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Development and Evaluation of Micro-sized Galactose conjugated microspheres for targeting Tuberculosis.

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ABSTRACT: Micro-sized Galactose conjugated microspheres (Ga-MSs) containing Isoniazid (INH) with targeting moieties on their surfaces were prepared by the solvent evaporvation technique. Since galactose is known to interact specifically with the asialoglycoprotein receptor on hepato-cyte, the galactose residues were introduced on the surface of MSs as the targeting moieties for hepatoma through polyethylene glycol (PEG) spacers. Therefore, the objective of the present study was to formulate and characterize microsphere of Isoniazid conjugated with Galactose and investigate in-vitro drug release profile for their potential against tuberculosis. The prepared microsphere were characterize for particle size, surface analysis (SEM) entrapment efficiency, in-vitro drug release and stability studies. The Micro-sized Galactose conjugated microspheres showed promising effects.

Keyword: Galactose, Microsphere, Tuberculosis, particle size, surface analysis.

I. INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by the bacterium called mycobacterium Tuberculosis. Tuberculosis generally affect in lungs. Mycobacterium tuberculosis is a constrained pathogenic bacteria species which belong to the family of mycobacteriaceae first discovered by Robert Koch in 1882.it can form acid stable complexes made up of peptidoglycan.[1-3] Tuberculosis is a transmissible airborne infection. it is transmitted by inhalation of infected droplet nuclei of 1-5 um diameter that remain suspended in the environment when an infectious person coughs,sneezes,laughs or sings .TB is one of the most common causes of death due to an infectious disease and is an important subject of medical research. Studies related to pathology, physiopathology, diagnosis, manifestation, treatment, prognosis, prevention,epidemiology and patient education are the most widely studied issues in TB.TB and high drug doses are required to be administered since only a small fraction of the total dose reaches the lungs after oral administered via the Pulmonary route can avoid the daily dosing, because they would help in; : (i) Direct drug delivery to the diseased organ;(ii) Targeting to alveolar macrophages which are used bythe mycobacterium as a safe site for their prolonged survival;(iii) Reduced systemic toxicity of thedrugs;and(iv) Improved patient compliance(v)Higher drug concentration at the site of infection,in contrast to the moral route of administration, in haled drug are not subjected to first-pass metabolism Isoniazid is the first line medication in prevention and treatment of tuberculosis. It inhibits the synthesis of Mycolicacid required for the mycobacterium cell wall.[4-6] NH is less permeated throughthe stomach and is mainly absorbed through the intestine because it occurs in the protonated form at acidic pH (PKa = 2). Therefore,it can be considered as a good candidate for the development of asite- specific release formulation especially in case of Tuberculosis to deliver it in lung.[11,12] Chitosan is a biodegradable,biocompatible, cationic hydrophilic polymer with low toxicity,mucoadhesive properties, biodegradability and ability to enhance the penetration of large molecules across mucosal surfaces obtained through de-acetylation of naturally occurring chitin. It is also hypo-allergenic and has natural antibacterial properties.[7] The release modifying and muco-adhesive property of chitosan appears to be a good choice for preparing sustained release formulation for lung delivery via inhalation Spontaneous emulsification method is a low energy emulsification method of adding a mixture of surfactant, oil and water miscible solvent into

aqueous phase. [8-9][11]. Suspensions are defined as heterogeneous system consisting of two phases. The continuous (or) semisolid (or) external phase and internal phase (or) dispersed phase which is made up of particulate matter i.e., insoluble in but dispersed throughout the continuous phase. The particle size of dispersed phase ranges from 0.5µm-5µm. To formulate any type of dosage form different excipients are essential apart from active therapeutic agent. The preparation of suspension also requires a Number of excipients (or) formulation additives so as to render it more stable and present it in desired form with desired properties Tubercular drugs is very effective, but is still associated with a number of significant drawbacks.

II. MATERIALS AND METHODS

2.1 Materials

Isoniazid (INH) was purchased from Sigma Aldrich, USA; N-Dicyclohexylcarbodiimide (DCC), and N-hydroxysuccinimide (NHS), were purchased from Sigma-Aldrich, India, and D-galactose was purchased from Hi Media Laboratories. Phosphate-buffered saline (PBS) (pH 7.4) was used for drug release. All other reagents used were of analytical grade.

2.2 Preparation of Isoniazid loaded microspheres [11][13,14][22]

Microspheres are small spherical particles with diameters in the micrometer range (typically 1 µm to 1000µm (1mm)). Microspheres are sometimes referred to as microparticles. Microspheres can be manufactured from various natural and synthetic materials. Microspheres were made by Solvent Evaporation Method. Galactose as a ligand was attached to the prepared micropsheres using EDC-NHS linkers. All the ingredient were weighed. 1.5 gm of Ethyl cellulose were dissolve in 15 ml of ethyl acetate and 5 ml of Acetone in 100 ml beaker. In another beaker 0.5 ml of Tween 80 and 500 mg drug was taken and dissolve in 300 ml of distilled water. With continues stirring at 700 RPM. Then solution of Ethyl cellulose were into the water phase by drop by drop wise mixing of after 40-50 min with continues stirring. The resulting oil-in-water **(**O/W) emulsion was stirred gently at room temperature with a magnetic stirrer for at least 4 h to evaporate the organic solvent.

2.3 Microsphere size and morphology[14,17]

The size of IZD microspheres with and without Isoniazid was measured by Optical microscope and the morphology of these microspheres was studied by scanning electron microscopy.

2.4 Fourier transform infrared spectroscopy (FTIR)

Pure drug of Isoniazid, Galactose conjugated microspheres with Isoniazid were examined by FTIR, using a spectrophotometer model ATR-FTIR Perkin-Elmer 100S. Samples were taken in a KBr pellet, and scanned in the IR range from 600 to 4000 cm–1.

2.5 Differential scanning calorimetry (DSC)

Pure drug of Isoniazid, Galactose conjugated microspheres with Isoniazid (Ga-MSs) were examined by DSC using DSC-60, Shimadzu. The instrument consists of the calorimeter (DSC 60), flow controller (FCL 60) and thermal analyzer (TA 60). It was operated by software TA-60 from Shimadzu Corporation, Japan. The sample was placed in a sealed aluminum pan, and then it was heated under nitrogen flow (30 ml/min) at a scanning rate of 5°C/min from 30 °C to 300 °C. Reference was the empty aluminum pan without the sample. The heatflow as function of temperature was measured for both the pure drugs and dug-excipient mixture

2.6 Efficiency of encapsulation

The entrapment efficiency (EE) and drug loading (DL) were determined by the amounts of galactose conjugated microspheres were dispersed in phosphate buffer solution (pH 7.4) containing 0.2ml tween 80. Then sampled were vortexed for 5 min and dissolve with the non-conjugated microspheres. The dispersion was centrifuged at 15 000 rpm for 30 min by centrifuge machine. The solution was filtered through filter paper. 0.1ml of this solution was diluted up to 10ml with phosphate buffer (pH-7.4) solution and the absorbance was measured spectrophotometrically at 255.6 nm against phosphate buffer (pH-7.4) solution as blank with the help of UV Shimadzu 1800 spectrophotometer.

2.7 Release of Isoniazid in-vitro [16-18]

About 10 mg of galactose conjugated microspheres (Ga-MSs) were taken in dialysis bag (10000 M.W. cut off). This dialysis bag was transferred to a vial containing 5 ml of phosphate buffer (pH 7.4; 100 mM) and the beaker was protected from light and kept horizontally on a shaking water bath rotating at 100 rpm for 48 hours. An aliquot (1 ml) of the release medium was withdrawn at predetermined interval time points and volume withdrawn was replaced with fresh

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release medium for 6 hr. Drug content in samples was analyzed by UV Spectrophotometer at 255.6 nm wavelength of Isoniazid as standard drug.

2.8. Statistical analysis [17][22]

Data are shown as means ± standard deviation (n = 5). Statistical data were analyzed by the Student's t-test at the level of P = 0.05.

III. RESULT

3.1 Morphological analysis and average size of microspheres

Scanning electron microscopy (SEM) has been extensively used to examine the morphology and size of polymer-based microspheres. Figures 1 show SEM images, respectively, In all cases, the microspheres were spherical and no drug crystals were observed. In the present study, the diameters of the Isoniazid based microspheres and galactose conjugated microspheres of Isoniazid were (in µm): 12.3 ± 1.5 and 15.4 ± 1.3 (mean ± SD; n=3), respectively, as determined by Optical microscopy i.e., well below the 250 µm limit indicated above.

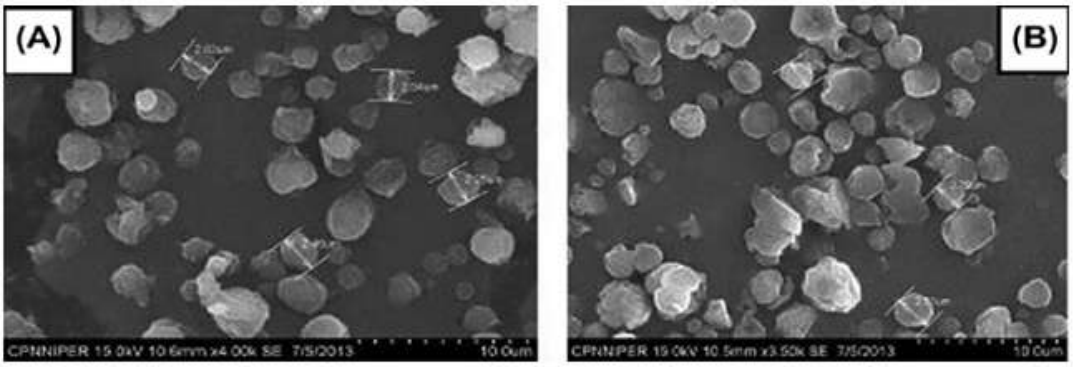

Figure 1: SEM Photomicrographs of galactose conjugated microspheres of Isoniazid (Ga-MSs).

3.2 Drug state in the microspheres

DSC is useful in the investigation of solid state interactions. DSC was performed using DSC-60, Shimadzu, Japan. DSC has been used to investigate drug-polymer interactions in microparticles and can provide information on the melting temperature, crystallization temperature, glass transition temperature, melting enthalpy, enthalpy of crystallization and degree of crystallinity. Figure 2 and 3 shows the DSC curves pure isoniazid, galactose conjugated microspheres of Isoniazid. The incorporation of isoniazid into the microspheres did not alter the thermal properties of galactose and ethylcellulose, as shown by the glass transition temperature (Tg) of the polymer ethylcellulose at 33-40 ℃.

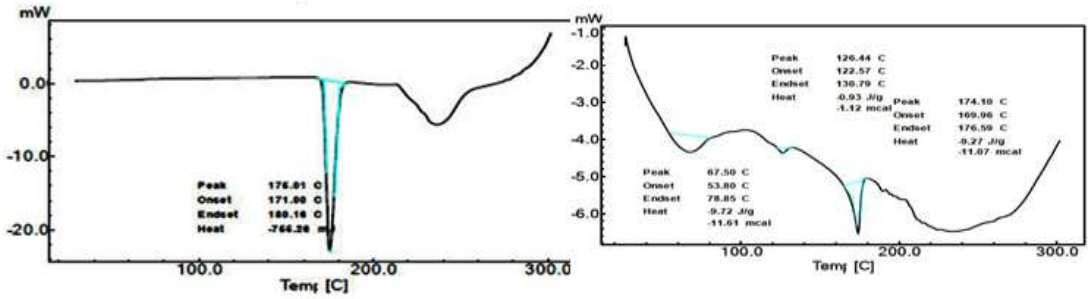

Figure 2: DSC spectra of Isoniazid

Figure 3: DSC spectra of Isoniazid with Galactose

3.3 Fourier infrared transmission spectroscopy (FTIR)

FTIR spectroscopy is widely used by researchers to verify the chemical characteristics of drug and polymer used in the preparation of polymeric microspheres. The FTIR spectrum of Isoniazid and galactose conjugated microsphere loaded Isoniazid (GLMI) is shown in Figure 4 A and Figure 4 B shows several of the characteristic functional group bands. The main bands are the

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carbonyl C=O; amino group NH2 ; and N–N single bond, C=C double bonds, and a C–H bond of the aromatic ring. The GLMI have characteristic infrared bands from 3,000 to 3,400 cm−1 due to the OH vibration. The GLMI contain almost all of the bands of the pure drug and the GLMI, with a slight shift in the wave numbers of certain peaks due to the drug interaction with the inorganic hosts. Such a shift can be observed for the carbonyl band, which is shifted to 1,600 from 1,660 cm−1 in galactose conjugated microspheres.

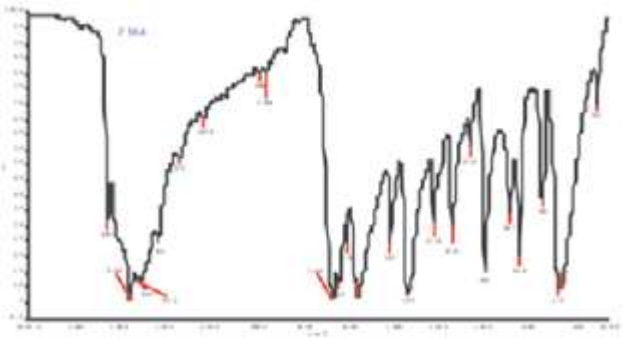

Figure 4 A : FTIR spectrum of Isoniazid

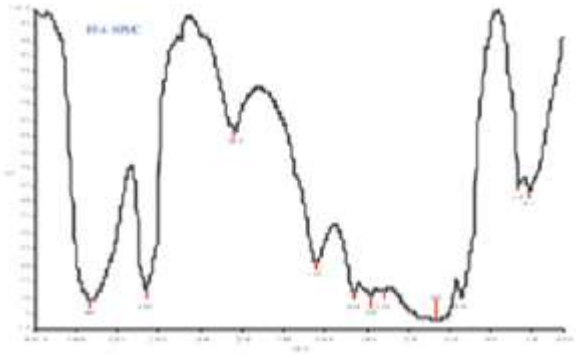

Figure 4 B : FTIR spectrum of galactose conjugated microsphere loaded Isoniazid (Ga-MSs).

3.4 Efficiency of encapsulation

The %EE of the prepared microspheres was found to increase with increase in drug/polymer ratio (Figure 5). The factors affecting particle size also influences %EE. It ranged from 46.30 to 82.38% for galactose conjugated microspheres (Ga-MSs). This could be recognized to more amount of drug present in the internal phase, enhanced viscosity of the medium, large particle size and fast solidification of the formed micro-particles. The increase in %EE was better of galactose conjugated microspheres.

3.5 Drug release in vitro [17][23]

The cumulative percent of drug released from microspheres in phosphate buffer pH 7.4 are shown in Figure 6. The drug release pattern showed from galactose conjugated microspheres was better. Although both series showed drug release extended up to 12 h, controlled drug release with less than 80% of drug release in 12 h. From it was found that microspheres with drug/ polymer ratio 1:1 showed optimum response in terms of in vitro drug release and as well as EE.

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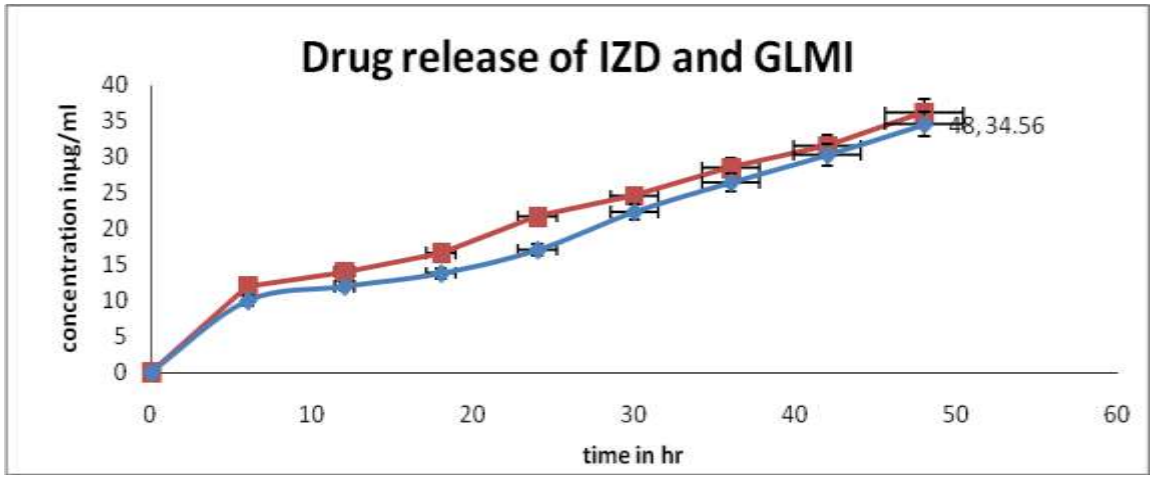

Figure.5. In vitro drug release of pure drug Isoniazid (Blue line) and Galactose conjugated microsphere loaded Isonizaid (Red Line) in phosphate buffer pH 7.4 at 255.6 nm. In vitro drug release of microspheres is expressed as mean ± S.D

Statistical analysis

Data are shown as means ± standard deviation (n = 5). Statistical data were analyzed by the Student's t-test at the level of P = 0.05.

IV. CONCLUSION

In this research, Galactose conjugated micropsheres containing antituberculosis agent Isoniazid were successfully prepared by Solvent Evaporation Method . The entrapment efficiency was higher in MSs. The use of Galactose in MSs as liquid was found to be advantageous in entrapment efficiency. The in vitro release of Isonizaid loaded MSs was time dependent So in Future it can help to formulate Galactose conjugated Isoniazide loaded MSs showed good targetibilty for the treatment of Tuberculosis.

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