

## Original Research Article

## Preparation and Characterization of Liposomes and Ethosomes Bearing Indomethacin for Topical drug delivery

Bindu Malla<sup>1</sup>, Komal Malla<sup>2\*</sup>, Ajay Kumar Sah<sup>2</sup>, Anjali Koirala<sup>2</sup>, Sarita Karki<sup>2</sup>, Daya Ram Prajuli<sup>2</sup>, Bindu Thapa<sup>2</sup>

<sup>1</sup>Department of Clinical Pharmacology, Gandaki Medical College, Kaski, Nepal

<sup>2</sup>Department of Pharmacy, School of Health & Allied Sciences, Pokhara University, Lekhnath-12, Kaski, Nepal

### ABSTRACT

Liposomes and ethosomes, the novel drug delivery system, are starting to be widely applied in topical preparation. Several studies showed that indomethacin, an anti-inflammatory drug loaded liposomes, when given transdermally reduced the side effects and enhanced its efficacy against rheumatoid arthritis and musculo-skeletal disorders. The antiinflammatory activity is directly proportional to the amount of drug that actually crosses the skin and particle size of vesicles directly determines the dermal delivery of drug substances. Indomethacin loaded liposomes and ethosomes were prepared by different methods and characterized by determining their size and entrapment efficiency. To improve the therapeutic outcome and prepare a formulation which is skin-friendly, liposomes and ethosomes were incorporated into the Carbopol gels. The results revealed that entrapment efficiency and size of liposomes and ethosomes varied according to drug:lipid ratio and method of preparation. The entrapment efficiency was higher for ethosomes than liposomes. Furthermore, the incorporation of the vesicles in carbopol gel increased viscosity and stability of the formulation. Hence, these finding suggested that indomethacin loaded ethosomes and liposomes prepared by appropriate method using optimum drug lipid ratio could be a novel and potent transdermal delivery system for safe and effective topical analgesics.

**Keywords:** Indomethacin, Liposomes, Ethosomes, Carbopol gels.

### BACKGROUND

Liposomes are tiny spheres ranging in diameters from 50 nm to several microns (1). The liposomal vesicles are unilamellar or multilamellar spheroid structure composed of lipid molecules assembled into bilayers and they can carry hydrophilic drugs by encapsulation in water phase or hydrophobic drugs by intercalation them into hydrophobic domains. Liposomes have been reported to increase drug stability, enhance therapeutic effects, prolong circulation time and promote uptake of the entrapped drugs into target site while drug toxicity is diminished (2). They have been shown to be interesting as drug delivery systems since they enhance the availability of compound, reduce their systemic toxicity and increase half life in vivo.

Due to their similarity to biological membranes, since the lipid bilayer contains natural phospholipids and cholesterol, liposomes theoretically don't present any risk of antigenicity (3). They have become a valuable

experimental and commercially important drug delivery system because of their biodegradability, biocompatibility, low toxicity and their ability to entrap both lipophilic and hydrophilic drugs. Unfavorable pharmacokinetic profiles of certain drugs can be altered by the entrapment of the drug in liposomes and the tissue distribution of the liposomes themselves can be influenced by varying the diameter, composition, and modifying the surface by attaching ligands that liposomes can be used for the sustained release of drugs into epidermis when applied topically (4). The major obstacle for topical drug delivery is the low diffusion rate of drugs across the stratum corneum. One of the approaches to increase permeation

\*Correspondence: Bindu Malla  
Department of Clinical Pharmacology, Gandaki  
Medical College, Kaski, Nepal  
E-mail: bindumalla8@hotmail.com

rate is the application of drugs in formulations containing vesicles (5). Topically applied liposomes can either mix with the stratum corneum lipid matrix or penetrate the stratum corneum by exploiting the lipid water interface of the intracellular matrix (6).

Ethosomal systems are vesicular systems composed mainly of phospholipid, ethanol and water. Unlike classic liposomes, they are known mainly to deliver drugs to the outer layers of skin. Ethosomes were shown to enhance permeation through the stratum corneum barrier (7,8). Ethosomal systems are easy to prepare, non-irritant and composed mainly of phospholipids (phosphatidylcholine, phosphatidylserine, phosphatidic acid), high concentration of ethanol and water (9). In ethosomal systems, ethanol has a dual function of fluidizing both the vesicle and the stratum corneum lipids, so that the skin is more penetrable, and the vesicles are more flexible (10). Moreover, oral therapy with indomethacin is very effective but its clinical use is often limited because of its potential to cause adverse effect such as irritation and ulceration of gastrointestinal mucosa. Also, it has a short elimination half-life and requires frequent dosing (11). Encapsulation of indomethacin into egg phosphatidylcholine not only reduces or eliminates the gastric and intestinal ulceration normally associated with the ingestion of indomethacin but also modify its pharmacokinetics and biodistribution parameters (12,13). Similarly, ethosomes (novel permeation-enhancing lipid vesicles) can penetrate the skin and exhibit enhanced delivery of various compounds to the deep layers of the skin (10). Therefore, in the present study, we aim to compare the liposomes by different method and ethosomes containing indomethacin by using different parameters like entrapment efficiency, particle size and stability in order to optimize the carrier system for the topical delivery of indomethacin in a suitable vehicle.

## METHODOLOGY

All the solvents and chemicals used were of analytical grade. Indomethacin IP was gifted from Time Pharmaceuticals Private Limited. Carbopol 940 (Goodrich, USA), Dialyses tubing/ membrane Molecular weight cut off (MWCO): 12,000-14,000 (Spectrum Laboratories, Japan), Cellulose nitrate membrane filter 0.20  $\mu\text{m}$  (Toyo Roshi Kaisha, Ltd., Japan) were used. We used double distilled water in all of our experiments.

### Preparation of liposomes and ethosomes

Liposomes and ethosomes were prepared by following methods using standard indomethacin as a material to be

entrapped.

### Proliposome Method

Phosphatidylcholine (100 mg Lipoid S-75) and indomethacin (25 mg) were mixed and dissolved in 2 ml concentrated ethanol and 1 ml PBS pH7.4. The sample was stirred in the magnetic stirrer at 60 °C for 5 min. The sample was left for cool at the room temperature. The proliposome mixture was finally converted to a liposome suspension by the drop-wise addition of 9 ml of PBS pH 7.4. The suspension was stirred for 1 hour in the magnetic stirrer at the rotation at 600 rpm and sonicated for 5 min in the bath sonicator. Then sample was left over night in refrigerator (4 °C) to stabilize prior to characterize (14).

### Ethanol Injection Method

The PBS pH7.4 (10 ml) was kept in a vial and stirred at the magnetic stirrer at 600 rpm. Phosphatidylcholine (100 mg Lipoid S-75) and indomethacin (25 mg) were dissolved in ethanol and rapidly injected into the PBS 7.4 (10 ml). The sample was stirred for 1 hour at 600 rpm and sonicated for 5 min in the bath sonicator (15).

### Thin film hydration method

Phosphatidylcholine (100 mg Lipoid S-75) and indomethacin (25 mg) were dissolved in chloroform and kept in the 250ml round bottom flask and evaporated in the rotary evaporator at the temperature 44 °C until complete evaporation of chloroform. The thin film of the lipid in the round bottom flask was hydrated by the 10 ml of PBS pH7.4. Then sample was hand shaken for 45 min and sonicated for 5 min in the bath sonicator and then stored at the refrigerator (16).

### Preparation of ethosomes

Ethosomes were prepared according Touitou et al., 2000. Phosphatidylcholine (100 mg Lipoid S-75) and 25mg indomethacin were dissolved in the concentrated ethanol and total volume was made 4 ml by adding ethanol and kept in vial. Under the constant stirring with magnetic stirrer, 6 ml of PBS pH 7.4 was injected slowly and left for 30 min under constant stirring (600rpm) and sonicated for 5 min in a bath sonicator.

### Particle size determination of liposomes and ethosomes

The appearance and particle size of liposome was determined using Olympus microscope BH-2 which contains nanometer scale. The samples diluted with PBS were observed under microscope and around 300 particles of each sample were measured manually. The mean diameter was calculated.

## Determination of entrapment efficiencies of liposomes and ethosomes

### Chromatographic condition:

The mobile phase consisted of Methanol: Water: Acetonitrile: Acetic Acid (55:35:10:1). The temperature of column was maintained at 35°C during the chromatographic separation. The flow rate was 0.5 ml/min and run for 20 minutes. Indomethacin was monitored at UV 234 nm (17).

### Separation of untrapped material:

Untrapped materials were separated from the liposomal and ethosomal suspensions by dialysis. Dialysis tube was soaked in PBS pH 7.4 for half an hour. Each liposomal and ethosomal sample (2 ml) were placed in a dialysis tube and extensively dialyzed against PBS pH 7.4 for 24 hours. After 24 hours PBS pH 7.4 was changed and dialysis continued for another 24 hours (New, 1990). The volume of PBS was adjusted to correlate with indomethacin solubility in PBS pH 7.4.

### Entrapment efficiency for ethosomes and liposomes:

After dialysis, lipids in dialyzed liposomal and ethosomal suspensions were dissolved by the addition of ethanol and indomethacin content in each sample was determined by the HPLC. Briefly, to 500 µl of each dialyzed liposomal or ethosomal suspension, 2 ml of ethanol was added and solution was filtered through the cellulose nitrate membrane filter of pore size 0.20 µm. The aliquots of each filtered solution were injected into the HPLC to determine the indomethacin concentration in the sample.

### Preparation of Carbopol gels

Carbopol gels (1 % w/w) were prepared according to Pavelic et al. (2001). Briefly, Carbopol 940 was dispersed distilled water. The mixture was stirred for 1 hour then glycerol was added. Finally the formulation was neutralized by drop-wise addition of 50 % (w/w) triethanolamine, until transparent gel appeared.

### Incorporation of liposomes and ethosomes into the 1 %

Carbopol gels For the incorporation of ethosomal and liposomal suspensions in the gel, at first liposomal and ethosomal suspension were freed from the unencapsulated material through the process of dialysis. Then, the liposomal or ethosomal suspensions were mixed into gel to result in the final concentration of liposomes/ethosomes in the gel being 15 % (w/w).

### Evaluation of gel

Each gel was evaluated visually for their appearance and integrity. Gels spreadability was evaluated on the upper hand skin.

## Stability studies of liposomes, ethosomes and carbopol gels

The empty gel, empty liposomes prepared from different methods, and ethosomes, liposomes and ethosomes without and with indomethacin were incorporated in the gels, and were put in glass vials. They were kept at elevated temperature for accelerated stability (40 °C) and in room temperature over a 3 month period. They were visually evaluated every week for their stability and appearance. The physical appearance and stability of these preparations were determined by organoleptic analysis.

## RESULTS

### Sizes of liposomes and ethosomes

Sizes of liposomes prepared by proliposomes method, ethanol injection method and modified film method and ethosomes were measured and presented in Table 1. Liposomes prepared by different methods and ethosomes have different size distribution. In addition to that, liposomes prepared by ethanol injection method and ethosomes were smaller than liposomes prepared by proliposomes and thin film method.

### Entrapment efficiency of liposomes and ethosomes

Entrapment efficiency of Liposomes and ethosomes were determined by using following formula.

$$\text{Entrapment efficiency} = \frac{\text{Total theoretical amount of drug}}{\text{Amount of entrapped drug}}$$

Ethosomes and liposomes prepared by different methods were destroyed and then amount of indomethacin was calculated by injecting into the HPLC. Quantitative analysis of indomethacin in ethosomes and liposomes were performed in triplicate. Peak identification was made by matching retention times with standard indomethacin which is further confirmed by injecting standard indomethacin together with destroyed liposomes and ethosomes. The chromatogram of a typical ethosomes and liposomes were shown in Figure 1.

In order to optimize the ratio between lipid and indomethacin content, liposomes and ethosomes were prepared by using different amounts of phospholipids and drugs which has different entrapment efficiency (Table 2). Both liposomal and ethosomal gels were stable for up to 2 months at accelerated stability testing at 40 °C. They were more stable than ethosomes and liposomes in the form of suspensions under the same conditions of accelerated stability testing at 40 °C, which supports the view that gel increases the stability of the liposomal dispersion (Pavelic et al., 2001), as well as ethosomal dispersions containing indomethacin.

**Table 1.** Sizes of ethosomes and liposomes prepared by different method

S. N.	Preparation Method	Lipid:Drug	Amount of Lipid (mg)	Amount of Drug (mg)	Size (nm)
1.	Proliposomes	10:1	100	10	174.37 ± 25.63
2.	Proliposomes	50:1	100	2	147.15 ± 23.30
3.	Ethosomes	10:1	100	10	77.66 ± 23.65
4.	Ethosomes	50:1	100	2	98.55 ± 28.89
5.	Thin Film	10:1	100	10	192.18 ± 28.72
6.	Thin Film	50:1	100	2	115.62 ± 13.25
7.	Ethanol Injection	10:1	100	10	76.56 ± 15.46
8.	Ethanol Injection	50:1	100	2	92.96 ± 62.97



**Figure 1.** Typical HPLC chromatogram of standard indomethacin, ethosomes and liposomes. (A) HPLC Chromatograms of Standard indomethacin, (B) Destroyed ethosomes containing indomethacin, (C) Destroyed liposomes containing indomethacin.

Conditions: column: fluofix (4.6X150mm); column temperature: 35 °C; mobile phase: Methanol: Water: Acetonitrile: Acetic Acid (55:35:10:1); detector: UV at 234 nm; flow rate: 0.5 ml/min; chart speed: 5 mm/min.

**Table 2.** Entrapment efficiencies of liposomes and ethosomes

S. N.	Preparation Method	Lipid:Drug	Amount of Lipid (mg)	Amount of Drug (mg)	*Entrapment efficiency (%)
1.	Proliposomes	10:1	100	10	3.49 ± 1.27
2.	Proliposomes	50:1	100	2	11.24 ± 1.04
3.	Ethosomes	10:1	100	10	5.04 ± 0.99
4.	Ethosomes	50:1	100	2	24.22 ± 4.46
5.	Thin Film	10:1	100	10	5.61 ± 2.94
6.	Thin Film	50:1	100	2	6.35 ± 3.19
7.	Ethanol Injection	10:1	100	10	2.53 ± 0.08
8.	Ethanol Injection	50:1	100	2	7.94 ± 1.49

\*Mean ± SD (n=3)

## DISCUSSION

Liposomes prepared from naturally occurring biodegradable and non-toxic lipids are good candidates for local delivery of therapeutic agents such as anti-inflammatory drugs. Our preliminary data indicated that liposomes prepared by different method have different

entrapment efficiency and size distribution. By adjusting the drug lipid ratio we can prepare vesicles having optimum entrapment efficiency and size distribution.

It has been shown that the particle size of liposomes directly influences the dermal delivery of substances into the skin. Confocal laser scanning microscopy study indicate that the large vesicles with a size  $\geq 600$  nm are not able to deliver their contents into the deeper layers



of the skin and stay in/on the stratum corneum, which after drying may form a layer of lipid, strengthening the barrier function of stratum corneum. The liposomes with size  $\leq 300$  nm are able to deliver their contents to some extent into the deeper layers of the skin. Most promising for dermal delivery seems to be liposomes with size  $\leq 70$  nm as they have been found both in viable epidermis as well as in dermis (18). All the liposomes and ethosomes were of sizes less than 300 nm. So they are able to deliver their contents to some extent into deeper layers of the skin. Among them, ethosomes and liposomes prepared by ethanol injection method were of small size enough to penetrate into the deeper layers of skin. This may be due to incorporation of high ethanol concentration in ethosomes and ethanol injection. Ethanol confers a surface negative net charge to the liposome which causes the size of vesicles to decrease (8,19).

It is possible to incorporate indomethacin into the ethosomes which has been recently developed showing enhanced skin delivery and are interesting and innovative vesicular systems in the fields of pharmaceutical technology and drug delivery (8). Among these vesicles prepared by different method, ethosomes has highest entrapment efficiency at drug lipid ratio 50:1, which can be very effective topical delivery system for indomethacin. Liposomal and ethosomal suspensions should be incorporated into the appropriate vehicle to achieve the suitable viscosity for the topical application because they are not applicable to the skin as they would be too leaky. A vehicle for incorporation of liposomal and ethosomal suspensions should provide adequate pH value, stability and rheological characteristics. Due to the good physical, chemical and biological properties of hydrophilic polymer gels carbopol resins are chosen to prepare hydrogels as an appropriate vehicle for incorporation of liposomes and ethosomes designed for topical application (16). Gels have advantages of being less greasy, easily spreadable, and stable over longer period of time as compared to creams or ointments. Hence liposomal and ethosomal suspensions were incorporated into Carbopol 940 gels. Moreover, gels are known to increase the stability of liposomal suspensions (14). To prove whether this applies to ethosomal suspensions as well, we tested liposomal and ethosomal gels for their stability. We found that the liposomal and ethosomal gel containing indomethacin were more stable than the liposomal and ethosomal suspension.

## CONCLUSION

It is concluded that liposomes prepared by different method have different entrapment efficiency and size distribution. Ethosomes and liposomes containing indomethacin of optimum size for penetration into the

skin can be readily prepared. Among these vesicles prepared by different method, ethosomes have highest entrapment efficiency. To obtain the suitable viscosity and attractiveness of formulation, as well as to increase the stability of liposomal or ethosomal suspensions, they were incorporated into the Carbopol gels. This type of formulation can be superior in enhancing the sustained delivery of indomethacin.

## COMPETING INTERESTS

The authors declare that they have no competing interest.

## ACKNOWLEDGEMENT

We would like to express our deep gratitude to Pharmacy Department of Pokhara University for providing the laboratory space, materials and equipments. We also want to extend our sincere thanks to Time Pharmaceuticals for providing indomethacin IP.

## REFERENCES

1. Fang JY. Nano- or submicron-sized liposomes as carriers for drug delivery. *Chang Gung Med J.* 2006;29 (4): 358-62.
2. Manosroi A, Wongtrakul P, Manosroi J, Sakai H, Sugawara F, Yuasa M and Abe M. Characterization of vesicles prepared with various non-ionic surfactants mixed with cholesterol. *Colloids and Surfaces B: Biointerface.* 2003;30: 129-38.
3. Cereda CMS, Araujo DRD, Brunetto GB and Paula ED (2004) Liposomal Prilocaine: preparation, characterization and *in vivo* evaluation. *J Pharm Pharmaceut Sci.* 2004;7(2):235-40.
4. Peschka R, Dennehy C, Szoka FC Jr. A simple *in vitro* model to study the release kinetics of liposome encapsulated material. *J Control Release.* 1998;56(1-3):41-51.
5. Bouwstra JA and Honeywell-Nguyen PL. Skin structure and mode of action of vesicles. *Adv Drug Deliv Rev.* 2002;54: 41-55.
6. Yarosh DB. Liposomes in investigative dermatology. *Photodermatol Photoimmunol Photomed.* 2001;17(5): 203-12.
7. Dayan N and Touitou E (2000) Carriers for skin delivery of trihexyphenidyl HCl: ethosomes vs. liposomes. *Biomaterials.* 2000;21(18): 1879-85.
8. Touitou E, Dayan N, Bergelson L, Godin B and Eliaz M. Ethosomes-novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. *J Control Release.* 2000; 65(3):403-18.
9. Touitou E, Godin B, Dayan N, Weiss C, Piliponsky A and Levi-Schaffer F. Intracellular delivery mediated by an ethosomal carrier. *Biomaterials.* 2001; 22(22):3053-9.

10. Godin B and Touitou E. Erythromycin ethosomal systems: physicochemical characterization and enhanced antibacterial activity. *Curr Drug Deliv.* 2005;2(3): 269-75.
11. Puglia C, Trombetta D, Venuti V, Saija A and Bonina F. Evaluation of in-vivo topical anti-inflammatory activity of indomethacin from liposomal vesicles. *J Pharma Pharmacol.* 2004;56(10): 1225-32.
12. Soehngen EC, Godin-Ostro E, Fielder FG, Ginsberg RS, Slusher MA and Weiner AL. Encapsulation of indomethacin in liposomes provides protection against both gastric and intestinal ulceration when orally administered to rats. *Arthritis and Rheuma.* 1988;31(3): 414-22.
13. Morilla MJ, Benavidez P, Lopez MO, Bakas L and Romero EL. Development and *in vitro* characterization of a benzonidazole liposomal formulation. *Int J Pharm.* 2002; 249(1-2): 89-99.
14. Pavelic Z, Skalko-Basnet N and Jalsenjak I. Liposomes containing drugs for treatment of vaginal infections. *Eur J Pharm Sci.* 1999;8(4): 345-51.
15. Skalko N, Cajkovic M and Jalsenjak I. Liposomes with metronidazole for topical uses: the choice of preparation method and vehicle. *J Liposome Res.* 1998;8(2):283-93.
16. Pavelic Z, Skalko-Basnet N and Schubert R. Liposomal gels for vaginal drug delivery. *Int J Pharm.* 2001;219(1-2):139-49.
17. O'Brien M, McCauley J, Cohen E. *Analytical Profiles of Drug Substances.* Volume 13. Academic Press INC, (London) LTD: USA; 1984: 211-235.
18. Verma DD, Verma S, Blume G and Fahr A. Particle size of liposomes influences dermal delivery of substances into skin. *Int J Pharm.* 2003;258 (1-2): 141-51.
19. Lopez-Pinto JM, Gonzalez-Rodriguez ML and Rabasco AM. Effect of cholesterol and ethanol on dermal delivery from DPPC liposomes. *Int J Pharm.* 2005;298 (1): 1-12.