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Research Article

Formulation and Characterization of Chitosan coated Liposome for Sustained Release of bio-actives

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ABSTRACT
The aim of current study was to assess the potential Chitosan coated liposomes formulation. Modified ethanol injection method was adopted and inspects the vesicle morphology. Average particle size was found to be 303.6 nm & entrapment efficiency 78±5 %. In vitro release pattern followed 63.5±0.18 in 24hrs. Prepared formulations have received considerable attention in pharmaceutical and biomedical application, specifically achieving sustained release controlled formulation. Thus, it is a useful method for prolonging drug release from dosage forms, reducing adverse effects and to deliver drugs in a controlled manner.

Keywords: Liposomes, Chitosan, Release rate, stability, sustain release

1. INTRODUCTION

Novel drug delivery systems is an essential requirements, which delivers drug against the causative agent of the disease being treated by carrier based drug loaded system and transported to site of action. The Chitosan coated drug delivery system like liposome decodes challenges against micro-organism related disease. Liposomes are bilayer round or oval vesicles composed of aqueous volume portion covered by lipid bilayered. They comprises of natural or synthetic phospholipids. Liposomes are colloidal, vesicular structure based on phospholipids bilayers. Their characteristics depend on the manufacturing protocol and choice of bilayer components. They range from 20 nm to 100 nm diameter[1].

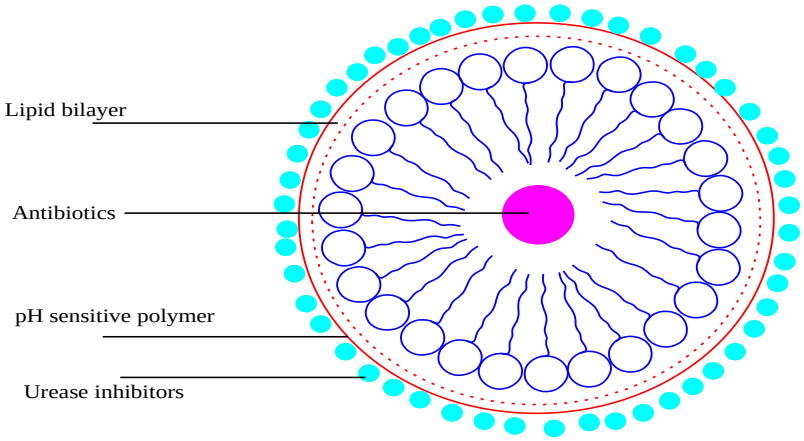


Fig.1 Schematic representation of liposome containing antibiotic at the cove and covered with pH sensitive polymer linked with Urease inhibitor

Liposomes has number of components however phospholipids and cholesterol being the main components. It is apparent form the above facts that liposome is a model drug delivery system for mucoadhesive preparations incorporating drug moiety. The coating by Chitosan is required for more adhesion to the membrane and Urease inhibitor use to inhibit Urease synthesis, finally stop the growth of bacteria. Chitosan is a polycationic, nontoxic, mucoadhesive polymer, which has been proven to be safe. [2-4] Chitosan proposed has gastric retentive property, linked with the electrostatic interactions between the cationic amine groups of chitosan and the gastric mucins that are negatively charged at the stomach acidic pH. [5, 6] Acetohydroxamic acid (AHA) displays bacteriostatic and bactericidal effects on H. pylori through competitive inhibitory

binding site [7, 8]. The present study was to prepare and investigated acquire particle size with the help of Zeta-seizer. Behaviour mechanism including Invitro drug release profile having highest regression coefficient values for Higuchi's model. Performance of prepared formulation was examined by stability testing. In future prepared formulation can be further used as medicated product.

2. MATERIAL AND METHODS

2.1 Materials

Generous gift of Chitosan (Fisher Company), & Amoxicillin (Ranbaxy, Dewas). Acetohydroxamic acid (AHA) & Lecithin was procured from Sigma-Aldrich USA. Cholesterol, Ethanol, Glacial acetic acid, all other chemical used in present work is of Analytical or HPLC grade.

2.2 PREPARATION OF LIPOSOMES

Chitosan coated sustained release preparation based on modified ethanol injection method were fabricated according to the procedure suggestive karn et.al. 2011[9] (fig2). Ethanolic solution was prepared and homogeneously mixed in Lecithin: cholesterol mixture. Simultaneously Chitosan solution was prepared in glacial acetic acid. Both solutions were slowly injected in PBS (pH7.4) buffer solution at stirring speed 300rpm for 4hrs using mechanical stirrer (Remi, India). Chitosan coated Liposomes suspension were collected by washing three times and dried at room temperature for 24 hrs.

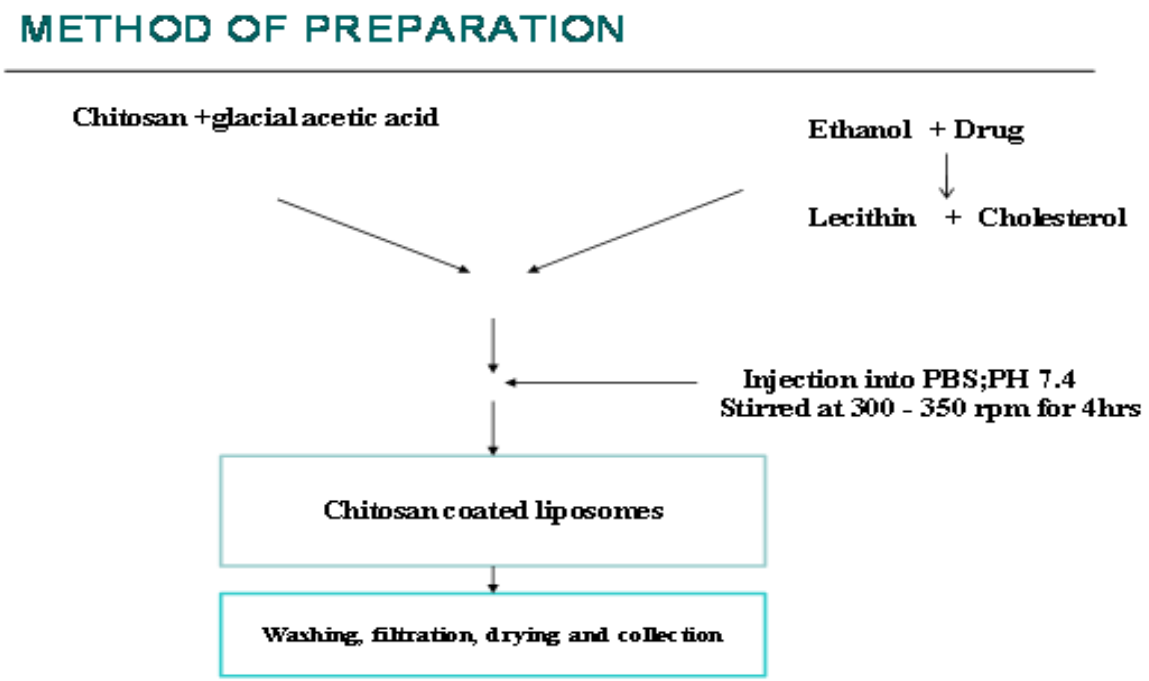


Fig .2-Schematic Presentation of Prepared Liposomes

2.3 Characterization of prepared liposomes

2.3.1 Particle size and size distribution

Particle size and size distribution were calculated microscopically with calibrated ocular micrometer. Least count of the ocular micrometer was considered as 16.2 µm around 100 particles from each formulation. Zeta potential was observed by Malvern Zeta seizer (Malvern Instrument, UK) and the observed data for each formulation were recorded.

2.3.2 Shape and surface morphology

Drops of prepared liposomes placed on glass slide were observed under optical microscopy (Leitz-Biomed, Germany) and scanning electron microscope (SEM, Hitachi, Japan) to examine their shape. In order to examine the surface morphology, the formulations were viewed under scanning electron microscope. Prepared Liposomes powders in double adhesive tape were lightly shake and then trapped to an aluminium stub. The stubs were then coated with gold to a thickness of about 300°A using a sputter water. The samples were then randomly scanned for studying surface morphology but show the images of coating to prove internal surface.

2.3.3 Entrapment efficiency

Prepared Liposome100 mg formulation was distributed in 100 ml in PBS solution; (pH 7.4) and shaken vigorously for 10 min. and supernatant was kept aside. Similarly, the

sediment was again treated in the same manner and second supernatant was mixed with first supernatant. Formulations were dissolved in 20 ml in PBS solution; (pH 7.4) for 2 hrs centrifuged at 3000 rpm for 5 min[5,6] and then filtered through 0.45μm syringe filter (Millipore Millex HN, USA) Sample withdrawn at regular interval and absorbance was taken on Shimadzu UV-Spectrometry at λmax 228nm. The free drug detected in supernatant and the percent drug entrapped was calculated.

2.3.4 Drug release In vitro study in biological fluids

The dissolution test of prepared Liposomes was conceded by the paddle type dissolution apparatus specified in USP XXIII[7, 8]. 10mg prepared formulation precisely mixed with 100 ml of dissolution medium maintain perfect sink condition. The content was rotated at 100 rpm thermostatically controlled at 37±0.5°C. Biological media SGF (pH 1.2) and PBS (pH 7.4) used to classify release rate of prepared formulation. The samples were withdrawn and equivalent amount of fresh medium was added to release medium. The collected samples were filtered through 0.45μm-syringe filter (Millipore millex HN) and analyzed spectrophotomatrically.

2.3.5 Stability studies

The Stability of Prepared liposomes is crucial for market point of view. The formulations developed were tested for their stability by storing them in amber coloured glass bottles at 4°C and 27±2°C for 90 days. Formulation was dissolved in PBS (pH 7.4) (1:1 v/v) solutions. The product was filtered and then it was analysed for drug content using spectrophotometric techniques.

RESULT AND DISCUSSION:

The surface and particle morphology of prepared formulation was evaluated. The particle attained plain & spherical surface morphology as depicted in figure 4.

The average Particle size attributes for Prepared Liposome was 303.6 nm in figure 3. Zeta potential factor contain Electrophoretic cell supporting with electric field were found 2.80±0.20mV indicate stability and mucoadhesive of the formulation.

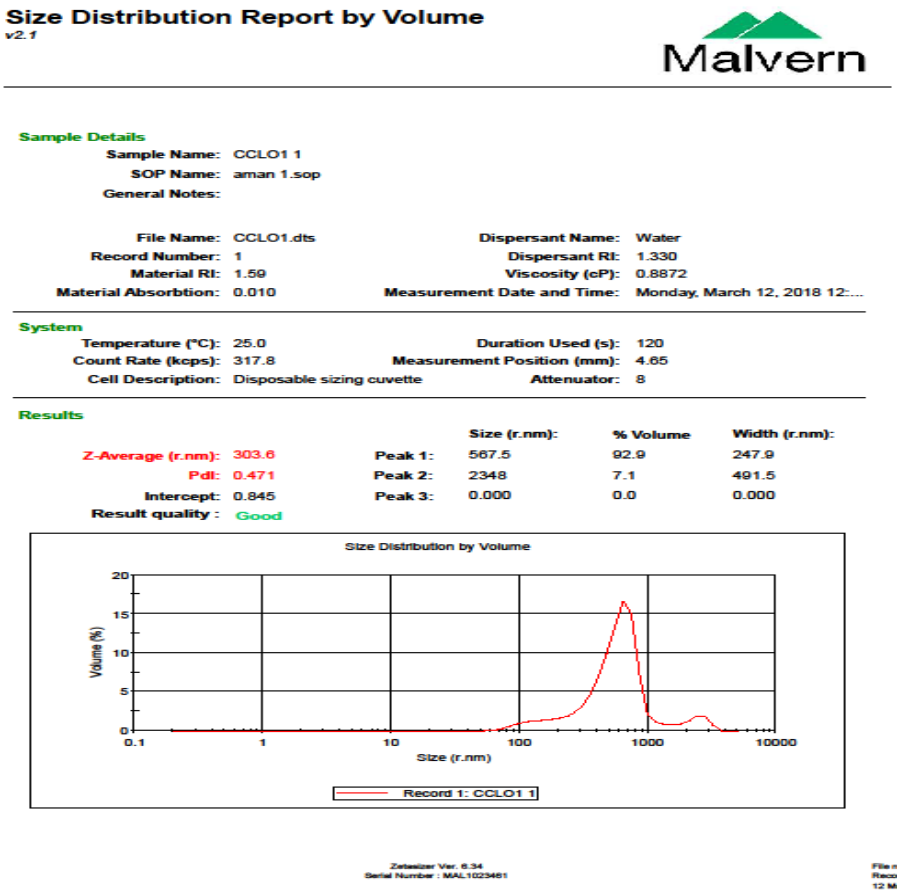
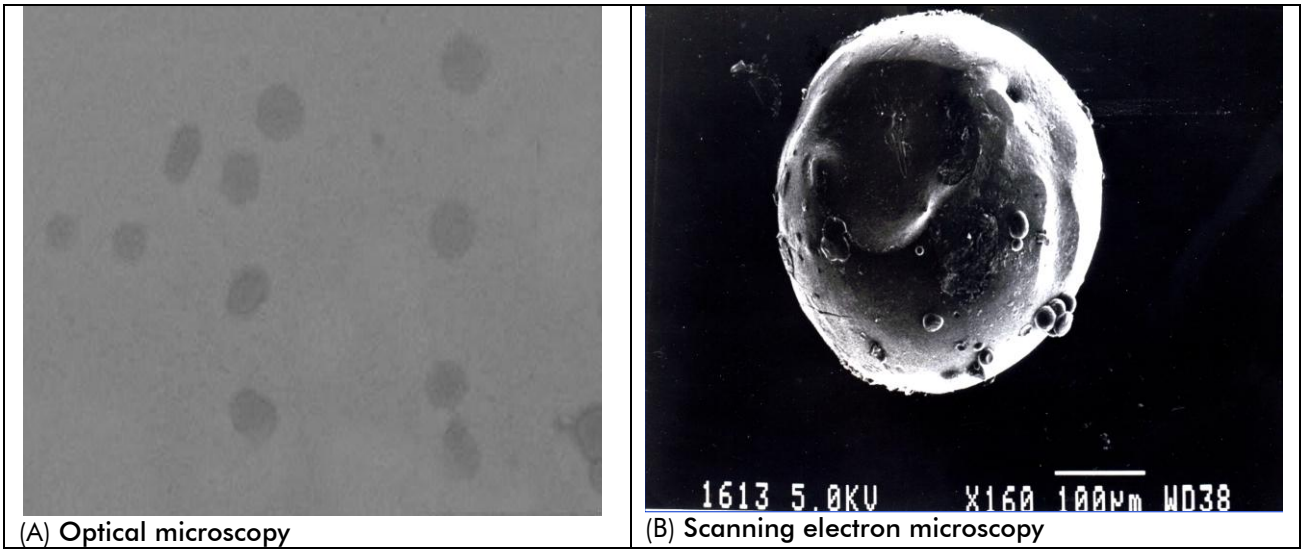


Fig.3:Average particle size assessment of prepared liposomes



(A) Optical microscopy (B) Scanning electron microscopy

Fig.4:Microscopic assessment of prepared liposomes

Entrapment efficiency:

The finding suggested physical mechanism for drug into Chitosan coated liposomes. The formulation were analyzed UV-Spectrometer and result was found to be 78± 5%.

In-vitro Drug Release:

In figure 5, in-vitro drug release profile of the prepared formulations was presented graphically. Dialysis method was used to conclude the drug release and it was found that at 6 hrs the percent of drug released of formulations was 48.3±0.23%. After 24 hrs the drug release was found to be 63.5±0.18%. In vitro drug release was carried out in simulated gastrointestinal fluids of different pH (1.2 & 7.4). It was observed that the release rate of Amoxicillin from the prepared formulation was significantly slower than the conventional dosage forms. The in vitro drug release study performed in (pH 1.2 & Ph 7.4) respectively to confirm that prepared formulation resulted in sustained and prolonged release of drug in the GIT fluids.

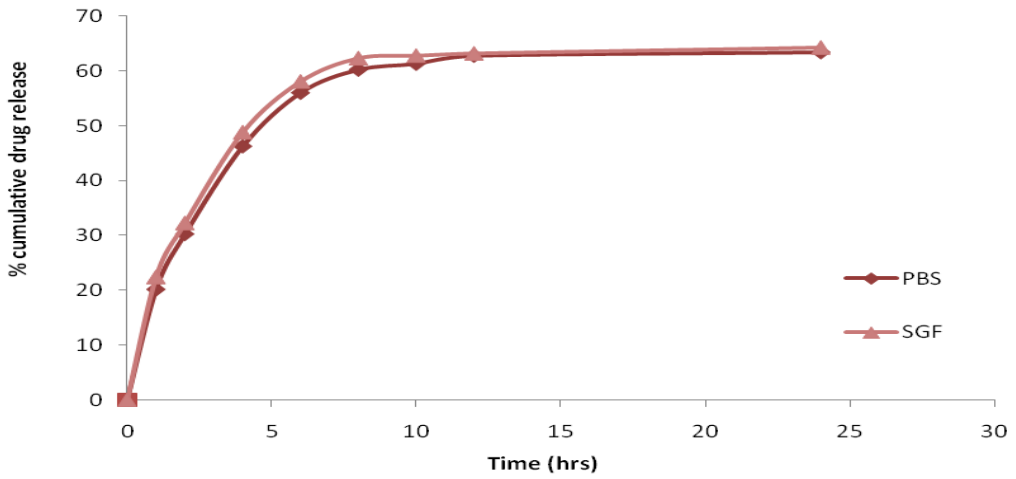


Fig.5: Percentage cumulative drug release of prepared liposomes

Stability study

Prepared formulation was stored at 04±1°C, 25±1°C and at 40±1°C and the residual drug content of the formulation was measured after 15, 45 and 90 days. The percent residual drug content of the selected formulation is presented in table 2. It was observed that the formulation stored at 04±1°C and 25±1°C was quite stable as very less drug was degraded on storage for 30days while it was quite unstable at 40±1°C as the residual drug content was

less after storage at 40±1°C for 90 days.

Table 2 Stability study of prepared liposomes

Parameters	Initial observation (0 day)			Initial observation (45 day)			Initial observation (90 day)		
	4^{0C}	25^{0C}	40^{0C}	4^{0C}	25^{0C}	40^{0C}	4^{0C}	25^{0C}	40^{0C}
Particle size(nm)	302± 04.23	302± 04.23	321± 03.42	303±03 .22	303± 04.32	332 ± 02.5 9	308± 04.23	329± 03.59	336± 02.49
Residual drug content (%)	NA	NA	NA	83± 09	82± 03	56± 08	83±0 9	69± 04	52± 07
Surface morphology	-	-	-	-	-	++	-	+	++

Note: - =No change, + =slight change ++= moderate change

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Authors contribution statement:
Mr. Mahendra Chouhan had collected the data, prepare a liposome novel drug delivery system and further estimation done with various techniques. Dr. Rajesh Sharma and Dr.Kamlesh Dashor reviewed these data and provided suggestions to improve the designing of coated liposome formulation. All authors collectively contributed to methodology and resulted in parts of the final manuscript.

Conflict of interest:
Conflict of interest declared none.

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