) is considered to have a better steric stability of the nanoemulsion system [6].

3.3 Drug content

The amount of NT loaded in the oil or lipid matrix depends upon the type of oil used [15]. Here, NE developed with castor oil and miglyol 812. The solubility of NT differs in both the oil & lipid matrix system, respectively. Considering the results in Table 4, the drug content in M3 (97.5 ± 1.9%) and C1 (101.2 ± 0.06%) were higher as compared to other NT-NE formulations. Moreover, the solubility of the NT with the lipids and oils depends upon the crystalline structural imperfections in the oil/lipid matrix system. Higher the structural imperfections, higher would be the solubility since it contains higher void spaces in between them. Also, castor oil containing hydroxyl functional group (-OH) is more polar than several other fatty acids. Higher the polarity, higher will be solubility of the drug compound. Thus, we can also conclude that NT being highly lipophilic in nature is more soluble in castor oil than Miglyol 812 which is why higher concentration of Miglyol 812 was required to dissolve higher drug load.

3.4. Stability studies

The NT-NE formulations (M3 and C1) selected were further analyzed for stability at refrigerator and room temperatures. The stability results are presented in Table 5 and Table 6. From the Table 5, it is appatent that there was no significant difference among particle size and

PDI from Day 1 to Week 3 in M3 NE. On the contrary, a significant difference in drug contents were observed from Day 1 until week 2.

For the C1 nanoemulsion formulation (Table 6), it was observed that there is a decrease in particle size at both temperatures and no particular difference was observed regarding PDI. The zeta potential for this nanoemulsion system remained same until week 2. The assay did not show much difference for the NT-NE kept in 4 °C but lowered by about 10-12% at 25 °C.

Table 5. Stability studies of NT-NE formulation (M3) at refrigerator and room temperature for one month (mean ± SD, n = 3). Refer to Table 2 for NT-NE compositions.

Duration	Condition	Size (nm)	PDI	Zeta Potential	Assay (%)
Day 1	**4 °C**	179.2 ± 0.6	0.29± .03	-46.1 ± 2.4	97.5 ± 1.9
	25 °C	170.8 ± 2.1	0.28±0.005	-50.8± 0.4	95 ± 0.2
Week 2	**4 °C**	191.3 ± 2.45	0.30 ± 0.03	-46.1 ± 2.45	81.5 ± 0.4
	25 °C	162.2 ± 1.41	0.17 ± 0.02	-56 ± 3.81	81.3 ± 0.4

Table 6. Stability studies of NT-NE formulation (C1) at refrigerator and room temperature for one month (mean ± SD, n = 3). Refer to Table 2 for NT-NE compositions.

Duration	Condition	Size (nm)	PDI	Zeta Potential	Assay (%)
Day 1	4 °C	212.7 ± 6.7	0.46 ± 0.04	-66.1 ± 0.3	101.2 ± 0.06
	25 °C	247.7 ± 10.1	0.44 ± 0.04	-64.7 ± 0.6	99.4 ± 0.3
Week 2	4 °C	204.8 ± 36.4	0.418 ± 0.02	-66.3 ± 1.8	93.8 ± 0.04
	25 °C	277.9 ± 136.1	0.505 ± 0.07	-62.8 ± 1.6	86.5± 0.02

3.5. *In vitro* release study

The *in-vitro* release studies were carried for both M3 & C1 for 24 hours. For M3 nanoemulsion formulation, the drug release at 24th hour was about 4.86% and that of C1, it was observed to be 14.3%. The control (0.3% w/v NT; 0.5% Sodium CMC; 10 ml water) was observed to have 80.38% of average release at 24th hour.

This suggests that with increase in drug load, there is an increment of sustain release actions. The average frequency of application of the marketed Natamycin suspension (Natacyn$^{VR)}$ is usually between 6 to 8 times daily. But with the developed NT nanoemulsion system, the dosing frequency could be reduced to once in a day thus reducing toxic effects, and increasing patient compliance as well. Table 7 represents the 24-hour study data for both the formulations developed.

Table 7: *In vitro* release profiles of NT-NE (M3 and C1) and NT-C formulations (mean ± SD, n = 3). Refer to Table 2 for NT-NE compositions. (NT-C: 0.3% w/v NT + 0.5% Sodium CMC + 10 ml water)

Time (hrs.)	**C1**	**M3**	**NT-C**
	%drug release	%drug release	%drug release
0.5	0.85 ± 0.05	0.24 ± 0.008	1.65 ± 0.09
1	1.14 ± 0.13	0.33 ± 0.01	2.59 ± 0.18
2	1.84 ± 0.11	0.54 ± 0.03	4.79 ± 0.50
4	2.99 ± 0.14	0.94 ± 0.08	9.86 ± 1.15
6	4.32 ± 0.29	1.32 ± 0.08	17.98 ± 0.43
8	5.36 ± 0.26	1.69 ± 0.07	21.28 ± 2.37
12	7.73 ± 0.41	2.55 ± 0.14	34.12 ± 3.60
24	14.30 ± 0.92	4.86 ± 0.43	80.78 ± 7.49

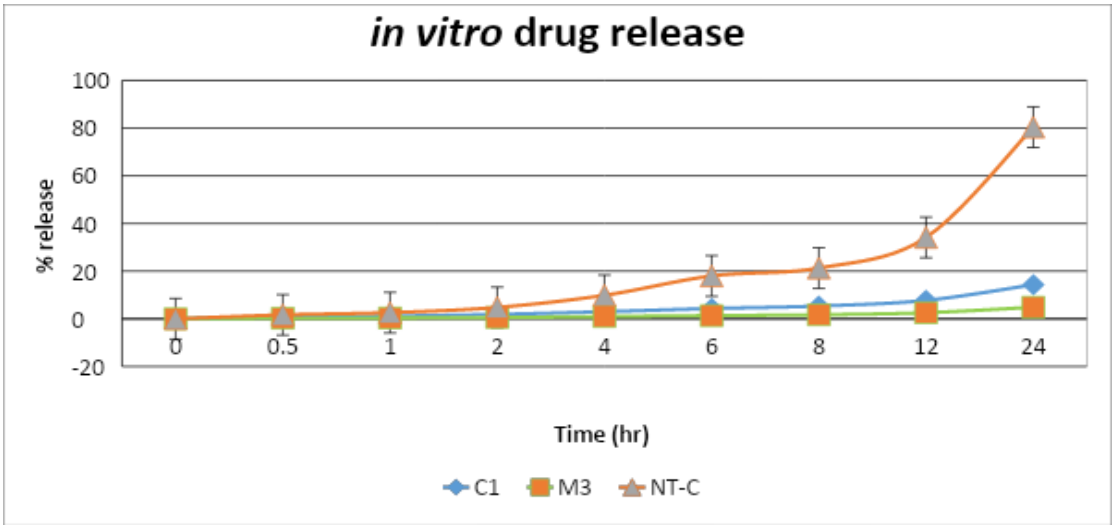

Figure 1: *In vitro* release profiles NT from NT-NE (M3 and C1) and NT-C formulations (mean ±SD, n = 3). Refer to Table 7 for values.

CHAPTER 5: CONCLUSION

NT loaded nanoemulsion with liquid lipid Miglyol 812 and castor oil (CO) were successfully developed and optimized. NT-NE with castor oil at 4 ºC showed small particle size with narrow particle distribution for upto 2 weeks. The drug content did not show much of the difference. Although, NT with Miglyol 812 showed stable particle size, PDI and ZP for upto two weeks, drug content decreased. The *in vitro* release study revealed the sustained release and dose-dependent effect of NT-NE compared with NT control formulation. Future studies require to study stability of the formulations for a longer duration of time to confirm the effectiveness of the formulation with its stability. Also, transcorneal permeation and in-vivo ocular distribution studies could confirm the efficacy and residence time of NT-NE on the ocular surface.

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PUBLICATIONS

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PRESENTATIONS AND ABSTRACTS AT SCIENTIFIC CONFERENCES

- Goel Kanika, Recent Advances in Cardiovascular Sciences, 7 th International Conference of International Academy of Cardiovascular Sciences (IACS), Amity University, Noida, Uttar Pradesh, 2015.
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