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PREPARATION AND CHARACTERIZATION OF L-ASPARAGINASE MICELLES FOR THE TREATMENTOF LEUKEMIA

[1]Bhavik Sharma,[2]Kush Biswas

[1]Assitant Professor, Department of pharmacy, Madhav University, Abu road, Rajasthan, 307026

[2] Assistant Professor, One Beat College of Medical Sciences, Bhira, Kheri,262901

ABSTRACT:

Hematologic cancer known as acute lymphoblastic leukemia (ALL) most frequently affects youngsters between the ages of 2 and 10. L-asparaginase is a crucial part of ALL treatment, and since it was included to pediatric treatment protocols in the 1960s, childhood survival rates have steadily increased to around 90%. The deamination and depletion of serum asparagine levels is the identical mechanism of action shared by all currently available asparaginases, yet each exhibits a distinctly different pharmacokinetic profile. The first-line treatment is a pegylated asparaginase generated from the bacteria E. coli, however up to 30% of patients experience a hypersensitive reaction that limits the efficacy of the medication. According to studies, individuals who were switched to L. asparaginase owing to hypersensitivity can achieve results that are comparable to those of patients who do not have a hypersensitivity reaction with the right dosage of the enzyme. To guarantee that therapeutic levels of asparaginase activity are maintained, therapeutic medication monitoring may be necessary.

Keyword: - Acute lymphoblastic leukemia, L-asparaginase

1. INTRODUCTION

Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells, which can result in death.[1,2] Cancer develops when cells in a part of the entire body start to grow out of control. Normal body cells grow, divide, and die in an orderly fashion. Throughout the early years of a person's life, normal cells divide more rapidly until the person becomes an adult. After that, cells in most parts of the body divide only to replace worn-out or dying cells and to repair injuries. Because cancer cells keep growing and divide, they are different from normal cells. Instead of dying, they outlive normal cells and continue to form new abnormal cells.[3,4]

An oncogene is a gene that has the potential to cause cancer. In tumor cells, they are often mutated or expressed at high levels.[5,6] Most normal cells undergo a programmed form of death (apoptosis). Activated oncogenes can cause those cells that ought to die to survive and proliferate instead. One of the fundamental traits of cancer cells is their ability to proliferate without the controlled signaling input.[7,8]

1.2. LEUKEMIA

It is derived from "leuk" (white) + "emia" (blood): referring to the color of the blood in leukemia when large numbers of white cells have accumulated. In leukemias the normal differentiation pathway gets blocked at a stage of differentiation at which the cells continue to proliferate, and most cells do not move on to terminal differentiation. Leukemia is a metastatic and malignant disease for blood-making organs which is resulted due to incomplete evolution and problematic growth of white blood cells (WBCs) and its substrates in blood and bone marrow. [9,10]

The origination of leukemia may be sudden (acute) or slow and gradual (chronic). This type of malignancy is also categorized based on the type of blood cell affected. Malignancy that involves myeloid cells, granulocytes (neutrophils, basophils, and eosinophils) and monocytes (macrophages) lead to myeloid leukemia whereas that involves T and B lymphocytes give rise to lymphocytic leukemia.[11,12]There are various classes of leukemia. The four main classes include Acute Myelogenous Leukemia (AML), Chronic Myelogenous Leukemia (CML), Acute Lymphocytic Leukemia (ALL) and Chronic Lymphocytic Leukemia (CLL). There are some other forms of leukemia which are normally rare; these are hairy cell leukemia, T-cell prolymphocytic leukemia, large granular lymphocytic leukemia and adult T-cell leukemia.[13,14]

2. MATERIALS AND METHODS

The drug and chemicals used for the research work are collected from Sigma-Aldrich. Chemicals used were of analytical grade, and drug was procured as gift sample from Sigma-Aldrich, Bangalore.

2.1 PREFORMULATIONSTUDIES

2.1.1 Organoleptic Properties

The drug sample was examined for its color, odor and appearance.

2.1.2 Melting PointDetermination

Thin-walled capillary melting point tubes were used to hold samples for determination of melting point.

2.1.3 Determination ofSolubility

Solubility is the property of a solid, liquid or gaseous chemical substance called solute to dissolve in a solid, liquid or gaseous solvent to form a homogenous solution of the solute in the solvent.

2.1.4 PARTITIONCOEFFICIENT

A partition or distribution coefficient is the ratio of concentration of a compound in a mixture of two immiscible phases in equilibrium. These coefficients are a measure of the difference in solubility of the compound in these two phases.

2.1.5 FOURIER TRANSFORM INFRA-RED (FT-IR)ANALYSIS

FT-IR analysis was carried out to study the chemical interaction between drug and polymer and if any degradation occurs during process of micelles preparation.

2.1.6 DETERMINATION OF λ_{max}OFL-ASPARAGINASE

Stock solution (100µg/ml) of L-asparaginase (L-ASNase) was prepared by dissolving accurately weighed 10 mg of the pure L-ASNase in 100 ml of PBS pH 7.4.

2.1.7 PREPARATION OF CALIBRATION CURVE OF L-ASNase

Stock solution (100 µg/ml) of L-ASNase was prepared by dissolving accurately weighed 10 mg of the pure L-ASNase in 100 ml of PBS pH 7.4. From the stock solution, aliquots of 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml, 5.0 ml, 6.0 ml, 7.0 ml, 8.0ml, 9.0 ml and 10.0 ml were transferred to 10 ml volumetric flasks and diluted upto 10 ml with same solvent to prepare subsequent solutions that contain 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 µg/ml of L-ASNase, respectively.

2.1.8 PREPARATION ANDOPTIMIZATION OFMICELLES

The different formulations were formed for the L-ASNase micelles. Formulation composed of L-ASNase (the drug), Chitosan-Stearic acid copolymer and EDC (the cross-linking agent).

2.2 PREPARATION OF L-ASNase CONTAINING MICELLES

All reagents and solvents were of analytical grade and were used without further purification. Deionized water was used for the preparation of all solutions.

2.3IN-VITRO RELEASESTUDIESAND EFFECT ONSTABILITY

The in-vitro release studies of L-ASNase loaded micelles were carried out at 37±2°C in phosphate buffer saline (PBS) pH 7.4 buffer media for a period of 24 hrs. Water bath shaker was used to conduct in-vitro release studies. The 30ml ambered colored screw capped bottles, containing L-ASNase polymeric micelles in 20ml of PBS pH 7.4 as release medium were fixed in holders on platform inside the water bath shaker.

2.4 STABILITYSTUDIES

To assess the stability of the micellar formulations, they were stored at room temperature and at 4°C. Entrapment efficiency of the formulations on day 1, day 30, day 60 and day 90 were compared as the measure of stability. The impact of storage on the entrapment efficiency on different time was calculated. On day 60, all the formulations stored at 4°C, remained as same without significant changes in the entrapment efficiency. The entrapment efficiency of the formulations did not show much difference at the end of 60 days when stored at 4°C but showed a decrease in 1-3% at the end of 90 days. When stored at room temperature, there was a decrease in entrapment efficiency of about 1-3% at the end of 90 days

3. RESULT AND DISCUSSION

3.1 Organoleptic propertiesof L-Asparaginase (L-ASNase) were found out as white lyophilized powder, colorless, odorless and bitter in terms of its appearance, color, odor and taste, respectively.

3.2 Melting pointof L-ASNasewas found out to be in between 232 - 235°C which lies in between of the reported melting point value of L-ASNase i.e. 234°C.

3.3 Solubility profileof L-Asparaginase (L-ASNase) was determined in different solvents such as water, ethanol, di-ethyl ether, formaldehyde and benzoyl chloride.

3.4 Partition coefficient of L-ASNase in n-octanol: water was determined and found to be -4.90, which lies around the reported the K_{OW} value of L-ASNase i.e., -4.96, commencing the drug is highly hydrophilic.

3.5 FTIR

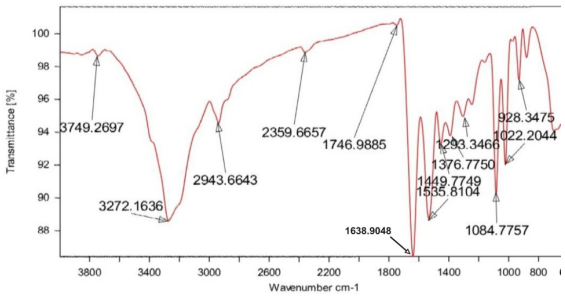

Figure 1. FT-IR of L-Asparaginase

3.6 λmax OFL-ASPARAGINASE

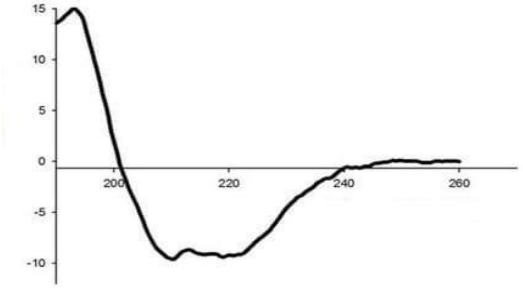

Figure 2. dictates λ_{max} of LASNase

3.7 CALIBRATION CURVE OF L-ASNase

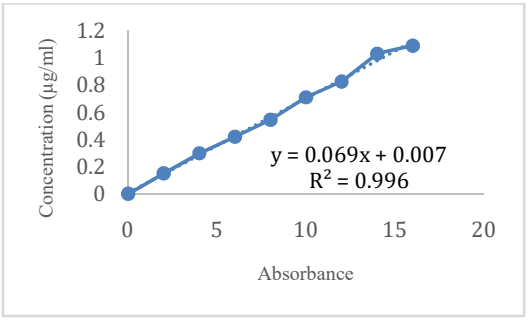

Figure 3. Calibration Curve of L-ASNase in PBS (pH 7.4)

3.8 SEM analysis

Figure 4. Microscopic Image of L-ASNase loaded Polymeric Micelles

3.9 *In-Vitro* Release Profile of L-ASNase loaded PolymericMicelles

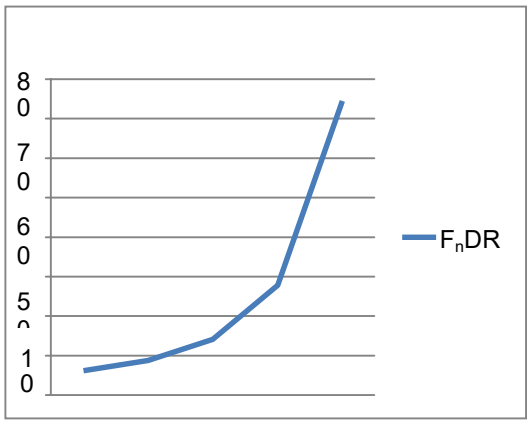

Figure 5. In-vitro Release Profile of L-ASNase

3.10 stability study

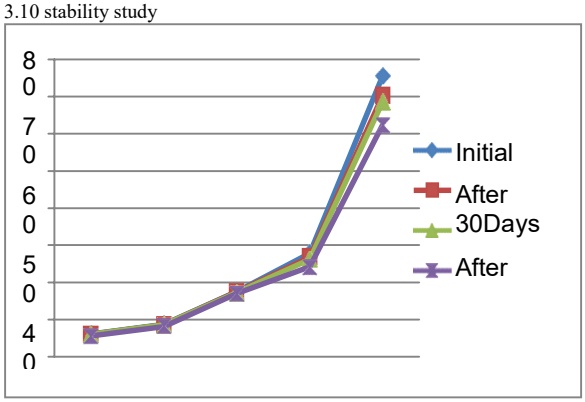

Figure 6. In-Vitro Release Profile of L-ASNase from Optimized Formulation Initially and During Stability

4. CONCLUSION

The aim of the present research work is to develop and characterize Copolymer based Polymeric Micelles. L-Asparaginase loaded Micelles were successfully prepared by self-assembly method. The analytical method was developed and standardized for L-ASNase in bulk as well as in formulations. The observed maximum absorbance peak at 222.0 nm has been considered for further studies. The standard curve of L-ASNase was found to be linear over a concentration range of 1 to 10 µg/ml with the correlation coefficient (R^2) value 0.9881, slope 0.008739 and intercept -0.015067. The developed analytical method based on UV-Spectrophotometry for L-ASNase was found to be sensitive and reproducible. FT-IR studies revealed that there was no change in the spectrum of the physical mixture and drug-loadedmicroparticles. L-ASNase were apparently cylindrical in shape with smooth surface. The particles were slightly aggregated

Zero-order and Krosmeyer-Peppas model gave a good fit for the drug release profiles of all micelles formulations with greater regression coefficients in comparison to other models, which demonstrated that drug release occurs mainly through diffusion and erosion process. Hence, sustained/controlled drug release was observed in prepared formulations when subjected for in in-vitro drug releasestudy.

Stability studies of L-ASNase loaded micelles were conducted over a period of 3 months. Drug stability in the micellar formulation was assessed by comparing the stored formulations with the control of its drug loading. Based on the in-vitro characterization, formulation F_n was found to be the most promising formulation with sustained release over a prolong period oftime.

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