

Preparation and characterization of chloramphenicol niosomes and comparison with chloramphenicol eye drops (0.5%w/v) in experimental conjunctivitis in albino rabbits

Muhammad Naveed Yasin¹, Shahzad Hussain*², Farnaz Malik², Abdul Hameed³, Tipu Sultan⁴, Fahim Qureshi⁵, Humayun Riaz¹, Ghazala Perveen⁶ and Amina Wajid⁶

¹Faculty of Pharmacy, University of Sargodha, Sargodha, Pakistan, ²Drugs Control and Traditional Medicines Division, NIH, Pakistan

³Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan

⁴Group General Manager, Wilson Group of Companies, Islamabad, Pakistan, ⁵Government College University, Lahore, Pakistan

⁶Quality Control Laboratories, NIH, Islamabad.

Abstract: Niosomes has gained tremendous popularity as ultimate drug carrier. Lot of research work is being carried out on preparation of niosomes for ophthalmic use having no significant effect on vision and its sustained release pattern. Chloramphenicol niosomes were prepared using two different ratios of cholesterol, drug and surfactant, termed as EIN-1, EIN-2 by ether injection method and their entrapment efficiency, particle size. The *in vitro* drug release pattern was observed for ten hours. The EIN-2 showed 90% entrapment and released 81% of entrapped drug after 10 hours. Zeta potential & viscosity were determined and in-vivo comparison was made with Chloramphenicol eye drops where it exhibited Cmax of 15µg/ml. Stability studies were done to determine shelf life. MIC of selected strain of *S. aureus* was also determined. EIN 2 niosomal suspension was compared with Chloramphenicol eye drops in experimental conjunctivitis in albino rabbits. In-vitro studies are encouraging as niosomes released about 75% of total entrapped drug by EIN-1 and 81% of total entrapped drug by EIN 2. *In vivo* study shows that niosomes released the drug in eye in acceptable range and showed a sustained release pattern without affecting the vision. Niosomes were found ultimate ophthalmic drug carriers capable to release drug in sustained and determined pattern.

Keywords: Niosomes, chloramphenicol eye drops, conjunctivitis, albino rabbits.

INTRODUCTION

Ocular infections caused by bacteria are very common and many patients are presented with ocular discharge, visual symptoms or red and painful eye. These infections accounts for most of visits to pharmacies and primary health care institutions and cost a lot on healthcare systems (Donahue *et al.*, 1996). Conjunctivitis is one of the painful and acute conditions which are inflammation of conjunctiva and *Staphylococcus aureus* is common cause of this acute bacterial disease (Jack, 2006). Approximately 55% of bacterial conjunctivitis is caused by *S. aureus* (Marvin, 2005.). Various antibiotics are available to treat *S. aureus* infections, but CHL (chloramphenicol) is first-line choice for acute bacterial conjunctivitis and its use is started prior to differential diagnosis (Practice Guidance, 2005). It is a broad spectrum antibiotic which acts by interfering with action of peptidyl transferase after binding to 50S subunit of ribosome and inhibit protein synthesis (Betram, 2007). Apart from *S. aureus*, CHL is also used for bacterial conjunctivitis caused by other organisms such as Gram-positive cocci including *S. epidermidis* and streptococci such as *S. pneumoniae*, *S. pyogenes* and the *Viridans streptococci*, gram-negative cocci such as *H. influenzae* and *Moraxella catarrhalis* (a Gram-negative aerobic

diplococcus) (Practice guidance, 2005; Betram, 2007). CHL ophthalmic drops (0.5% w/v) and ophthalmic ointment (1% w/w) are commonly available preparations and confronted with problems like frequent administration and lower bioavailability (Patton, 1976). On the other hand ointment cause blurred and unstable vision for a definite period after application and these problems can affect patient adherence and compliance (Lee, 2006).

Niosomes are non-ionic surfactant vesicles, have shown more penetration and promising progress in ophthalmic drug delivery because of their better penetration and more bioavailability (Karthikeyan, 2008). They are non-toxic, osmotically stable, non-irritant and hydrophilic and hydrophobic both drugs can be loaded in Niosomes (Dubey *et al.*, 2010). Positively charged Niosomes show better binding with corneal surface and more bioavailability (Rabino *et al.*, 2004). Niosomes are also better drug carriers than liposomes and other nano drug carriers, easy to prepare and stable over time (Biswajit *et al.*, 2007). They released the drug independent of pH resulting in significant enhancement of ocular bioavailability. Furthermore, a new drug delivery system is prepared from niosomes which are termed as disomes having large size and more retention time in cul-de-sac, they have Solutan C24 as surfactant (Ripal *et al.*, 2009). The aim of the present study was to develop a better dosage form of CHL having better bioavailability and

*Corresponding author: e-mail: shshaikh2001@yahoo.com

sustained release of drug without any interference to vision.

METHODS AND MATERIALS

CHL (Chloramphenicol) Niosomes were prepared by modified ether injection method. Span 60, CHL and dialysis sacks were from Sigma (Germany) and all other chemicals and solvents were from Merck (Germany).

Preparation of niosomes

Niosomes were prepared by a little modification of Ether Injection Method. Cholesterol and surfactant were dissolved in ether and mixed with methanol having dissolved drug in a ratio of 4 parts of ether and 1 part of methanol, due to high solubility of drug in alcohol. Niosomes having drug: cholesterol: surfactant ratio of 1:1:1 respectably, were termed as EIN 1 (ether injection Niosomes 1) and Niosomes having drug: cholesterol: surfactant ratio of 1:1:2 respectably, were termed as EIN 2 (ether injection Niosomes 2 having double surfactant ratio). The sterile mixture was injected with the help of 14-guage needle in phosphate buffer solution (PH 7.4) at 60-65 °C and this mixture was stirred continuously with magnetic stirrer (BP, 2007). Ether and methanol evaporated at this temperature, leaving behind suspension of CHL niosomes (Baillie et al., 1985; Srinavas et al., 2010).

Entrapment efficiency

The niosomes were kept at room temperature for 1 hr to get mature, and then centrifuged at 9000 rpm for 30 mins. The sediment having vesicles was separated from decanted fluid and resuspended in phosphate buffer. Vesicles were lysed with 1-propanol (50%) and checked for CHL concentration on UV spectrophotometer (Shimadzu Co., Japan) at 280nm against phosphate buffer as blank (Hu, C and Rhodes, DG, 2000). Then entrapment efficiency was calculated by formula:

$$\text{Entrapment efficiency} = \frac{\text{Amount of entrapped drug}}{\text{Total drug}} \times 100$$

Particle size

Particle size was determined by Laser scattering spectroscopy using Horiba Partica LSA-300 (Horiba Co., Japan) (Glive, 2005). Samples were checked at 25±2°C and average particle size was calculated (table 1).

Table 1: Comparison between EIN1 & EIN2

Formulation	Entrapment Efficiency	Average particle size (µm)
EIN 1	83.7% ± 0.5	4.12 ± 0.49
EIN 2	90.2% ± 0.7	4.69 ± 0.75

In vitro drug release

Both formulations were studied for in vitro drug release, Dialysis bag method was used. Niosomes suspensions

were placed in dialysis bag and kept in a 200ml of phosphate buffer stirred at 100 rpm using magnetic stirrer kept at 37 ± 2°C. Samples were drawn at intervals of 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 hours replaced with fresh buffer solution. Samples were checked at 280 nm on UV spectrophotometer (Bhaskaran, 2009; Vijay et al., 2009). Graph was plotted according to results, showing release of drug from niosomes. EIN 2 showed better entrapment as well as better release of drug, so it was selected for further study (fig. 1).

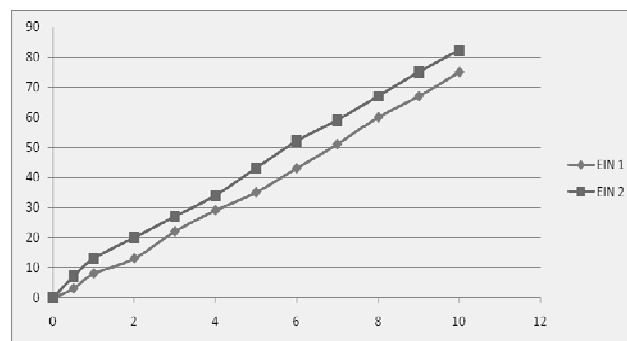


Fig. 1: Percentage of drug release vs time (hours)

Zeta potential and viscosity

Zeta potential tells us about the charge on surface of vesicles which in turn indicates whether vesicles will like to agglomerate or stay separate. It is a very important property of suspension. For determination of zeta potential Zeta probe DT300 (Horiba Co., Japan) was used. Zeta potential of EIN 2 was +31 indicating moderate stability of suspension.

Viscosity is important to know as it indicates the rheology of suspension as well as its thickness. It can determine the effect on the vision. Viscosity was determined by Brookfield viscometer DV 2 (Brookfield Engineering, USA). Viscosity of EIN 2 was 1.35 centi poise at 25±2°C showing that it will not affect vision on administration (Amélie et al., 1998; Arunothayanun et al., 1999).

Determination of MIC

MIC (minimum inhibitory concentration) of CHL against the selected strain of *S. aureus* was determined by Kirby Bauer disc diffusion method (Tao et al., 2003). Discs of various concentrations of CHL were applied on agar plates containing Muller-Hinton agar (Merck, Germany). Very small numbers of *S. aureus* colonies were already present on these plates. They were incubated at 37±1°C for 24 hours. Then zone of inhibition was measured for each disc and minimum concentration of CHL that inhibited the growth of bacteria was determined. The MIC which inhibited bacterial colony growth was 4.5µg/ml.

In vivo study

Twelve healthy rabbits weighing 2.5-3 kg were selected. Rabbits were kept under anesthesia during in-vivo study using 1:1 Ketamine hydrochloride (30mg/kg) and

Xylazine hydrochloride (10mg/kg) injected through IM route. Six rabbits were given CHL ophthalmic drops (0.5%w/v) and other six rabbits were given CHL niosomes suspension (standardized to 0.5%w/v CHL solution). Aqueous humor was drawn after 5 mins, 0.5, 1, 2, 3, 4, 5, 6, 7 & 8 hrs with help of 26 gauge needle, with extra care was exercised to avoid any irritation or lacrimation. Samples were analyzed on HPLC (1200 series Quaternary LC, Agilent tech., USA) and results were recorded in duplicate (Deepika *et al.*, 2007, Martin *et al.*, 1991, Faruk *et al.*, 2000, McCurnin, 2002).

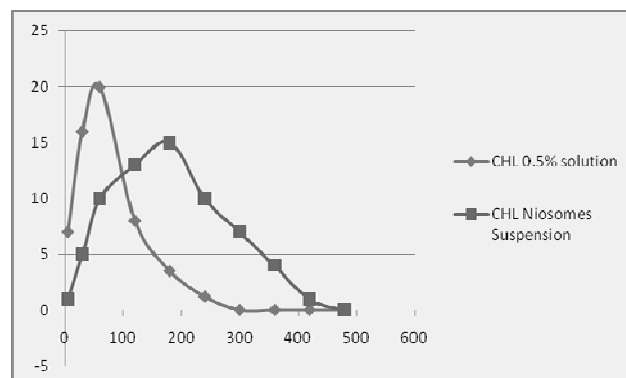


Fig. 2: Concentration of CHL in Aqueous humour (µg/ml) Vs Time (minutes).

Comparison in experimental conjunctivitis

Twelve healthy albino rabbits weighing 2.5-3.5 kg were selected and conjunctivitis was induced with the help of *S. aureus* (incubated in broth at 37°C). The treatment was started with CHL after the appearance of symptoms of conjunctivitis. Six rabbits were treated with CHL ophthalmic drops (5% w/v) and other six were treated with CHL niosomal suspension (standardized to 0.5% w/v CHL solution). Based on data from in vivo study, CHL drops were administered two drops every two hrs (from 8am-8pm) for first two days and then two drops every four hrs (from 8am-8pm) for further three days. Two drops of niosomal suspension were administered (standardized to 0.5% w/v CHL) every six hrs (8am-8pm) for first two days and then two drops of niosomal suspension every twelve hours (8am, 8pm) for further three days. Results of therapy were recorded by observing signs of conjunctivitis and recovery of rabbits from disease. All in-vivo experiments were in accordance with guidelines of NIH animal laboratory regulations and conducted after approval from ethical committee.

RESULTS

Both groups of rabbits showed same pattern of recovery from conjunctivitis. Sterile niosomal preparation showed comparatively better cure to signs and symptoms of conjunctivitis with less frequent administration thus

reducing toxic accumulation of drug which can be expected from commercial CHL drops thus enhancing compliance by less frequent administration. After treatment aqueous humor was collected using 26-gauge syringe and incubated on agar to see the presence of any organism the colony count was normal when compared to incubated aqueous humor of another healthy albino rabbit (Collart *et al.*, 1974). This shows that both group received appropriate treatment and no further *S. aureus* is present in eye to cause any infection.

Stability studies were carried out by keeping the sterile CHL Niosomal suspension at 2-8°C and at various intervals samples were drawn (Ahmed *et al.*, 2005). After 15 days 97.8% of total entrapped drug was present in niosomes. After 30 days there was 95.6% of the entrapped drug was present in niosomes. After 45 days 92.5% drug was present in niosomes and after 57 days 89.7% of the drug was present. So the shelf life came out to be around 55days. The leakage of the drug may be was due to less concentration of cholesterol that rendered the niosomes leaky. But still it is acceptable shelf-life. The shelf-life can be improved with more incorporation of cholesterol but it makes the niosome rigid and drug loading and release is reduced. Proniosomes are the solution for this problem, which can be reconstituted with buffer before administration (Sankar *et al.*, 2010).

DISCUSSION

The zeta potential was +31 which shows that suspension is moderately stable and no rapid flocculation is there. The viscosity of 1.35 centi poise at 25±2°C shows the preparation will not affect the vision and is unlikely to produce any blurredness or lacrimation. Niosomal suspension showed good physical characteristics.

The *in vitro* studies were also quite encouraging that niosomes released about 75% of total entrapped drug by EIN 1 and 81% of total entrapped drug by EIN 2. The in-vivo study shows that niosomes released the drug in acceptable range in eye and showed a sustained release pattern without affecting the vision. Niosomes during the treatment were administered only 2-3 times daily and similar pattern of recovery was observed as compared to commercially available CHL eye drops, which proves that niosomes are ultimate ophthalmic drug carriers capable of release the drug in sustained and determined pattern. The rabbits were monitored after the treatment by veterinarian to check any signs of irritancy or inflammation i.e. redness or increased tear production for 4 months. There were no signs of irritancy or damage to the cornea of rabbit's eyes. It can be predicted that niosomal suspension is not irritant to eye but still needs further studies to determine the minimum toxic concentration of this new formulation.

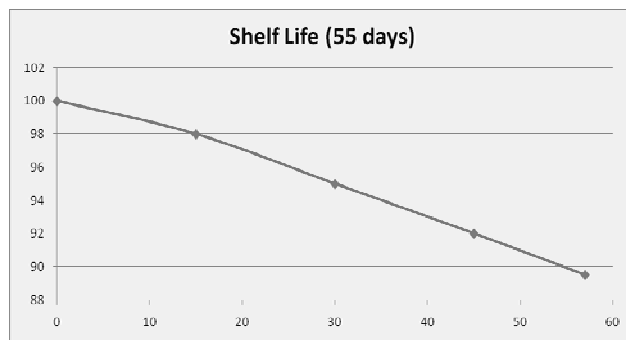


Fig. 3: Time in days Vs %age of drug remained entrapped in niosomes.

CONCLUSION

EIN 2 is ultimate drug carrier of CHL for ophthalmic infections caused by organisms susceptible to CHL. It showed good drug entrapment, better drug release profile and sustained release in eye. It cured the conjunctivitis with very less frequent administrations as required by CHL 0.5% w/v commercially available drops. Zeta potential and viscosity was acceptable as well as the shelf life. Further studies are needed to develop methods to prepare niosomes which can entrap more drugs and are more stable with good release characteristics. Methods should be developed for the cheap production of niosomes on industrial scale so that this ultimate drug carrier can be used in everyday life.

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