

## SHORT COMMUNICATIONS

### Formulation and Evaluation of Niosomes Using Different Non-Ionic Surfactants

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Niosomes containing salbutamol sulphate were prepared using different non-ionic surfactants like Tween 20, 40, 60, 80, Span 20, 40, 60, 80 and Brij 35 by transmembrane pH gradient method. The drug encapsulation efficiency varied from 28% to 79%. The vesicles have been characterized by infrared spectroscopy. *In vitro* drug release studies were carried out using dialysis bag and phosphate buffer, pH 7.4 as a dissolution medium for 24 h. The formulation exhibited retarded release for 24 h and Span 60 was found to be the most satisfactory surfactant which released 78.4% of drug in 24 h. Particle size distribution studies were carried out by optical microscopy technique. Most of the niosomes were found to be spherical in shape. Thermal stability studies were carried out at 4°, 25° and 50° for one month. The product was lyophilized. Tissue distribution studies were carried out on rabbits. The maximum concentration was seen in lungs.

Controlled release drug products are often formulated to permit the establishment and maintenance of drug concentration at target site for longer intervals of time<sup>1</sup>. One such technique of drug targeting is niosomes. These are microscopic lamellar structures formed on admixture of non-ionic surfactant, cholesterol and diethyl ether with subsequent hydration in aqueous media. They behave *in vivo* like liposomes prolonging the circulation of entrapped drug and altering its organ distribution<sup>2</sup>.

Niosomes are biodegradable, biocompatible and non-immunogenic<sup>3</sup>. Niosomes can entrap both hydrophilic and lipophilic drugs and can prolong the circulation of the entrapped drug<sup>4</sup>. Rifampicin<sup>5</sup> and silboglucuronate<sup>6</sup> were entrapped in niosomes for targeted delivery. To improve bioavailability, flurbiprofen and piroxicam were entrapped into niosomes<sup>7</sup>. Niosomes can also be used as a novel peroral vaccine delivery system<sup>8</sup>.

Antiasthmatic drugs have high potential to induce toxic side effects. Therefore, it is desirable to deliver them to

target tissue in the right manner at the right time. Salbutamol sulphate is a selective  $\beta_2$  adrenergic agonist. The initial oral dose is 2-4 mg given 3-4 times a day i.e., a total dose of 32 mg should not be exceeded<sup>9</sup>. The plasma half-life is 2-7 h. So in the present study salbutamol sulphate was encapsulated in niosomes using different non-ionic surfactants to attain greater therapeutic efficacy, to increase half-life and to target delivery.

Salbutamol sulphate was a generous sample from M/s Juggat Pharma, Bangalore. Tween 20, 40, 60, 80, Span 20, 40, 60, 80 and Brij 35 were purchased from S.D. Fine Chemicals, Boisar. Sephadex G-50 was purchased from Aldrich Thomas Co. USA, and cholesterol and ether was procured from Loba Chemie, Mumbai.

Niosomes were prepared by transmembrane pH gradient method<sup>11</sup>. Twelve milligram each of surfactant and cholesterol (1:1) were dissolved in chloroform. The solvent was then evaporated under reduced pressure to get a thin film on the wall of the round bottom flask. The film was hydrated with 300 mM citric acid (pH 4.0) by vortex mixing. Then the product was frozen. To this niosomal suspension,

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5 ml aqueous solution containing 1.2 mg/ml salbutamol sulphate was added and vortexed. The pH of the sample was then raised to 7.0-7.2 with 1 ml of disodium hydrogen phosphate. The mixture was later heated at 60° for 10 min. The prepared niosomes were purified to remove unentrapped drug by passing through sephadex G-50 column. Elution was taken to be complete when no difference in absorption could be detected between the eluent and the blank.

Two millilitres of niosomes were taken in a small test tube. Then they were sonicated for 5 min. using a needle probe type sonicator (Braun Sonic 1410) set at 400 watts. One ml of the above solution was pipetted into 50 ml volumetric flask and the volume was made up with distilled water. From the stock solution 5 ml was pipetted into a 25 ml volumetric flask. The color was developed using 4-aminophenazone and absorbance was measured at 505 nm using Spectronic-20. The entrapment efficiency was also calculated. The results are given in Table 1.

For *in vitro* release studies, a dialysis bag containing 2 ml of niosomes were tied at both the ends and was placed in a 100 ml beaker containing 50 ml of isotonic phosphate

buffer solution of pH 7.4. The beaker was placed on a magnetic stirrer and stirred continuously at 50 rpm at 37° ± 1°. Five milliliters of the sample was withdrawn from the beaker at different time intervals and replaced with fresh buffer solution. The sample was analyzed after necessary dilution and treatment with reagents.

Optical microscopy technique was used to study the particle size and shape. Ten millilitres of the sample was sealed in vials (niosomes suspended in phosphate buffer pH 7.4). They were stored at 4°, 25° and 50° for one month. The percentage of drug release with time was estimated and shelf life was calculated.

Lyophilisation was carried out for formulation F<sub>7</sub> containing Span 60 as this showed best release rate. One millilitre of the niosome formulation was added in to the tube, 10% w/v of mannitol was added as cryoprotective agent. The tubes were well stirred under closed conditions and frozen in the cooling bath of the lyophilizer at a temperature of 40° and a pressure of 10<sup>-2</sup> torr for 5-6 h. Then they were lyophilized by subjecting to a high vacuum cooling trap, where the sample was dried to a powder. Then they were kept in a desiccator till use. The drug content was estimated and an IR spectrum was determined.

TABLE 1: PARTICLE SIZE AND ENTRAPMENT EFFICIENCY OF THE NIOSOME FORMULATIONS.

| Formulation Code | Surfactant used | Entrapment efficiency mg ± S.D. |
|------------------|-----------------|---------------------------------|
| F <sub>1</sub>   | Tween 20        | 64.87±0.056                     |
| F <sub>2</sub>   | Tween 40        | 58.80±0.046                     |
| F <sub>3</sub>   | Tween 60        | 72.96±0.208                     |
| F <sub>4</sub>   | Tween 80        | 62.50±0.015                     |
| F <sub>5</sub>   | Span 20         | 63.70±0.078                     |
| F <sub>6</sub>   | Span 40         | 67.70±0.023                     |
| F <sub>7</sub>   | Span 60         | 87.51±0.239                     |
| F <sub>8</sub>   | Span 80         | 73.82±0.320                     |
| F <sub>9</sub>   | Brij 35         | 62.50±0.078                     |

F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub>, F<sub>4</sub> are niosomal formulations containing Tween 20, Tween 40, Tween 60 and Tween 80, respectively whereas F<sub>5</sub>, F<sub>6</sub>, F<sub>7</sub>, and F<sub>8</sub> containing Span 20, Span 40, Span 60 and Span 80. F<sub>9</sub> Contains Brij 35 as the surfactant prepared by transmembrane pH gradient technique.

Wistar rats weighing around 150-180 g were divided in to 3 groups comprising of 3 animals each to carry out tissue distribution profile. The rats were injected with free drug in group 1 and niosome formulation-F<sub>7</sub> in group 2. Group 3 was treated as control, which received free niosomes without the drug. The rats were sacrificed 1 h after the injection of the drug. Various tissues such as liver, lungs, spleen, kidney and heart were removed and washed with phosphate buffer pH 7.4, homogenized and centrifuged. The supernatant liquid was taken for the determination of drug content.

Salbutamol sulphate, cholesterol and surfactant were used in equal ratios and niosomes were prepared using transmembrane pH gradient method. The drug content was found to be 28-79%. The release rate was found to be 70% after 24 h (fig.1) All the formulations exhibited retarded release rate for 24 h. Span 60 was found to be the most satisfactory surfactant which released 78.4% of drug in 24 h. Most of the niosomes were found to be spherical in shape, few being either triangular or slightly elongated. The mean diameter is 3.6  $\mu\text{m}$ . One millilitre of the formulation gave 150 mg of lyophilized product. After reconstitution the product in saline, the niosomes were found to be spherical in shape.

The IR spectra of lyophilized product, formulation and pure drug gave the same kind of peaks proving the intactness of the drug in the formulation. From tissue distribution data it is seen that 75% of the formulation concentrates in the lungs. This shows that targeting can be achieved. Thus the niosomal formulations can increase the half-life of the drug and helps in prolonged action with targeting. As a result the frequency of administration can be decreased to improve patient compliance.

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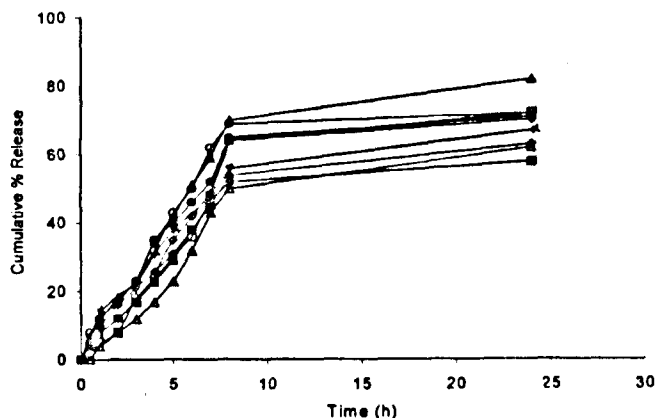


Fig. 1: *In vitro* release from niosome formulations.

Release of salbutamol sulphate from various niosomal formulations made up of Tween 20 (F<sub>1</sub>-x-), Tween 40 (F<sub>2</sub>-□-), Tween 60 (F<sub>3</sub>-Δ-), Tween 80 (F<sub>4</sub>-◇-), Span 20 (F<sub>5</sub>-●-), Span 40 (F<sub>6</sub>-■-), Span 60 (F<sub>7</sub>-▲-), Span 80 (F<sub>8</sub>-◆-) and Brij 35 (F<sub>9</sub>-○-) was studied using transmembrane pH gradient technique.

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