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Development of respirable rifampicin-loaded nano-lipomer composites by microemulsion-spray drying for pulmonary delivery

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ABSTRACT

The purpose of this study was to develop respirable rifampicin (RIF) loaded nano-lipomer (lipid/polymer) composites for pulmonary delivery to increase the availability of drug at local site, and enhance the residence time of drug in the lungs and thereby improving the therapy. Rifampicin-loaded nano-lipid polymer (nano-lipomer) composites were prepared by a microemulsion-spray dry technique and were characterized for particle size, thermal stability, polymorphic transitions and chemical integrity. Furthermore, the lipomer were screened for their surface morphology and *in vitro* rifampicin release behavior in simulated lung fluid (artificial lysosomal fluid (ALF) at pH 4.5 and Gamble's solution at a pH of 7.4) represent different interstitial environment in the lung. The particle sizes of the lipomer were ranged between 382.5 ± 6.033 to 561.8 ± 4.965 nm with a narrow polydispersity index (0.315 ± 0.023 to 0.424 ± 0.033) and zeta potential (−32.5 ± 1.206 to −26.5 ± 1.211 mV). Rifampicin entrapment efficiency was between 61.25 ± 1.049 to 73.14 ± 1.048% and SEM images revealed well-separated, sphere-shaped and smooth surface lipomer. DSC and XRD analysis of lipomer corroborated that the formulations were in an amorphous state. New nano-lipomer formulated with different ratios of lipid and polymer exhibited a rapid dissolution by an initial burst release of RIF followed by a controlled release profile.

1. Introduction

Tuberculosis (TB), where the lungs are frequently involved is chiefly caused by *Mycobacterium tuberculosis* [1]. Rifampicin (3-(4-methyl-*l*-piperazinyl-irninomethyl) rifampicin) (RIF) is a highly potent and broad spectrum antibiotic used against bacterial pathogens. The drug diffuse easily into tissue, living cells and bacteria, making it highly effective against pathogens such as *M. tuberculosis,* and often used for pulmonary diseases. In addition, it symbolizes the primary choice of antibiotic when drug resistance grows in chronic chemotherapy of infectious diseases such as TB [2,3].

Drug delivery directly to the lungs is advantageous compared the other routes of administration such as oral. Pulmonary drug delivery has shown promise as the lungs have significant permeability for the bioactives and a large surface area for absorption [3,4]. Drugs can be targeted and delivered for local action in the lungs for the treatment of various respiratory diseases such as TB, asthma or cystic fibrosis. This results in a significant reduction in the overall dose and adverse effects may occur from high level of systemic drug exposure. In addition, systemic absorption can be accomplished by targeting drug delivery to the alveolar area where drug can be easily absorbed *via* the lining of epithelial cells [4]. Recently, several studies have demonstrated for the delivery of *anti*-TB agents to the lungs as the primary site for infection. Specifically, RIF has been used as the first choice of drug. RIF has many limitations associated with oral delivery including acidic-degradation. Reports revealed that researchers have been exploring a respirable form of RIF delivery *via* nano and microparticles, liposomes, and liquids for lung targeting. The microparticles and liposomes possess a drug clearance limitation due their higher particle size compared to their polymeric counterparts. Nevertheless, the safety assessment of polymeric nanocarriers still

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under investigation [5–10].

Currently, attention has been drawn towards integrating lipids and polymers for exploitation of their combined properties. These hybrids/composites can be used for targeting, pH-specific; and controlled drug release to the lung. The drug release performance of lipid matrices can be attuned by the addition of polymers. The presence of the lipid can influence the solid state properties of the polymer and can provide further drug stability for direct lung delivery [11–13].

Therefore, the present study focused on the development of a respirable RIF-loaded lipomer nano-composites using microemulsion-spray dry technique for pulmonary delivery. Herein, a novel synthesis method for RIF-loaded nano-lipomer composites by microemulsion-Spray drying for pulmonary delivery approach is reported. The nano-lipomer composites were prepared employing palmitic acid as the lipid component and polycaprolactone (PCL) as the polymeric component. Palmitic acid is the most commonly found saturated fatty acid in nature while PCL was chosen for its easy processability for nanoparticle formation *via* emulsification. The nano-lipomer composites were characterized for their solid-state properties and controlled drug release in simulated lung fluid (Artificial Lysosomal Fluid (ALF) and Gamble's solution were prepared and employed to mimic the interstitial environment within the lung. ALF, being similar to the lung fluid with a pH of 4.5 where once nanoparticles are respired, it will come in contact followed phagocytosis through the alveolar and interstitial macrophages in the lung. Gamble's solution with a pH value of 7.4 represents the interstitial fluid present within the lung. The significance of the current work is the combination of both lipid and polymer carriers for controlled release and increased pulmonary residence time of RIF.

2. Materials and methods

2.1. Materials

Rifampicin was procured from Sigma Aldrich, China. Palmitic acid was purchased from Sigma Aldrich, Malaysia. Polycaprolactone (MW = 85000) was acquired from Sigma Aldrich, UK. Polyvinyl alcohol was obtained from Sigma Aldrich, USA. MilliQ water was used from MilliQ gradient water purification system (Millipore SAS, Molsheim, France). All other solvents and reagents employed in the current study were of analytical grade.

2.2. Preparation of the rifampicin-loaded nano-lipomer composites

RIF-loaded nano-lipomer composites were prepared by a microemulsion-spray dry method. To study the effect of lipid and polymer on the properties of nanocomposites, the ratios were selected as 1:1, 1:2, and 2:1 weight-by-weight ratios of PA:PCL. Briefly, palmitic acid and polycaprolactone were dissolved in a closed container with 10 mL of dichloromethane at room temperature (Table 1). To this, 50 mg of RIF was added with continuous agitation. The entire mixture was added with a rate of 5 ml/min to an (100 ml) aqueous phase containing 1% $^{w}/_{v}$ PVA and stirring continued (500 rpm) for 1 h to remove excess organic solvent. The resultant mixture was sprayed dried using a nozzle with a spray-mesh size of 4.0 μm in diameter (NanoSprayDryer B-90, Büchi, Switzerland). The instrumental conditions were maintained as: inlet temperature = 50 °C, outlet temperature = 31 °C, spray head temperature = 56 °C, drying gas 148 L/min and a relative spray rate of 85%. Dried nano-lipomer composites were then collected from the collecting drum. The spray drying flux rate was automatically adjusted according the above conditions.

2.3. Measurement of particle size, polydispersity index and zeta potential

All nano-composites formulations were screened for size, polydispersity index (PDI) and zeta potential using a ZetaSizerNano ZS (Malvern Instruments Ltd, UK). The composites were diluted with deionized water (composite:deionized water:1:10) to maintain the number of counts per second in the region of 600. The samples were then analyzed using disposal cuvettes for particle size analysis and capillary cells to determine the zeta potential. Measurements were undertaken in triplicate, with size intensity and zeta potential distribution profiles used to assess the average particle size and surface charge (N = 3).

2.4. Estimation of the rifampicin entrapment efficiency

The entrapment efficiency of RIF loaded into the nano-lipomer composites were calculated by dissolving a weighed quantity of nano composites in dichloromethane. The solution was filtered and the quantity of RIF entrapped was determined by UV spectrophotometer (PerkinElmer, MA). The RIF entrapment efficiency was calculated using Equation (1).

$$EE\ (\%) = \left(\frac{W_{initial} - W_{actual}}{W_{initial}}\right) \times 100 \quad (1)$$

Where EE (%) = entrapment efficiency (%), $W_{initial}$ = weight of drug loaded initially (mg) W_{actual} = weight of actual loaded drug (mg).

2.5. Determination of the chemical structure integrity of rifampicin

Dry powder samples of native RIF, palmitic acid and polycaprolactone as well as RIF-loaded nano-lipomer (F1, F2 and F3) composites were screened for chemical structure integrity using FTIR spectroscopy (PerkinElmer Spectrum, UK). FTIR spectra were recorded over a wavenumber range between 650 and 4000 cm^{-1} with a resolution of 4 cm^{-1} and 10 scans per spectrum.

2.6. Determination of the thermodynamic stability of RIF

Dry powder samples of native RIF, palmitic acid and polycaprolactone as well as RIF-loaded nano-lipomer (F1, F2 and F3) composites were screened for their melting transitions and change in heat capacity using Differential Scanning Calorimetry (Mettler Toledo, Switzerland). DSC thermograms were obtained at a heating rate of 10 °C/min from 20 to 250 °C under a constant flow of N_2 as the purging gas in order to reduce sample oxidation.

2.7. Determination of polymorphic transitions

The crystalline and amorphous nature of the RIF-loaded nano-lipomer (F1, F2 and F3) composites and their native components were examined using a Rigaku MiniFlex600 Benchtop X-ray Diffractometer (Rigaku Corporation, Tokyo, Japan) fitted with; a 600 W (40Kv-15 mA) X-ray generator, a counter monochromator to cut X-rays other than Cu Kα X-rays and a high intensity D/tex ultra-high speed 1D detector. Experimental temperature was maintained at 19 °C. Measurements were performed by scanning each sample at 2°/min over a diffraction angle range of 0°-90°, 2θ. The XRD diffractograms generated were used to compare and evaluate the crystallinity of the respective samples.

2.8. Surface morphology assessment of nano-lipomer composites

Surface morphology of RIF-loaded lipid/polymer nan-