



PREPARATION AND CHARACTERIZATION OF L- ASPARAGINASE MICELLES FOR THE TREATMENT OF LEUKEMIA (ACUTE LYMPHOBLASTIC LEUKEMIA)

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ABSTRACT

Acute lymphoblastic leukemia (ALL) is a hematologic malignancy that predominantly occurs in children between 2 and 10 years of age. L-asparaginase is an integral component of treatment for patients with ALL and since its introduction into pediatric treatment protocols in the 1960s, survival rates in children have progressively risen to nearly 90%. All currently available asparaginases share the same mechanism of action - the deamination and depletion of serum asparagine levels - yet each displays a markedly different pharmacokinetic profile. Pegylated asparaginase derived from the bacterium *E. coli* is used as first-line therapy; however, up to 30% of patients develop a treatment-limiting hypersensitivity reaction. Studies have shown that with adequate dosing, therapeutic levels of L. asparaginase activity can be achieved, and patients switched to L. asparaginase due to hypersensitivity can obtain outcomes similar to patients who do not experience a hypersensitivity reaction. Therapeutic drug monitoring may be required to ensure that therapeutic levels of asparaginase activity are maintained.

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 PREFORMULATION STUDIES

2.1.1 Organoleptic Properties

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

2.1.2 Melting Point Determination

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

2.1.3 Determination of Solubility

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 PARTITION COEFFICIENT

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} OF L-ASPARAGINASE

Stock solution (100 μ g/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 μ g/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, 8.0 ml, 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 μ g/ml of L-ASNase, respectively.

2.1.8 PREPARATION AND OPTIMIZATION OF MICELLES

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.

2.3 IN-VITRO RELEASE STUDIES AND EFFECTON STABILITY

The in-vitro release studies of L-ASNase loaded micelles were carried out at 37 \pm 2 $^{\circ}$ 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 STABILITY STUDIES

To assess the stability of the micellar formulations, they were stored at room temperature and at 4 $^{\circ}$ 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 $^{\circ}$ 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 $^{\circ}$ 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 properties of 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 point of L-ASNase was found out to be in between 232 - 235 $^{\circ}$ C which lies in between of the reported melting point value of L-ASNase i.e. 234 $^{\circ}$ C.

3.3 Solubility profile of L-Asparaginase (L-ASNase) was determined in different solvents such as water, ethanol, diethyl 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

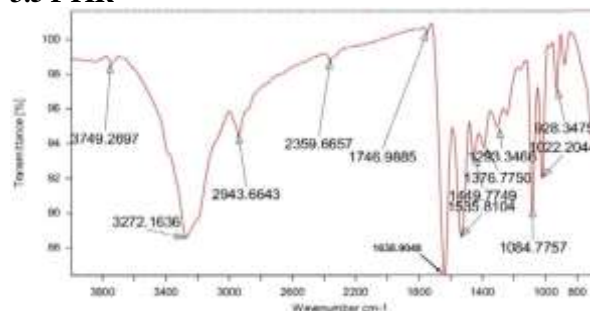


Figure 1. FT-IR of L-Asparaginase

3.6 λ_{max} OF L-ASPARAGINASE

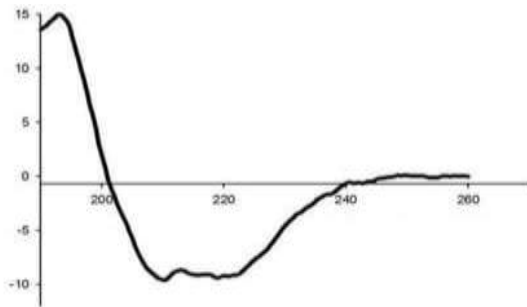


Figure 2. dictates λ_{max} of LASNase

3.7 CALIBRATION CURVE OF L-ASNase

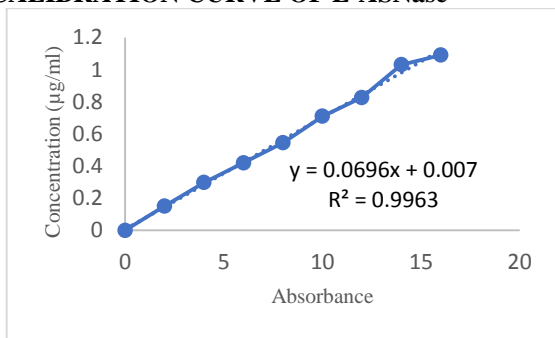


Figure 3. Calibration Curve of L-ASNase in PBS (pH 7.4)

3.8 SEM analysis

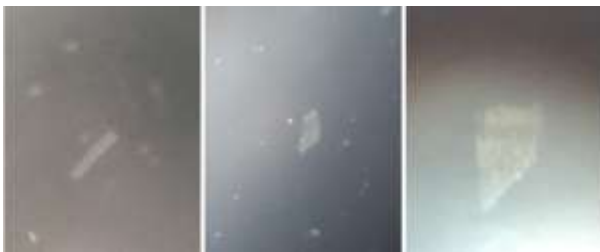


Figure 4. Microscopic Image of L-ASNase loaded Polymeric Micelles

3.9 In-Vitro Release Profile of L-ASNase loaded Polymeric Micelles

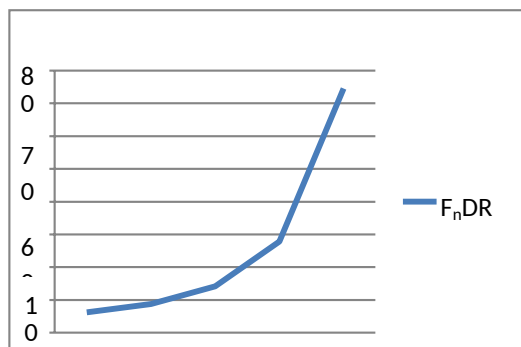


Figure 5. In-vitro Release Profile of L-ASNase

3.10 stability study

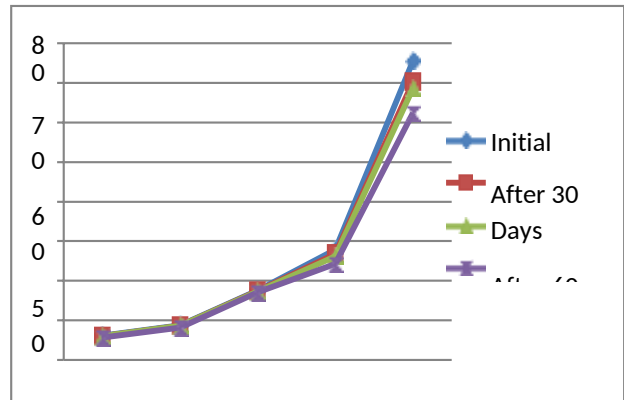


Figure 6. In-Vitro Release Profile of L-ASNase from Optimized Formulation Initially and During Stability

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 $\mu\text{g/ml}$ 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-loaded microparticles. L-ASNase were apparently cylindrical in shape with smooth surface. The particles were slightly aggregated

Zero-order and Krosmeier-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 release study.

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 of time.

5. REFERENCES

1. Jordan VC. "The development of tamoxifen for breast cancer therapy: Long-term tamoxifen treatment for breast cancer." University of Wisconsin Press, Madison. 1994; 3-26.
2. Langan Fahey SM, Jordan VC, Fritz NF, et al. "Clinical pharmacology and endocrinology of long-term tamoxifen therapy: Long-term tamoxifen treatment for breast cancer." University of Wisconsin Press, Madison. 1994;



27-56.

3. Osborne CK, Elledge RM and Fuqua SW. "Estrogen receptors in breast cancer therapy." *Sci Med.* 1996; 3: 32-41.
4. Lien EA, Solheim E and Ueland PM. "Distribution of tamoxifen and its metabolites in rat and human tissues during steady-state treatment." *Cancer Res.* 1991; 51: 4837-4844.
5. Horwitz KB, Jackson TA, Bain DL, et al. "Nuclear receptor coactivators and corepressors." *Mol Endocrinol.* 1996; 10: 1167-1177.
6. Sunderland MC and Osborne CK. "Tamoxifen in premenopausal patients with metastatic breast cancer: a review." *J Clin Oncol.* 1991; 9: 1283- 1297.
7. Haouas H, Haouas S, Uzan G, et al. "Identification of new markers discriminating between myeloid and lymphoid acute leukemia." *Hematology.* 2010 Aug; 15(4): 193-203.
8. Laneville P, Barnett MJ, Bélanger R, et al. "Recommendations of the Canadian Consensus Group on the Management of Chronic Myeloid Leukemia." *Curr Oncol.* 2006 Dec; 13(6): 201-221.
9. Friedreich N. "A new case of leukemia [in German]." *Virchows Arch Pathol Anat.* 1857; 12: 37-58.
10. Minot GR, Buckman TE and Isaacs R. "Chronic myelogenous leukemia, age, incidence, duration and benefit derived from irradiation." *JAMA.* 1924; 82: 1489-1494.
11. George P, Hernandez K, Hustu O, et al. "A study of total therapy of acute lymphocytic leukemia in children." *J Pediatr.* 1968; 72: 399-408.
12. Smith M, Barnett M, Bassan R, et al. "Adult acute myeloid leukaemia." *Crit Rev Oncol Hematol.* 2004; 50: 197-222.
13. Kantarjian HM and Talpaz M. "Chemo-therapy and bone-marrow transplantation in the treatment of chronic myelogenous leukemia." *Semin Oncol.* 1994; 21: 8-13.
14. Moricke A, Zimmermann M, Reiter A, et al. "Long-term results of five consecutive trials in childhood acute lymphoblastic leukemia performed by the ALL-BFM study group from 1981 to 2000." *Leukemia.* 2010; 24: 265-284.