DESIGN AND CHARACTERISATION OF ANIONIC SURFACTANT SPAN 20 NIOSOMES CONTAINING RIFAMPICIN

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INTRODUCTION

Niosomes are novel drug delivery vesicles composed of non-ionic surfactant bilayers with their size lies in the nanometric scale [10]. Niosomes are developed on the addition of cholesterol and non-ionic surfactant of the alkyl or dialkylpolyglycerol ether class upon hydration in aqueous media [1]. Vesicles may be unilamellar or multilamellar depends on the method of preparation [2]. The niosome is made of a surfactant bilayer with its hydrophilic ends exposed on the outside and inside of the vesicle while the hydrophobic chains face each other within the bilayer [3]. Hence, the vesicle holds hydrophilic drugs within the space enclosed in the vesicle while the hydrophobic drugs are embedded within the bilayer itself. As the niosomes are chemically, it stable can be stored for longer periods. The application of niosomal technology is widely varied and can be used to treat a number of diseases.

Localisation of drugs encapsulated in niosomes is utilized to treat tumors of liver and spleen, parasitic infection of the liver and antibodies to specific organs [4]. Niosomes can also be utilized for targeting drugs to organs other than RES.

MATERIALS AND METHODS:

Materials: Chemicals used as Rifampicin (Lupin), Cholesterol ‘Excels R’ (Qualigen), Span 20 (Sigma), Dicetyl phosphate (Sigma). Instruments: Rotary flash evaporator (Roteva), UV-Visible Spectrophotometer (Shimadzu UV-1700), Ultra Sonic processor (Vibronics), Scanning Electron Microscopy (Shimadzu S-3000H).

Preparation of Standard Curve for Rifampicin:
Weigh accurately about 0.1 g of rifampicin and dissolve it to sufficient methanol and make up to 100 ml with phosphate buffer.
From the stock solution a series of rifampicin solutions of known concentrations i.e. 5, 10, 15, 20 and 30 µg/ml in phosphate buffer pH 7.4 were prepared and the absorbances were measured in order to generate a standard curve on a spectrophotometer at a λ of 475 nm. The values are given in the Table 1 and Graph I [16, 19].

### Table 1: Standard Curve of Rifampicin

| S. No. | Concentration µg/ml | Absorbance at 475 nm |
|--------|---------------------|----------------------|
| 1.     | 5                   | 0.075                |
| 2.     | 10                  | 0.163                |
| 3.     | 15                  | 0.240                |
| 4.     | 20                  | 0.322                |
| 5.     | 30                  | 0.472                |

### Preparation of Niosomes

Surfactant (47.61, 48.38, 48.78 mg), cholesterol (47.61, 48.38, 48.78 mg) and dicetyl phosphate (4.76, 3.22, 2.43 mg) to give a micro molar ratio of 10:10:1 (S1); 15:15:1 (S2) and 20:20:1 (S3) respectively were dissolved in diethyl ether (10-15 ml) [5, 8]. The solvent was evaporated under reduced pressure at a temperature of about 60°C in a boiling water bath using a rotary flask evaporator, leaving a thin layer of solid mixture deposited on the wall of the round bottom flask [6]. The 10 ml of 1 mg/ml drug solution was added to the flask heated to about 50°C on the water on a vortex, until a good dispersion of the mixture was obtained. The suspension was then sonicated to form unilamellar Niosomes [7, 9]. Table 2 showed that the different formulations of niosomes were prepared.

### Table 2: Different formulations of niosomes

| S. No. | Niosomes                  | Product Name |
|--------|----------------------------|--------------|
| 1.     | Rifampicin niosomes with 10:10:1 molar ratios | S 1          |
| 2.     | Rifampicin niosomes with 15:15:1 molar ratios  | S 2          |
| 3.     | Rifampicin niosomes with 20:20:1 molar ratios  | S 3          |

### Particle size measurement:

The particle sizes of prepared niosomes were analyzed by Scanning Electron Microscopy (SEM) because it has high resolution and the sample preparation is relatively easy. Samples were scanned at different areas using Hitachi S-3000 H scanning. Probe microscope/ Scanning Electron microscopy for high resolution, was used for all analyses. Digital images were stored in computer and processed.

The scanning frequency of the instruments is 1-3 Hz. From the SEM photographs, the mean particle diameter analysis was performed by the standard scale value of SEM.

### Determination of Entrapment Efficiency:

The entrapment efficiency was determined by using the direct method. The detergents are used to break the membranes, 1 ml of 1% Triton X-100 solution (Triton X-100 dissolved in distilled water) [15] was added to 9 ml of niosomes was incubated at 37°C for 1.5 hours to complete breakup of the noisome membranes and to release the entrapped material. The sample was filtered through a Sigma dialysis membrane fitter (0.25 nm) and the filtrate was measured at suitable Nano meter (for Rifampicin 475 nm). The amount of drug was derived from the calibration curve [12].

### Stability Studies of Niosomes

The formulated niosomes were divided into groups and one group was kept at 4°C, second portion at room temperature and third at 45°C. Each and every week 0.1 ml of the niosomes were withdrawn and made up to 1 ml and dialyzed separately with phosphate buffer saline pH 7.4 [13, 17].

### IN-VITRO STUDIES

### Diffusion study

The in vitro release of rifampicin and from the niosomes formulations were studied by open cylinder method. This diffusion cell apparatus consist of a glass tube with an inner diameter of 2.5 cm, open at both ends. One end of the tube tied with sigma dialysis membrane, which serves as a donor compartment.

### Diffusion Study for Rifampicin Niosomes

Rifampicin niosomes, equivalent to 10 mg of rifampicin was taken in this compartment and placed in a breaker containing 400 ml of phosphate buffer saline pH 7.4 stirred at moderate speed, maintaining the room temperature. Periodically 5 ml of samples were withdrawn and after each withdrawal same volume of medium was replaced. Then the samples were assayed by UV Spectrophotometrically at 475 nm using phosphate buffer saline pH 7.4 as Blank [16].

### RESULTS AND DISCUSSION:

Encapsulation of a drug in vesicular structures can be predicated to prolong the existence of the drug in the systemic circulation and thus enhance
penetration into target tissue and perhaps reduce toxicity if selective uptake can be achieved.

Niosomes have a relatively long half-life in blood circulation and show an altered biodistribution in vivo [18]. Hence niosomes of rifampicin, were formulated, evaluated, studied and concluded that long circulation time of the formulation accounts for its superior therapeutic effectiveness.

Niosomes of rifampicin were prepared by lipid hydration technique using Rotary Flash Evaporator. The particle size distribution of niosomes, percentage drug entrapment, vesicle stability and compatibility of drug with excipients were evaluated. Also, the invitro release study using sigma dialysis membrane was evaluated.

Characterization of Niosomes:

Particle Size Analysis of Niosomes:
The niosomes were subjected to microscopic examination (SEM) for characterizing size and shape of Niosomes [11]. Microscopic examination revealed spherical small unilamellar vesicles of 175 ± 12.3 nm size range for rifampicin niosomes of all three formulations (S 1, S 2, S 3) and it is shown in Table 3 and photo graph of optimized formulation (S 3) based on invitro release were given in Figure I remaining two formulations (S 1 and S 2) were disclosed.

| S. No. | Product Name | Size of Niosomes (nm) |
|--------|--------------|----------------------|
|       | S 3          | 175 ± 12.3           |

Table 3: Particle Size Distribution of Formulated Niosomes

Entrapment Efficiency:
The entrapment efficiency of rifampicin niosomes using various molar ratios of 10:10:1 (S1); 15:15:1 (S2) and 20:20:1 (S3) were measured. The percentage entrapment of rifampicin niosomes was found to be 66%, 68% and 73% respectively and it is shown in Table 4. The Graph 5 shows that the higher entrapment efficiency present in the rifampicin niosomes using 20:20:1 molar ratio (S 3). Increasing the sonication time resulted into reduction in percent drug entrapment; the decrease in percent drug entrapment is due to leakage of the drug during sonication. Cholesterol provides endurance against mechanical strain during sonication and centrifugation.

Sonication brings about size reduction by breaking large niosomes to smaller ones and in doing so, leakage of small quantities of drug from the niosomes occur. Hence the sonication time was optimized to 15 min, and further reduction in the size by increasing sonication time was not attempted.

Stability studies:
The stability studies of optimized (S 3) niosomal formulation were carried out at refrigeration temperature (4º C), room temperature and at elevated temperature (45º C). Leakage of the drug from the prepared niosomes were analyzed in times of percent drug retained on storage under refrigerated condition showed promising results of 94% of the drug retained after 3 months. At room temperature the percentage retained after 3 months was 81% and at 45º C the percentage of drug retained was 59%. These results showed that the formulations were found to have more stable at refrigeration temperature, where as good stability at room temperature and the drug degradation increased at 45º C. It is shown degradation increased at 45º C. It is shown in the Table 5 and Graph 6.

Stability data clearly indicates that niosomes encapsulation gives protective effect at refrigerated condition. It is observed that at higher temperature, rate of degradation is higher. This may be due to the presence of lipid materials like cholesterol.

Invitro Release Study:
The invitro release study is carried by diffusion method using sigma dialysis membrane as a barrier. From this study we evaluated the percentage of drug diffused in the medium. The percentage drug diffused in the rifampicin niosomes at various molar ratio of 10:10:1 (S1); 15:15:1 (S2) and 20:20:1 (S3) were 95.47%, at the end of 17 hours, 96.53% at the end of 17 hours and 98.13% at the end of 17 hours, respectively. It is shown in the Tables 6 and Graph I, II, III.

The results showed that rifampicin niosomes with 20:20:1 (S3) having highest entrapment and maximum longevity of release. The comparative values are shown in the Graph IV.

CONCLUSION:
The report describes the efficiency of stealth niosomes to control the release for a prolonged period of time and their ability to maintain rigidity throughout their stability studies offers improved storage and shelf life [9, 10].

These are significant findings that further support the objective that appropriate niosomal formulations can be used as the means of better treatment.

These findings are encouraging and demonstrative that niosomal technology can be safely used in antimicrobial therapy.
### Table 4: Percentage Drug Entrapment of Formulated Niosomes

| Formulations | Trial 1 | Trial 2 | Trial 3 | Mean ± SD, n = 3 |
|--------------|--------|--------|--------|-----------------|
| S 1          | 66.66  | 66.71  | 66.51  | 66.62 ± 0.1     |
| S 2          | 68.54  | 68.58  | 67.70  | 68.27 ± 0.49    |
| S 3          | 72.53  | 73.65  | 73.67  | 73.28 ± 0.65    |

### Table 5 Drug Content during Stability Study of S 3 Formulation: (n=3)

| Temperature | Initial | I | II | III |
|-------------|---------|---|----|-----|
| 4°C         | 100     | 99 | 96 | 94  |
| Room Temp.  | 100     | 96 | 88 | 81  |
| 45°C        | 100     | 92 | 75 | 59  |

### Table 6 Comparative *In vitro* Release Study of Rifampicin Niosomes: (n=3)

| Time in (hrs) | S1 | S2 | S3 |
|---------------|----|----|----|
| 1             | 9.60 | 10.67 | 12.80 |
| 2             | 15.47 | 17.60 | 19.20 |
| 3             | 19.73 | 22.40 | 25.07 |
| 4             | 26.13 | 28.27 | 29.87 |
| 5             | 32.53 | 34.67 | 35.73 |
| 6             | 37.33 | 40.53 | 41.60 |
| 7             | 43.20 | 45.33 | 45.87 |
| 8             | 48.00 | 49.07 | 51.73 |
| 9             | 52.80 | 56.00 | 58.13 |
| 10            | 62.40 | 63.47 | 65.07 |
| 11            | 68.27 | 69.87 | 72.00 |
| 12            | 72.53 | 73.60 | 77.33 |
| 13            | 77.33 | 79.47 | 83.20 |
| 14            | 82.13 | 84.80 | 86.93 |
| 15            | 88.00 | 89.60 | 90.67 |
| 16            | 91.73 | 94.40 | 93.87 |
| 17            | 95.47 | 96.53 | 98.13 |
Graph I: Standard Curve of Rifampicin

Graph II: Percentage of Drug Entrapment

Graph III: Amount of Drug (%) retained after months

Graph IV: Invitro Release Study of S1
Comparative *In vitro* Release Study of Rifampicin Niosomes S 1, S 2, and S 3

Graph VII: Comparative *In vitro* Release of Rifampicin Niosomes

Figure I: Photo graph of optimized formulation (S 3)
With the addition of the results presented in this report, it seems apparent that niosomal technology can offer an alternative if not a better solution for improving antimicrobial therapy.

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