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SYNTHESIS OF NIOSOMES OF DICLOFENAC SODIUM AND THEIR MICROSCOPIC EVALUATION

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ABSTRACT
Objective: Incorporation of drugs into non-ionic surfactant vesicles (niosomes) during their manufacture affords a possible method of achieving controlled release. The aim of this study was to formulate niosomes as carriers for delivery of diclofenac sodium (DCS) to achieve controlled release. **Method:** Niosomes were prepared by Ether injection method using non-ionic surfactant (Span 80) and cholesterol in ratio of 1:1, the organic solvent (dichloromethane) and phosphate buffer. **Results**: The prepared niosomes were then evaluated for physical appearance, light microscopy, optical microscopy, transmission electron microscopy and entrapment efficiency. **Conclusion:** Diclofenac sodium niosomes were prepared successfully without using charge inducing surfaces and were confirmed by microscopic evaluation.

Keywords: Niosomes, Diclofenac sodium, Transmission electron microscopy, Entrapment efficiency.

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INTRODUCTION
Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) taken or applied to reduce inflammation and as an analgesic reducing pain in certain conditions. It is a Cox-2 inhibitor. Diclofenac is used to treat pain, inflammatory disorders, and dysmenorrhea. It may also help with actinic keratosis, and acute pain caused by minor strains, sprains, and contusions (bruises). A niosome is a non-ionic surfactant-based Vesicle (biology and chemistry). Niosomes are formed mostly by non-ionic surfactant and cholesterol incorporation as an excipient. Other excipients can also be used. Niosomes have more penetrating capability than the previous preparations of emulsions. They are structurally similar to liposomes in having a bilayer; however, the materials used to prepare niosomes make them more stable [**1, 2**].

Niosomes of Diclofenac Sodium prepared by ether injection method without charge inducing surfaces are easy to prepare, economical and are not time consuming and have distinct advantages over conventional dosage forms because the particles can act as drug containing reservoirs and slowing drug release rate may reduce the toxicity of drug [**3**]. The bilayer membrane of niosomes mimics biological membranes giving them increased stability and residence time in circulation. From a technical point of view, niosomes are promising drug carriers for Diclofenac Sodium as they possess greater stability and lack of many disadvantages associated with liposomes, such as chemical instability, high cost, variable purity of natural phospholipids and special requirements for handling and storage [**4-6**].

METHOD
Preparation
First the phosphate buffer saline (PBS) was produced by dissolving potassium dihydrogen phosphate in water and adjusting the pH with 0.1 N sodium hydroxide and 0.2 M hydrochloric acid to 7.4. Then lipid mixture was prepared by mixing cholesterol and span 80 and dissolving them in dichloromethane. Then lipid mixture was injected slowly at the rate of 0.25ml/min in 5ml PBS containing 5mg/ml of the drug and maintained at temperature 60^0 C. The organic solvent evaporated and lead to the formation of large unilamellar vesicles [**7**].

Evaluation of Niosomes of Diclofenac Sodium
Physical Appearance
The prepared niosomes were evaluated for color, homogeneity, clarity and foreign particles.

Light Microscopy
The prepared niosomes were observed under light microscope. A slide was prepared by adding 1 drop of niosomal preparation on slide and then cover slip

was placed. The slide was then observed under microscope.

Shape and Morphology

Shape and morphology of niosomal formulations were determined by optical microscopy.

Transmission Electron Microscopy (TEM)

The prepared niosomes were analyzed by electron microscopy through negative staining using 2% ammonium molybdate solution. 0.5 ml negative stain was mixed with 0.5ml niosomal solution and 1 drop of this mixture was placed on carbon coated grid. The grid was then dried and washed with water to remove excess stain and then examined under electron microscope [**8**].

Entrapment Efficiency

Entrapment efficiency was determined by exhaustive dialysis method. The niosomal suspension was taken in a dialysis tube which has a membrane attached to its one side called as dialysis membrane. A 100ml phosphate buffer solution (PBS) having methanol 10% v/v was stirred on magnetic stirrer and then dialysis tube was suspended in it. The un-entrapped drug moves from the niosomal suspension into the medium through dialysis membrane. After every 1 hour the entire medium (100 ml PBS) was replaced with fresh medium (for about 6-7 hours) until there is constant absorbance which indicates no drug is available in un-entrapped form. The withdrawn sample were then analyzed at 270nm by spectrophotometer using PBS (having pH 7.4) as blank. Entrapment efficiency was calculated by formula [**9, 10**]:

EE= [(total drug – unentrapped drug)/ total drug] *100

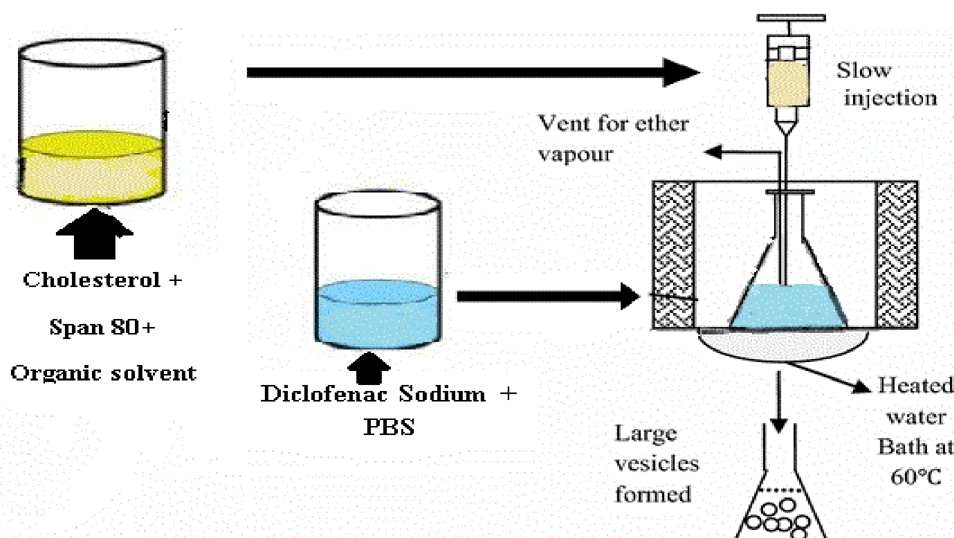

Figure 1: Niosomes preparation flow chart.

RESULT

Physical Appearance

Physical appearance is presented in **Table 1**.

Table 1: Physical appearance of niosomes.

Evaluation	Results
Color	Colorless
Homogeneity	Good
Clarity	Clear
Foreign Particles	None

Light Microscopy

The prepared niosomes were observed under light microscope to check the bubble formation or foreign particles. There was no bubble formation and no foreign particles were present in it.

Shape and Morphology

The formation of vesicles was confirmed by optical microscopy. One drop of niosomal preparation was placed over glass slide and was dried at room temperature for the purpose of fixing. The dry thin film is formed which is then observed under optical microscope. By using Digital camera, micro-photography of niosomal preparation was done as shown in **Figure 2**.

Entrapment Efficiency

Entrapment efficiency test was performed by using dialysis method in phosphate buffer solution having pH 7.4. Entrapment efficiency increases with the increase in the concentration of surfactant. Here the surfactant:drug concentration was 1:1. Entrapment

efficiency was determined by separating unentrapped drug using dialysis method. Here the entrapment efficiency was 55.14 ± 2.24.

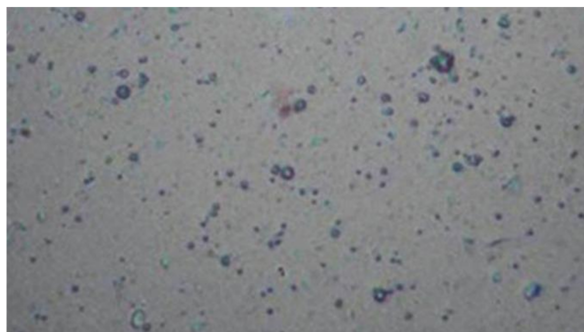

Figure 2: Optimal microscopic image of niosomes.

Transmission Electron Microscopy

For the purpose of detailed study and particle size evaluation, transmission electron microscopy was done. Negative staining technique was used. It was found that large unilamellar vesicles of size 2 μm having spherical shape were obtained as shown in **Figure 3**.

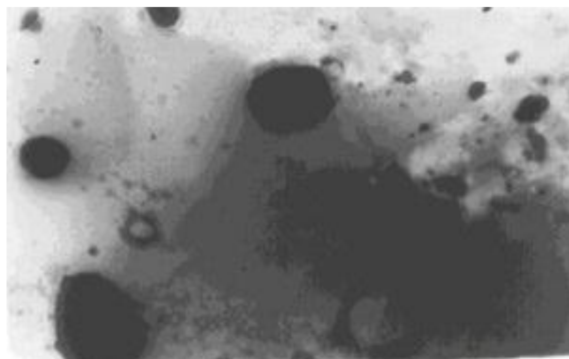

Figure 3: Negative stain micrograph of niosomes in TEM.

DISCUSSION

Physical Evaluation

Niosomes cannot be seen with naked eye but physical evaluation was done for the color, homogeneity, clarity and foreign particles. The niosomes were colorless and clear which indicates that the preparation was done correctly and under hygiene conditions. The presence of foreign particle and non-homogeneity can cause instability of the preparation and may impart harmful effect when used. There was no foreign particle and the homogeneity was good.

Light Microscopy

Light microscopy was performed on preparation to check any bubble formation. The presence of bubble formation can affect the efficiency of the preparation. There was no bubble formation in the preparation.

Shape and Morphology

To check the shape of prepared niosomes, optical microscopy was done by fixing the preparation and observing it under optical microscope. Spherical shape vesicles were obtained. The slow injection of organic solution into the aqueous phase causes formation of vesicles of almost same size and shape. The injection rate is main factor for formation of vesicles of same size and shape [**11**].

Entrapment Efficiency

Entrapment efficiency means how much drug is entrapped within the bilayer of niosomes. It depends upon the amount of surfactant used and charge inducing agent [**12**]. The entrapment efficiency was 55.14% with standard deviation of ±2.24. This decreased entrapment efficiency was due to lack of charge inducing agents.

Transmission Electron Microscopy

Transmission electron microscopy was done by negative stain technique for the purpose of particle size evaluation. It was found that the particle size of 2μm was prepared which are large unilamellar vesicles. The smooth shape vesicle formed was due to the slow injection technique and due to the ratio of cholesterol:span-80 (1:1). The conventional spherical shaped vesicles can be obtained if the ratio of cholesterol and span-80 is equal [**13**].

CONCLUSION

Niosomes of diclofenac sodium were successfully prepared by using ether injection method using cholesterol and non-ionic surfactant using ration 1:1. Non-ionic surfactant used in this method was span-80. Niosomes were prepared without using charge inducing agent like stearyl amine and dicetyl phosphate. The prepared niosomes were then microscopically evaluated for different tests to confirm that niosomes were actually prepared. The prepared niosomes were evaluated for physical appearance, light microscopy, shape and morphology by optical microscopy, transmission electron microscopy for further details and entrapment efficiency and the results were discussed above.

Niosomes could be prepared without using charge inducing surfaces and the method was easy to perform and was economical. But the niosomes produced had less entrapment efficiency as that of niosomes produced by charge inducing surfaces. Large unilamellar vesicles were obtained.

REFERENCES

1. Saeid Moghassemi, Afra Hadjizadeh. Nano-niosomes as nanoscale drug delivery systems: An illustrated review. Journal of Controlled Release. 185: 22–36, 2014.
2. G Puras, M Mashal, J Zárate, M Agirre, E Ojeda, S Grijalvo, R Eritja, A Diaz-Tahoces, G Martínez Navarrete, M Avilés-Trigueros, E Fernández, J L Pedraz. A novel cationic

niosome formulation for gene delivery to the retina. Journal of Controlled Release. 174: 27–36, 2013.
3. Hao Y, Zhao F, Li N, Yang Y, Li K. Studies on a high encapsulation of colchicine by a niosome system. Int J Pharm., 244, 73-80, 2002.
4. Blazek-Welsh AI, Rhodes DG. Maltodextrin -based proniosomes. AAPS Pharm Sci., 3(1), E1, 2001.
5. Abu-Zaid SS, El-Ghamry HA, Hammad M, Mokhtar M. Effect of some formulation parameters on encapsulation release profile biological activity of liposomes containing certain drugs. MJPS 19(2), 76-89, 2003.
6. Mokhtar M, Sammour OA, Hammad MA, Megrab NA. Effect of some formulation parameters on flurbiprofen encapsulation and release rates of niosomes prepared from proniosomes. Int J Pharm., 361, 104-11, 2008.
7. Ridy MS, Abdelbary A, Essam T, Abd El-Salam RM, Kassem AAA. Niosomes as a potential drug delivery system for increasing the efficacy and safety of nystatin. Drug Dev Ind Pharm., 37 (12), 1491-508, 2011.
8. Namedeo A, Jain NK. Niosomal delivery of 5-flurouracil. J Microencapsul., 16(6), 731-40, 1999.
9. Darwish IA. Preparation characterization of niosomes formed from mixed non-ionic surfactant Cholesterol. Alex J Pharm Sci., 12(1), 33-8.
10. Popli H, Nair MS. Niosomal delivery of tenoxicam. Indian J Pharm Sci 1996; 58(4):163-6.
11. Omaima NE, Ahmed HH. Preparation and evaluation of acetazolamide liposomes as an ocular delivery system. Int J Pharm, 158,121-5, 1997.
12. Hathout RM, Mansour S, Mortada ND, Guinedi AS. Liposomes as an ocular delivery system for acetazolamide: in vitro and in vivo studies. AAPS PharmSciTech, 8(1), 1-12, 2007.
13. Sammour OA, Hassan HM. Enhancement of the antibacterial activity of ampicillin by liposome encapsulation. Drug Delivery, 3, 273-8, 1996.