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Formulation development and characterization of silybin loaded nanoparticles

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ABSTRACT

The objective of the study is to prepare silybin loaded nanoparticle and to characterize it for particle size, zeta potential, surface morphology and to estimate its drug release behaviour. Emulsion (o/w) solvent evaporation method was employed in the preparation of silybin nanoparticles. Particle size, polydispersity indices of silybin nanoparticles and zeta potential were measured by dynamic light scattering using a 90 plus particle sizer. Transmission electron microscopy (TEM) was performed using a Philips CM 10 transmission electron microscope. *In vitro* release of silybin from the nanoparticles was performed by dialysis method. The result showed that silybin nanoparticles prepared gave rise to the Entrapment efficiency of 88%, Drug loading of 15%, mean diameter of hsylibin. Silybin nanoparticles showed optimum particle size and zeta potential with improved entrapment efficacy and a better drug release profile.

Keywords: Silybin; Nanoparticle; Drug delivery, Drug release behviour.

1. INTRODUCTION

Silybin is one of the oldest drugs very well considered for cancer.[1-3] Although it is considered to be ideal for the treatment of cancer, delivery to the tumor still needs improvement. Silybin needs to be administered daily to achieve its effects. Nanosized carriers encapsulating silybin can be taken up passively and can result in increased drug concentration, thus increasing therapeutic efficacy. [4-6] They can result in sustained systemic release of sylibin for more than a week, depending on various factors. Thus, repeated daily administration for sylibin can be avoided.

Thus, with this type of formulation, sustained release, improvement in bioavailability as well as enhancement of biochemical protection can be achieved. Together, these mechanisms lead to increase in effectiveness of therapy. Thus, the objective of this study was to prepare biodegradable nanoparticles of silybin, and to evaluate their characteristics like particle size, surface morphology, zeta potential, entrapment efficiency and drug loading efficiency and as well as the drug release behaviour.

2. METHODS

Silybin, Poly-ε-caprolactone (mol wt., 14,000), Polyvinyl alcohol (PVA, cold-water soluble) and Dichloromethane were procured from Sigma-Aldrich, Germany. All other reagents were of analytical grade.

2.1. Preparation of silybin nanoparticles

Emulsion (o/w) solvent evaporation method was employed in the preparation of silybin nanoparticles using poly-ε-caprolactone as the polymer. [7,8] For the preparation, silybin (100 mg) and polycaprolactone (100, 200, 300 or 400 mg) was dissolved in 15 ml of dichloromethane by vortexing. The mixture (organic phase) was added drop-wise to 50 ml of 2 % PVA solution under probe sonication at 40 w for 10 min to obtain a w/o emulsion. This emulsion was placed on a magnetic stirrer to ensure complete evaporation of dichloromethane, leaving nanoparticle suspension. The suspension was centrifuged at 10,000 rpm for 20 min, resulting in the formation of a pellet at the bottom of the tube. This pellet was washed with phosphate buffered saline (PBS), re-suspended and again centrifuged. The pellet was collected and allowed to dry completely. The powdered particles were collected, weighed and used for further evaluation.

2.2. Characterisation

2.2.1. Particle size, polydispersity index and zeta potential measurement

Particle size and polydispersity indices of silybin nanoparticles were measured by dynamic

light scattering using a 90 plus particle sizer (Master sizer, Malvern instruments) equipped with MAS OPTION particle sizing software. The measurements were made at a fixed angle of 90° for all samples. The samples were suitably diluted with Milli Q water for every measurement. Zeta potential measurements were also made using an additional electrode in the same instrument. For zeta potential determination, samples of all formulations were diluted with 0.1 mMKCl and placed in the electrophoretic cell, where an electric field of about 15 V/cm was applied. The mean hydrodynamic diameter (Dh) and polydispersity index (PI) of the particles were calculated using the cumulative analysis after averaging the three measurements. [9, 10]

2.2. Transmission electron microscopy

Transmission electron microscopy (TEM) was performed using a Philips CM 10 transmission electron microscope. The sample was prepared by a formvar resin grid method. Briefly, a 0.5 % w/v suspension of silybin nanoparticles was sprayed on a formvar resin coated TEM grid and air dried for 10 min before observation. Contrast enhancement and particle measurement were performed using the NIH image software.

2.3. Determination of drug loading and entrapment efficiency

Drug encapsulation efficiency and drug loading of the prepared silybin nanoparticles were determined by the following procedures. Firstly, a certain volume of nanoparticle suspension was accurately taken, dissolved and diluted with anhydrous methanol. Then, drug content in the resultant solution was determined by HPLC method and the calculated drug amount was designated as W_{total}. To determine the unencapsulated drug, equal volume of nanoparticle suspension was accurately taken and ultra-filtered by a filter membrane with molecular weight cut-off (MWCO) of 12 kDa (Reili Separation Instrument Factory, Shanghai, China). The ultra-filtrate was diluted with anhydrous ethanol and drug content in the resultant solution was analyzed under the same HPLC condition. The amount of free drug was designated as W_{free}. Consequently, the drug encapsulation efficiency (EE) and drug loading (DL) could be calculated by the following equations. [11, 12]

$$EE(\%) = (W_{total}-W_{free})/W_{total} \times 100 \quad \text{.... Equation 1}$$

$$DL(\%) = (W_{total}-W_{free})/W_{polymer} \times 100 \quad \text{.... Equation 2}$$

Where W_{total} was the total amount of drug, W_{free} was the amount of unencapsulated drug, and $W_{polymer}$ was the weight of the polymer.

2.4. *In vitro* release studies

In vitro release of silybin from the nanoparticles was performed by dialysis method. Silybin nanoparticles were dissolved in deionized water at a concentration proportionate to 2 mg/mL silybin. Pure silybin was dissolved in little methanol, then diluted with more deionized water (2 mg/mL), and used as a control. Five millilitres of the samples was transferred immediately to the dialysis bags. The bags were promptly put in 500-mL glass beakers containing 400 mL of the disintegration medium maintained at 37°C. The outer phase was stirred continuously with a magnetic stirrer and samples (1 mL) were taken at specific time intervals followed by renewal with 1 mL of new disintegration medium. The measure of drug in the samples withdrawn from the outer phase over a 12-hour period was determined by HPLC to describe the release of silybin. The disintegration medium was recreated gastric fluid (pH 1.2) and mimicked intestinal fluid (pH 6.8). [13-15]

2.5. Statistical analysis

ANNOVA was used to analyse the data for statistical differences followed by Bonferroni's modified t test for multiple comparisons using GraphPad Prism. To determine the statistical significance, the confidence interval was set at 95%.

3. RESULTS AND DISCUSSION

3.1. Characterization

3.1.1. Particle size, polydispersity index and zeta potential measurement

The mean particle size of silybin nanoparticles was 216 nm with a polydispersity index of 0.193 ± 0.026 (Figure 1). A narrow PI means that the colloidal suspensions are homogenous in nature. The Zeta potential of the silybin nanoparticle was found to be -15 mV, (Figure 2) and it is sufficiently high to form stable colloidal nanosuspension.

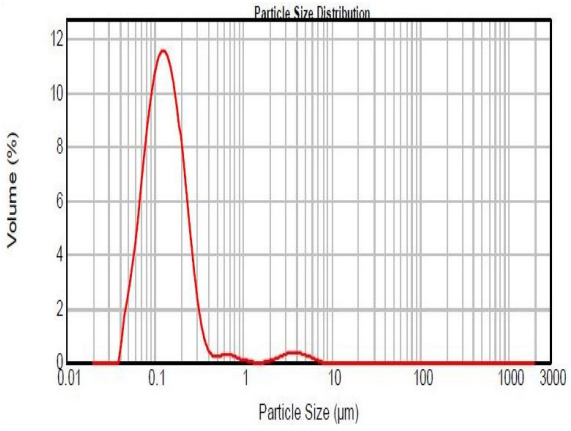

Figure – 1: Particle size distribution and zeta potential of silybin nanoparticle.

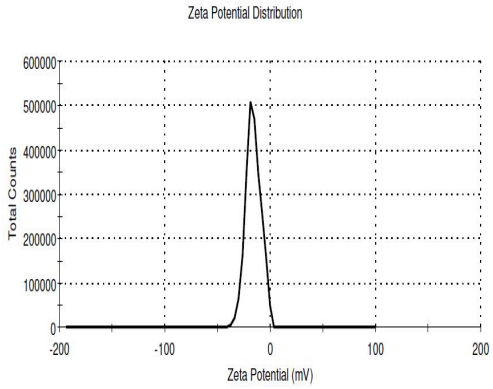

Figure – 2: Zeta potential of silybin nanoparticle.

3.1.2. Transmission electron microscopy

In order to provide information on the morphology and size of the optimal silybin nanoparticle, TEM was used to take photos of the optimal silybin nanoparticle, as shown in figure 3. The silybin nanoparticles are spherical. The size is about 216 nm.

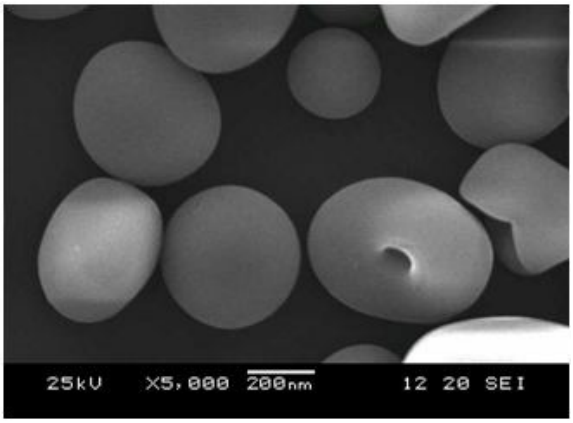

Figure – 3: TEM of silybin nanoparticle.

3.1.3. Drug release characteristics

The strength and stability of the drug-nanoparticle complex were investigated using *in vitro* release studies in simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 6.8).Because the external electrostatic interaction was found to be the major mechanism for drug complexation by nanoparticles, we can expect that the strength of electrostatic interaction determines the drug release behavior from nanoparticles. The release of silybinfromnanoparticle matrixes should be faster in lower pH conditions. As it can be seen from Figure 4 and 5, the lower the pHvalues the faster the release rate of silybin. This is due tothe availability of positively charged proton to interact with the phenolic hydroxyl group of silybin molecules,which reduces the electrostatic interactions between the nanoparticle matrix and the drug, thereby increasing the release rate of silybinfrom nanoparticles. Alternatively, the positive charge of nanoparticles, which increase thepolarity of the interior cavities of nanoparticles, wouldcontribute to the distinct release behavior of silybin indifferent pH conditions. The differences of drug releaserate in different dissolution media can be correlated witha combination effect of the ionization state of the drugand the nanoparticles. These results strongly suggested that electrostatic interaction might play an important role in release of drugs from nanoparticle matrixes.

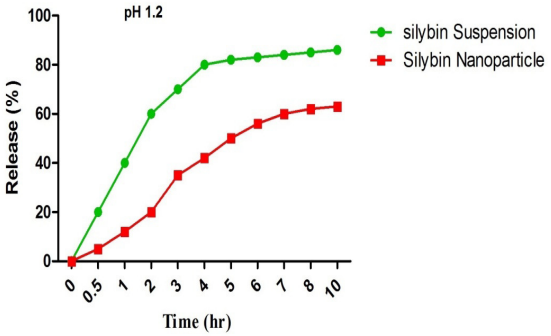

Figure – 4: *In vitro* release of silybin from nanoparticle compared with the diffusion of a silybin suspension in simulated gastric fluid, pH 1.2.

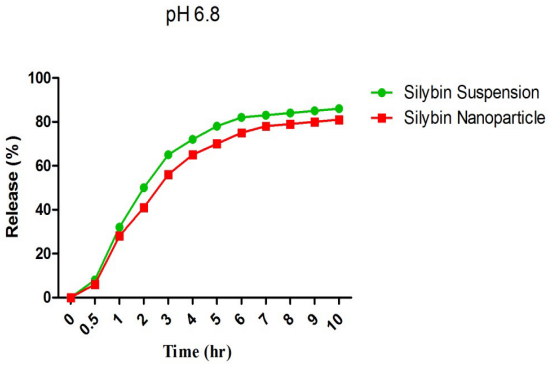

Figure – 5: *In vitro* release of silybin from nanoparticle compared with the diffusion of a silybin suspension in simulated intestinal fluid, pH 6.8.

4. CONCLUSION

Silybin nanoparticles can be suitably prepared by emulsion solvent evaporation technique using polycaprolactone as a biodegradable polymer. The particles showed good encapsulation efficiency and sustained drug release in vitro. Silybin nanoparticles offer an effective approach for drug targeting of the liver.

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