Toxicity and drug resistance are major obstacles in the therapy of tuberculosis. Tuberculosis an intracellular bacterial parasitic disease common in rural areas of developing countries, affecting around 10 million people caused by (*Mycobacterial tuberculai)* and is highly prevalent in many parts of Northern and Eastern India. The disease is endemic in several states, i.e. Assam, Bihar, Bengal, Sikkim and some other parts of Southern region of the country The major hindrance to obtain the potential drug delivery system in order to eradicate tuberculosis parasite is its ability to rapidly replicate in different macrophage cells. Development of drug resistant strains causes the delivery system to be modest and short-lived, having relatively weaker potency, rapid development of resistance; and the toxicities of drugs. Therefore, it is required to develop such delivery system, which facilitates the targeting of drugs to the infected sites.

The newer approach of drug delivery is to deliver drug into systemic circulation at a predetermined rate. The primary objectives of controlled drug delivery are to ensure safety and to improve efficacy of drugs as well as patient compliance. This is achieved by better control of plasma drug levels and less frequent dosing. The impetus for the development of newer novel drug delivery system apart from therapeutic efficiency is cost. The development cost of a new drug may be about $250 million (Rs. 1250 Crores) and takes about 12 years to reach the market place. Whereas an existing drug molecule can get a second life with newer drug delivery system that can be developed in half of the time with 20% cost of the new drug discovery. In the recent past, controlled release concept and technology have received increasing attention in the face of growing awareness to toxicity and ineffective drug administration in the form of tablets, capsules, injectables and ointments etc. which usually produce fluctuations in drug concentration in the blood stream and tissues and consequently undesirable toxicity and poor efficiency. This as well as other factors such as repetitive dosing and unpredictable absorption led to the concept of the controlled drug delivery system or therapeutic system.

Many carrier systems such as nanoparticles, resealed erythrocytes, microspheres, liposomes as well as microemulsion have been utilized to prolong the circulation time of certain molecules, to deliver them at the appropriate sites, and to protect them from degradation in the plasma. Nano Lipid Carrier (NLC)s offer the potential to deliver drugs to desired targets within the body and to reduce systemic toxicity. By incorporating drugs into (NLC)s, the toxicity of the drug also reduced (patients may experience fewer side effects), the therapeutic efficacy or potency of the drug may stay the same or get better.

The localization of (NLC)s in organs rich in macrophage cells such as liver, spleen, lungs and kidney may provide a mechanism for improving the therapeutic index of anti-tubercular agents. One particular method exploits the mechanism of sugar recognition that specific cell types possess. Protein-carbohydrate interactions mediate critical biological recognition processes, such as those involved in cell signaling, organogenesis, fertilization, and inflammation. Additionally, carbohydrate-protein interactions facilitate the initial attachment of pathogens to host cells. Macrophage of RES is important host for parasite and plays a key role in the pathogenesis of Tuberculosis by providing long-term reservoirs for the parasite. As Nano Lipid Carrier (NLC)s are preferentially taken up by cells such as macrophages, monocytes and lymphocytes, their use as carriers of anti-tubercular agents represents an interesting approach.

Coating the (NLC)s formulation with charge inducer e.g. Sterile Amine or using surfactant would efficiently target the Nano Lipid Carrier (NLC)s to the site of action. This modification can enhance (NLC)s specificity and reduce the undesired delivery associated with toxic effects. Targeting specific tissues by altering the physical properties of Nano Lipid Carrier (NLC)s can also be combined for optimum targeting outcomes.

Rifabutin was selected for the present study because of the several limitations associated with these drugs. The various adverse effects such as toxicity, peripheral neuropathy, headache, nervousness, diarrhea, nausea, vomiting, nephrotoxicity, hepatotoxicity, weight loss and myopathy etc., are associated with the use of RIF. Hence it is aimed to design such Rifabutin (RIF) delivery system, which could reduce above mentioned side effects.

The samples of drug was identified and characterized as per requirement of official compendia. The absorption maxima were found to be maximum at 242.0 nm in PBS pH 7.4. The obtained data i.e. concentration *vs.* absorbance were linearly regressed and equation of line was determined. The estimation procedure was found to be fairly reproducible and sensitive for the concentration range 10-100 μg/mL. Correlation coefficient value was observed near 0.98, which indicated that drug follow Beer-Lambert law in the concentration range 10-100 μg/mL. The functional groups in the drug samples were identified by infrared spectra, which were in agreement with the standard spectrum. Drug was insoluble in PBS, Distilled water, and soluble in Benzene, Chloroform, Methanol and Diethyl Ether. The selected drug i.e. Rifabutin was identified by the methods reported in the official books. Standard curve of Rifabutin was prepared in PBS at 242.0 nm. This was subsequently

subjected to linear regression. HPLC method was developed and all parameters found according to ICH guidelines.

Drug loaded Nano Lipid Carrier (NLC)s were prepared by Ether Injection method using Capmul MCM EP, Capmul MCM C-8, Precirol ATO-5 and Poloxamer 188. The screening and optimisation of selected defined variables and dependent response on the bases of design experiment by using the Minitab-17 software. Screening process has been done through Placket barman design significant factors were selected for the further study. In this study four factors are screened out as a selected factors i.e. Homogenization time, quantity of Poloxamer-188, organic & aqueous ratio and ratio of solid lipid & liquid lipid. Mathematical equation was developed for entrapment efficiency and particle size.

At next stage box Behnken design applied from RSM for optimisation of selected variables and determined the value of these variables for this value is further used for preparation of NLCs batch. The optimum value was found with a maximum entrapment efficiency of 67.45±3.40 % and minimum particle size is 111±7.24 nm

The (NLC)s were characterized for shape, size, encapsulation efficiency and in vitro drug release. Shape and size of Nano Lipid Carrier (NLC)s was evaluated by Scanning Electron Microscopy, which revealed the Nano Lipid Carrier (NLC)s to be spherical in shape. Whereas, The increase in size for the Nano Lipid Carrier (NLC)s can be attributed to Entrapment. Particle size is increases with increasing the ratio of Precirol ATO-5 but entrapment efficiency is decreases because long chain of carbon molecule is increasing the rigidity of particle and give the better stability of the particle.

The Entrapment efficiency was determined by method reported by A.Abd-Elbary. It was found to be 67.32±3.3% for NLC capmul MCM C-8 and NLC capmul MCM EP is 61.33±3.0. The Entrapment efficiency is increases when the increasing the Precirol ATO-5 at optimum level after that it was decreases in (NLC)s. This may be tends to aggregation or disruption of Particles. Nano Lipid Carrier (NLC)s may be due to greater membrane integrity of Nano Lipid Carrier (NLC)s and chemical nature of Capmul MCM EP and Capmul MCM C-8 the corresponding lipophillicity of is 4.7 and 6.6 respectively the lower HLB number is more lipophillicity is the compound thus the Capmul MCM EP has highest lipophillicity; therefore the maximum drug was entrapped in Capmul MCM C-8. NLC Capmul MCM C-8 is the best fit combination of capmul mcm C-8 and Precirol ATO-5 ratio it gives the better entrapment and particle size compare to others

The *in-vitro* drug release from Nano Lipid Carrier (NLC)s was studied in PBS (pH 7.4) using dialysis tube. The percentage drug release was found to be 63.17±1.90% for NLC capmul mcm C-8, 79.4±1.72% for NLC capmul mcm C-8 while after 60 hours. The reduction in the drug release for liquid lipid capmul mcm c-8 formulation as compared to capmul mcm c-8 formulation can be attributed to enhancement of membrane integrity and the layer of rigid Precirol ATO-5 on the (NLC)s surface, which have an impact on drug diffusion kinetics.

The lower values of drug release in case of capmul mcm c-8 Nano Lipid Carrier (NLC)s as compared to capmul mcm EP. In-vitro release rate studies revealed that the cumulative % of Rifabutin released was maximum for capmul mcm EP based (NLC)s and minimum for capmul mcm c-8 based (NLC)s, these differences in release rate is assumed to be based on lipophillicity of the capmul mcm c-8. The capmul mcm EP being least lipophillic would provide easy access of released media to the drug; whereas capmul mcm c-8, being relatively lipophillic, impedes the easy permeation to the aqueous phase.

The stability analysis for various (NLC)s formulations was conducted with respect to particle size and residual drug content. The average particle size was found to increase on storage, which was least in case of formulations stored at 4°C than those, stored at 30±2^{o}C and 45±2^{o}C for 06 months. By keeping the initial drug content 100%, percentage of residual drug lasting in (NLC)s exposed that significant percent of drug was lost (4-7%) from the formulation within 06 months, which were stored at 4±2^{o}C only (11-19%) drug was lost from those stored at 30±2^{o}C and (32-40%) drug was also lost those stored at 40±2^{o}C, which could be due to more leaching of the drug from (NLC)s at temperature 40±2^{o}C. Hence, the study favors 4°C as optimum temperature for the storage of (NLC)s formulations efficiently

Ex-vivo cellular uptake studies were carried out in order to explore about the uptake behavior of the (NLC)s by the living cells J744A.1. Study shows the significant structured changed in nucleolus and monocytes, which reflect an increased uptake of (NLC)s. Cell uptake studies were done to measure the cell uptake of NLC capmul mcm c-8 formulation. Uptake of calcein loaded (NLC)s is seen by florescent die technique. Intensity of fluorescence was exhibited maximum. The uptake of the calcein loaded (NLC)s by J744A.1 cell lines were visualized using fluorescence microscopy. The intense cell-associated fluorescence in the sample containing coupled (NLC)s and drug coupled with calcein revealed that calcein loaded (NLC)s formulation was more taken up by the J744A.1 macrophage cell line than that of plain drug as the intensity of fluorescence was shown maximum by the calcein loaded

(NLC)s formulation. Thus based on the above study it can be concluded that (NLC)s can deliver drug to the macrophage in an effective way and hold great potential for RES targeting, in the treatment of Tuberculosis. NLC Capmul MCM C-8 showed better Antitubercular compared to pure drug (Rifabutin) in 60 hours when given by the IV route.

Conclusion

From the results of present this study, it is concluded that the investigated system has potential to remain in the treated site for prolonged periods and is capable of maintaining constant drug concentration through a longer duration of time due to it's sustained action. The pharmacokinetic results were inconclusive in confirming the extent of duration of action the formulation. This can be expected to reduce the frequency of administration and decrease the dose dependent side effects associated with repeated administration of conventional Rifabutin loaded dosage forms, thereby improving patient compliance.

We can reveal that a sustain release Rifabutin formulation with satisfactory release characteristics was successfully prepared with (NLC)s technique.

FUTURE SCOPE

The aging population, the high expectation for better quality of life and the changing lifestyle call for improved, more efficient and affordable health care. In the present study, Rifabutin is used specifically for this model and has been entrapped by (NLC)s particle. For future study, repeated investigation is necessary when using a chosen drug, and depending on a molecular structure, as well as physical and chemical properties of the drug and their respective synthesis techniques, characterization protocols and quantitative measurements may be necessary. The (NLC)s NLC Capmul mcm C-8 (size range 112.12±3.22nm) shows good entrapment, drug release and cell uptake response with extended duration of action. New strategies and opportunities in the design of novel drug delivery system could be adapted today's demands. Different biodegradable polymer could also be used that might increase the drug entrapment. Higher efficacy and minimizing undesirable side effects of the present study may be utilized alone or in combination with other biodegradable polymer to produce novel dosage forms which may make better products.