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FORMULATION AND EVALUATION OF RIFAMPICIN AND GATIFLOXACIN NIOSOMES ON LOGARITHMIC-PHASE CULTURES OF *MYCOBACTERIUM TUBERCULOSIS*

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ABSTRACT

Niosomes are vesicles mainly consisting of non-ionic surfactants that encloses and encompasses the drug molecules. Niosomes of rifampicin and gatifloxacin were prepared by lipid hydration technique using rotary flash evaporator. The prepared rifampicin and gatifloxacin niosomes showed a vesicle size in the range of 100-300nm, the entrapment efficiency were 73% and 70% respectively. The *invitro* release study showed that 98.98% and 97.74% of release of rifampicin and gatifloxacin niosomes respectively. The bactericidal activities of the niosomal formulation were studied by BACTEC radiometric method using the resistant strains (RF 8554) and sensitive strains (H37Rv) of *Mycobacterium tuberculosis* which showed greater inhibition and reduced growth index.

KEY WORDS

Niosomes, Rifampicin, Gatifloxacin, BACTEC, *Mycobacterium tuberculosis*.

INTRODUCTION

Tuberculosis (TB) is an important public health problem; about two billion people (one third of the world's population) were infected with TB[1]. The number of new cases each year was raising, mainly as a result of the increasing burden of HIV infection. Rifampicin used as first-line drug for the treatment of tuberculosis, showed various side effects such as immunological disturbances, rheumatoid or lupoid syndrome, allergic rashes, eosinophilia and other hepatotoxic manifestations. Fluroquinolones were novel anti-TB drugs to be used when a patient is infected with a Multidrug Resistant - Tuberculosis (MDR-TB) strain[2]. Gatifloxacin is a synthetic broad spectrum 8-methoxy quinolone anti bacterial agent, active against both typical and atypical bacterial respiratory pathogens. Drug delivery through niosomes was one of the approaches to improve the therapeutic performance of drug and their distribution in body. Niosomes may reside in lung due to alveolar and effect of alveolar phagocytic cells with small sized vesicles, which can pass through small capillaries[3]. Niosomes were taken up by liver and breaks down substantially to release the free drug which eventually re-enters the circulation and maintain the plasma drug level. Rifampicin and gatifloxacin niosomes showed extended release of the drug which suffices to decreased dose, lesser days of treatment. The duration of treatment under DOTS strategy may also be reduced to the greater extent thereby improves patient compliance.

MATERIAL AND METHODS

Rifampicin was obtained as a gift sample from Lupin pharmaceuticals. Gatifloxacin was obtained as a gift sample from Panacea Biotech Ltd. Surfactants such as Span & Tween, Dicetyl phosphate and sigma dialysis membrane obtained from Sigma Aldrich. Cholesterol 'Excela R' was obtained from Qualigens. All other chemicals used were of analytical grade, obtained from local suppliers.

(i) Preparation of Niosomes :

To prepare niosomes, the method followed was a slight modification of the procedure previously adopted by Chandraprakash[4] et al., and Raja Naresh[5] et al. Rifampicin and gatifloxacin niosomes were prepared by using surfactant, cholesterol and dicetyl phosphate in the ratio of 47.5: 47.5: 5 and dissolved in diethyl ether (10-15ml). The solvent was evaporated under reduced pressure at a temperature of about 60°C using a rotary flask evaporator, leaving a thin layer of solid mixture deposited on the wall of the round bottom flask. The 10ml of 1mg/ml drug solution was added to the flask heated to about 50°C on the water bath on a vortex, until a good dispersion of the mixture was obtained. The suspension was cooled in ice bath and was sonicated using probe sonicator to form unilamellar niosomes.

CHARACTERIZATION OF NIOSOMES

(i) Particle Size Measurement:

The particle sizes of prepared niosomes were analysed by Atomic Force Microscope (AFM). For this study, approximately 2-5 ml of the filtered niosomes were mounted on the cover glass and allowed to dry in air. This was mounted directly on the specimen metal disc using double adhesive tape. Samples were scanned at different areas using Shimadzu SPM 9500-2J. Atomic force microscope employed for high resolution, contact mode micro cantilever 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 AFM photographs, the mean particle diameter analysis was determined by the standard scale value of AFM.

(ii) Drug Entrapment Efficiency:

The entrapment efficiency was determined by using the direct method[6]. The detergents were used to break the vesicles; 1ml of 2.5% Sodium Lauryl Sulphate solution (SLS dissolved in phosphated buffer saline) was added to 0.1 ml of noisome which was incubated at 37^0C for 1.5 hours to complete the break up of the niosome and to release the entrapped material. The sample was filtered through a millipore membrane filter (0.25 µm), and the filterate was measured at suitable wavelength (for Rifampicin 475 nm, Gatifloxacin 394 nm). The amount of drug was derived from the calibration curve.

(iii) Compatibility Study:

The stability of a formulation depends upon the compatibility of the drug with the excipients. Differential scanning calorimetry is a fast and reliable method to screen drug-excipient compatibility and provide maximum interactions. In this study Differential Scanning calorimeter (Perkin-Elmer DSC-7) was used for analysis.

STABILITY STUDIES

Freshly prepared niosomal formulations were divided into 3 groups and one group was kept at 4^0C, second group at room temperature and third stored at 40^0C and 75% RH[7]. Every week 0.1ml of the niosome was withdrawn and dialysed separately with phosphate buffer saline pH 7.4.

IN-VITRO STUDIES

(i) Diffusion Study:

The *in-vitro* release of rifampicin and gatifloxacin from the niosome formulations were studied by open ended 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[8].
Rifampicin and gatifloxacin niosomes, equivalent to 10mg of drug was taken in this compartment and placed in a beaker containing 250 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. The samples were assayed by UV Spectrophotometer at 475nm for rifampicin niosomes and at 394nm for gatifloxacin niosomes using phosphate buffer saline pH 7.4 as blank[9].

BACTERIOLOGICAL STUDY

(i) BACTEC radiometric method[10, 11]:
Inoculating 0.1 ml of broth culture of the strains (H37 Rv & RF 8554) from 12B medium of Growth Index (GI) 300-500 in all drug containing 12B medium. The same broth was diluted 1/100 times with diluting fluid, and 0.1 ml of this diluted sample was inoculated in the drug free medium and incubated at 37^0C. Drugs were added to 12B medium to give final concentration as single drug with and without niosome. The readings were taken for all the vials daily for 5-7 days. When the reading of the control vial reached more than a GI of 30, then the ΔGI of the control reading was compared with the ΔGI of the test medium[11,12] (ΔGI is the difference between two consecutive days readings).

RESULT AND DISCUSSION

Niosomes of rifampicin and gatifloxacin were prepared by lipid hydration technique using rotary flash evaporator. The niosomes were subjected to microscopic examination (AFM) for characterizing size and shape of Niosomes. Microscopic examination revealed, spherical small unilamellar vesicles of 100-300 nm size range for rifampicin niosome (RIF-S60) and 150-350nm size range for gatifloxacin niosome (GAT – T40) are given in Fig. 1&2.

Fig.1
AFM Photograph of Rifampicin Niosomes

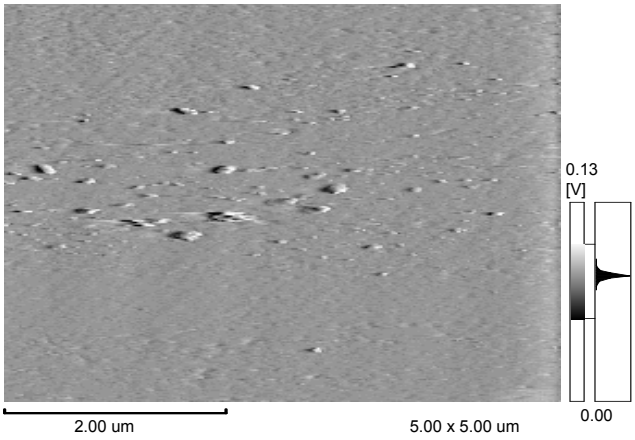
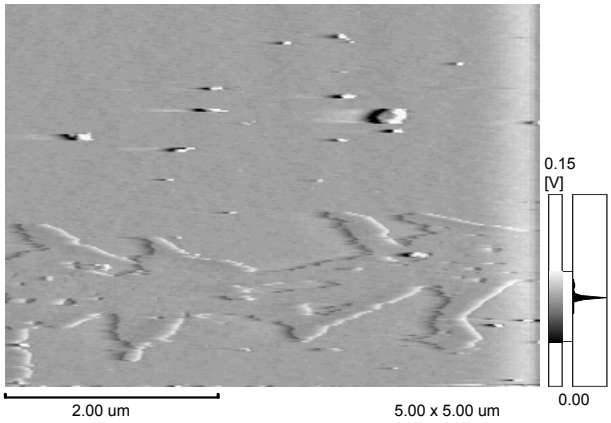
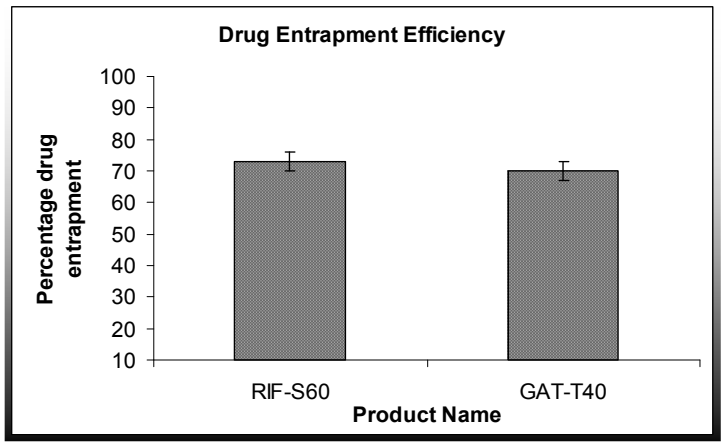


Fig.2
AFM Photograph of Gatifloxacin Niosomes



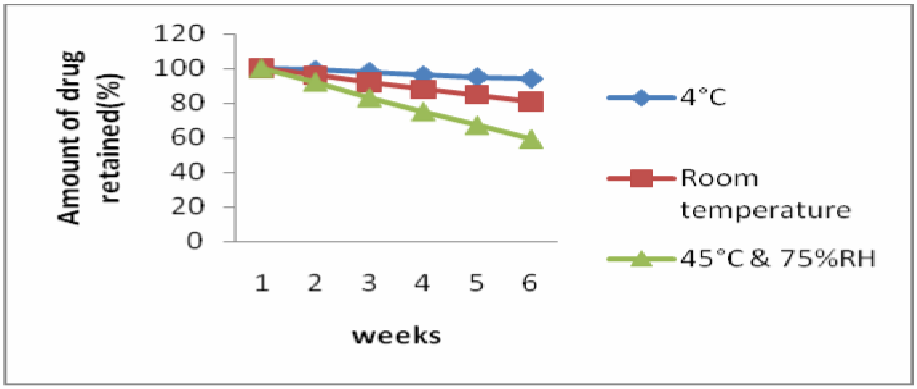
The percentage entrapment of rifampicin niosomes (RIF – S60) and gatifloxacin niosomes (GAT-T40) were 73% & 70% respectively. It is shown in the Fig 3.

Fig.3
Drug Entrapment Efficiency



The stability studies of Niosomal formulation were carried out at refrigeration temperature (4^0C), room temperature and 45°C and 75% RH. Leakage of the drug from the prepared niosomes was analyzed in terms of percent drug retained and storage under refrigerated condition showed promising results of 94% of the drug retained after 5 weeks. At room temperature the percentage retained after 5 weeks was 81% and at 45^0C and 75% RH 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 was observed at room temperature and the drug degradation was increased at 45^0C. It is shown that degradation was increased at 45^0C and 75% RH. It is shown in the Fig 4.

Fig.4
Stability Studies



The DSC thermograms of rifampicin, gatifloxacin and 1:1 drug-excipients physical mixtures were obtained and compared. In this study DSC revealed the compatibility of rifampicin with Span 60 and cholesterol, gatifloxacin with Tween 40 and cholesterol were compatible with the drug.

The *invitro* release study was carried by diffusion method using sigma dialysis membrane as a barrier. The percentage drug diffused in the rifampicin niosomes using Span 60 (RIF – S60) were 98.98% at the end of 16 hours. The percentage drug diffused in gatifloxacin niosomes using Tween 40 (GAT – T40) were 97.74% at the end of the 14 hours. After that the steady state of release were obtained. It is shown in the Fig 5&6.

Fig. 5
Invitro release study for RIF-S60

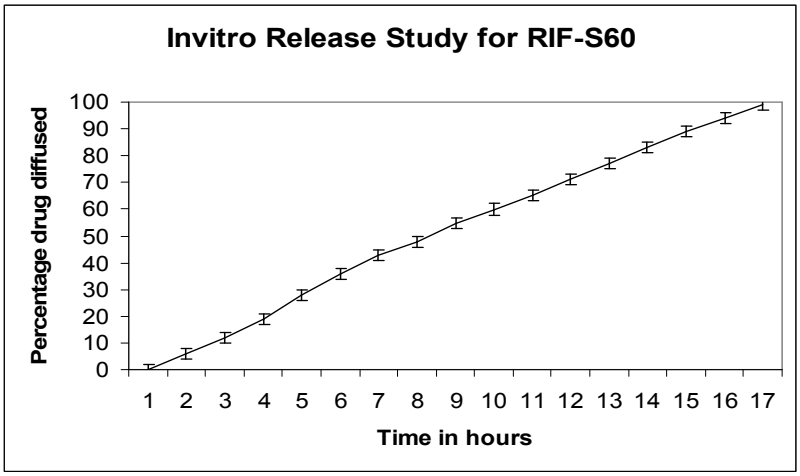
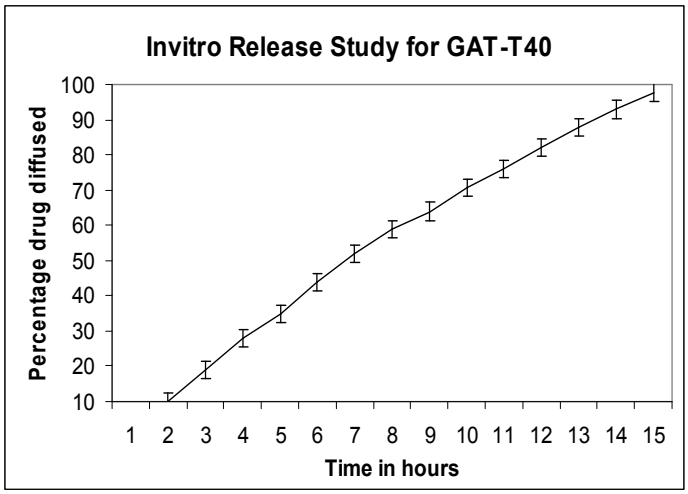


Fig. 6
***Invitro* release study for GAT-T40**



The Bactericidal Activity of the drugs (Rifampicin and Gatifloxacin) and in niosomal formulations (RIF-S60 and GAT-T40) were studied by BACTEC radiometric method using the resistant strains (RF 8554) and sensitive strains (H37Rv) of *Mycobacterium tuberculosis*.

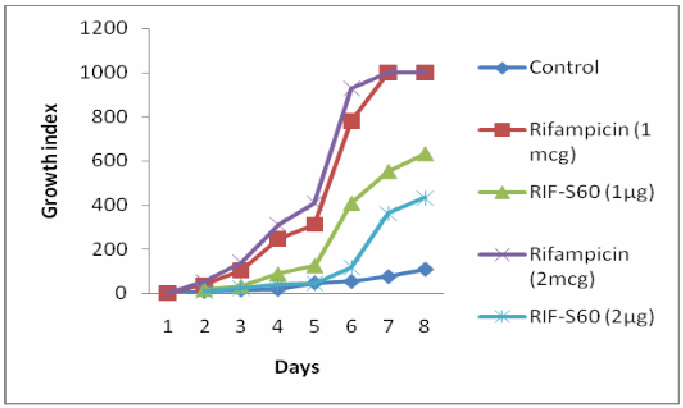
Rifampicin drug 5 mg/5ml solutions were prepared in 0.5ml of DMSO and 4.5 ml of sterile distilled water. From the stock solution 80 µg/ml & 40 µg/ml is prepared, so that 0.1 ml (8 µg/ml) corresponds to 2 µg/ml and 1 µg/ml in 4 ml of BACTEC medium.

Gatifloxacin drug 10mg/10ml solution was prepared in sterile 0.1 N NaOH. Subsequent dilutions were made with sterile distilled water and aseptically added to the BACTEC media to give the desired final concentrations as 0.125 µg/ml and 0.25 µg/ml. Equivalent dilutions were made for rifampicin niosomes and gatifloxacin niosomes separately. The result of Daily Growth Index (GI) readings were taken for 7 days. The control reading of resistant strain (RF 8554) of *M. tuberculosis* showed a marked multiplication from the 4^{th} day onwards.

Rifampicin (1µg& 2µg) and Gatifloxacin (0.125µg and 0.25µg) drug showed that the strain is resistant to the drug and reduced the activity by the increased Growth Index and attained a maximum of 999 on 6^{th} day.

Fig.7

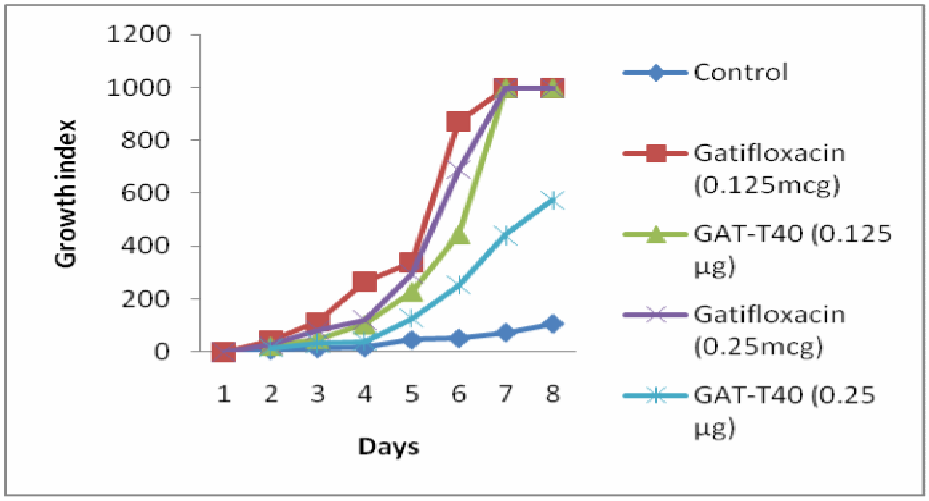
Comparative Bactericidal activity of Rifampicin (1µg& 2µg) and RIF-S60 (1µg& 2µg) using Resistant strain (RF 8554) of M. tuberculosis



Rifampicin niosomes (RIFS60) (1µg& 2µg) and gatifloxacin niosomes (GAT-T40) (0.125µg and 0.25µg) showed that the Growth Index were increasing uniformly for 5 days and it reached a maximum of 999 indicating the resistance, but greater activity for niosomes were shown with relatively less Growth Index and reached a maximum of 999 on the 6th day, which was equal to the drug alone. (Fig. 7 and 8)

Fig.8

Comparative Bactericidal activity of Gatifloxacin (0.125µg and 0.25µg) and GAT-T40 (0.125µg and 0.25µg) using Resistant strain (RF 8554) of M. tuberculosis



For sensitive strain H37Rv same concentrations of pure drug and niosomes were used in BACTEC medium. (Fig. 9 and 10) It also showed that the formulations of niosomes having greater inhibition and reduced Growth Index than the drug alone for sensitive strain.

Fig.9

Comparative Bactericidal activity of Rifampicin (1µg& 2µg) and RIF-S60 (1µg& 2µg) using Sensitive strain (H37Rv) of M. tuberculosis

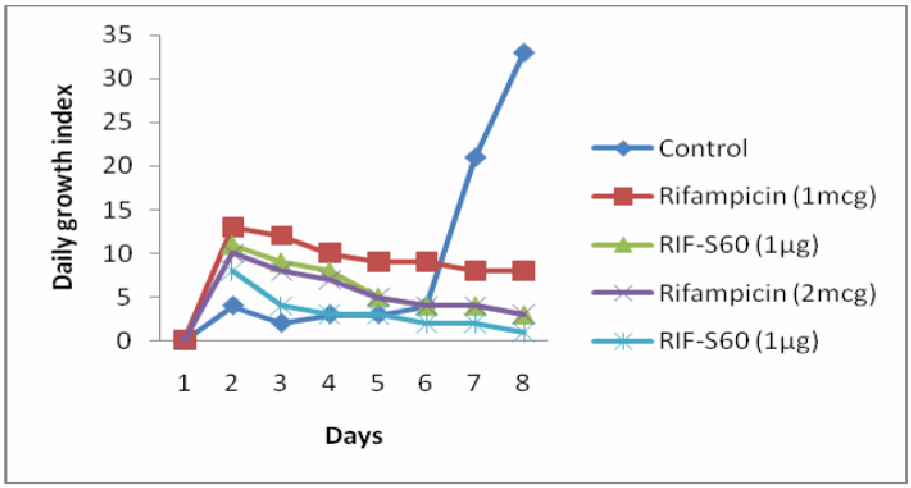
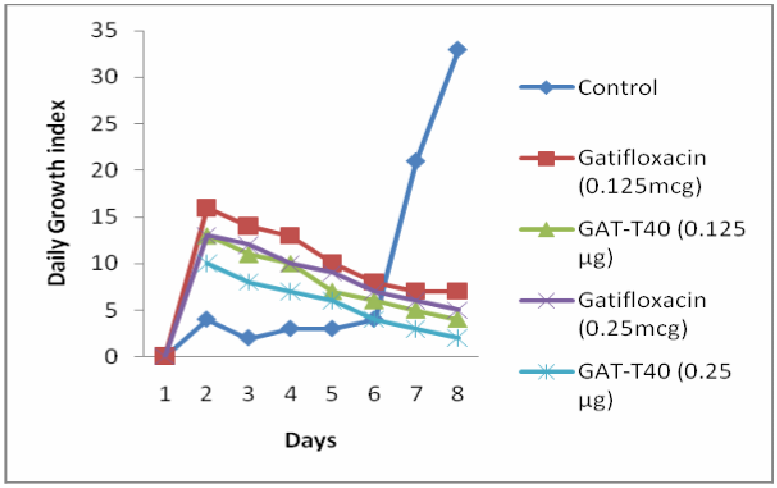


Fig.10

Comparative Bactericidal activity of Gatifloxacin (0.125µg and 0.25µg) and GAT-T40 (0.125µg and 0.25µg) using Sensitive strain (H37RV) of M. tuberculosis



CONCLUSION

The study showed that the drugs loaded in niosome vesicles exhibited improved bactericidal activity against the tubercle bacilli. The diffusion study of the rifampicin niosome and gatifloxacin niosomes gave extended release of the drug, which suffices to decreased dose, lesser days of treatment and more patient compliance. The duration of treatment under DOTS strategy may also be reduced to the greater extent.

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