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# Formulation and Characterization of Alginate Chitosan Nanoparticlas of L- Asparginase

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**Abstract:** If any disease is much cautious after heart and infectious diseases, cancer is currently leading cause of death in the world. Researchers have vigorously searched for proper treatment to destroy this disease but precisely the treatment was not found to be enough for the said disease. Administration of potent anticancer bioactive by i.v. route and intense side effects due to action of the drugs on non-target sites. Ultimate cancer therapeutics is desired for successful cancer management. Before the past few decades conventional treatment options such as chemotherapy and radiation have experienced many advances in cancer treatment with lot of limitations and side effect.. With such nonspecific drug action, the concentration of drug rendered available at the tumor site itself is low on the other hand drug entering into a healthy tissue leads to produce-toxicity and ultimately the effective dose became an ineffective dose.

**Introduction**: Cancer has been considered as one of the most common diseases worldwide, for which the new cases are promptly rising every year[1]. In spite of hasty advancements in investigative procedures and therapies, the global survival rate from cancer has not been upgraded significantly over the previous 30 years[2]. Cancer is undoubtedly acknowledged as one of the most prominent basis of mortality in national as well as global scene. More than 15.5 million children and adults with a history of cancer were alive on

1 January, 2016, in the United States. By 1 January 2026, it is estimated that the population of cancer survivors will increase to 20.3 million almost 10 million males and 10.3 million females[3].

If any disease is much cautious after heart and infectious diseases, cancer is currently leading cause of death in the world. Researchers have vigorously searched for proper treatment to destroy this disease but precisely the treatment was not found to be enough for the said disease[4]. Ultimate cancer therapeutics is desired for successful cancer management[5]. Before the past few decades conventional treatment options such as chemotherapy and radiation have experienced many advances in cancer treatment with lot of limitations and side effect[6]. Administration of potent anticancer bioactive by i.v. route and intense side effects due to action of the drugs on non-target sites[7]. With such nonspecific drug action, the concentration of drug rendered available at the tumor site itself is low on the other hand drug entering into a healthy tissue leads to produce-toxicity and ultimately the effective dose became an ineffective dose[8]. To improve this complexity, researchers have been paying attention on fabrication of tumor-specific drugs or delivery systems that can selectively localize existing agents to the tumor sites[9]. Recent advances in nanotechnology promises further developments in target-specific drug delivery systems[10].

**Material & Methods** :

**Preparation of Alginate –chitosan nanoparticles**

Finely requisite quantity of Alginate (10 mg) was dissolved in 10 ml PBS (pH 6.0)with continuous stirring and the reaction was performed at room temperature for 12hrsunder magnetic stirring at 3000 rpm. This solution was added drop wise in above solution with continuous stirring. Alginate –chitosan nanoparticles were formed then solvent was evaporated at room temperature. The resulting suspension of nanoparticles was filtered through 0.45 µm membrane filter (Millipore) and centrifuged for 45 min at 10,000 rpm (Remi, Mumbai, India). The supernatant was discarded and Alginate –chitosan nanoparticles were kept for lyophilization.

**1.Characterization of Nanoparticles**

The optimized formulation chitosan nanoparticles ACHNP were selected and characterized for various paramters size, shape, surface morphology, zeta potential, PDI, % drug entrapment& Cumulative release of drug..

**1.1 Shape and surface morphology**

The topographical study of CHNP was carried out using a Scanning Electron Microscope(SEM, Jeol Philips, MANIT, Bhopal). A tiny drop of the diluted nanoparticles suspension was placed onto a carbon coated copper grid and kept for 15min for drying. Grids were then coated with gold to a thickness of about 300A by using a sputter coater. All samples were examined under a SEM at an accelerated voltage of 10KVand magnification of2500X.

### 1.2 Particle size and zeta potential determination

The average particle size, zeta potential and polydispersity index (PDI) of ACHNP were determined by a Zeta sizer (Malvern Instruments, England, SAILSOPS, RGPV Bhopal). Briefly, nanoparticulate sample dispersion was added in polystyrene cuvettes diluted with ultra-pure deionized water and analyzed at a 90°fixed angle. The zeta potential of NPs formulation was measured by determining electrophoresis mobility with a laser-based multiple angle particle electrophoresis analyzers, Malvern Zeta sizer (DTSVer.4.10, Malvern Instruments, England).

The nanoparticles were suspended in ultra-pure de-ionized water and kept in an electrophoresis cell with an electric field of 15.24 V/cm and the zeta potential was measured.

### 1.3 **Estimation of drug entrapment efficiency**

The drug entrapment efficiency was determined using a dialysis method for separating unloaded L-Asparaginase from the NPs. It is a method to estimate indirectly determined the amount of drug bound with the NPs. Taken 5 ml of the L-Asparaginase –loaded Alginate chitosan NPs dispersion was placed into a dialysis bag of MWCO (Himedia, Mumbai, India) and dialyzed against 50 ml of 0.1N NaOH for 15 minutes with magnetic stirring (50 rpm). After 15 minute 5ml sample withdrawn and replaced with fresh solvent and drug were estimated spectrophotometrically (Noquera*etal*., 2013). ). So as to quantify indirectly the amount of entrapped L-Asparaginase within the NPs.

### 1.4 *In-vitro* release study

*In-vitro* release profile of optimized formulation L-Asparagianse Alginate-Chitosan Nanoparticle (ACHNP) was determined using dialysis tube. 5 ml L-Asparaginase loaded chitosan NPs was placed into dialysis bag of MWCO 12000KDa, (Himedia,Mumbai India), tied at both the ends are placed in a beaker containing 1000 ml of phosphate buffer (pH 7.4). The beaker was placed over a magnetic stirrer at 100 rpm and the temperature maintained at 37±1ºC throughout the procedure. At specific time intervals, 5ml sample was collected and replaced with fresh PBS and sink condition was maintained (Garg*et al*., 2015; Kesharwani *et al*., 2015). Samples were quantified spectrophotometrically at λ max 303 nm.

**Result & Discussion:**

Characterization of Nanoparticles

**Table 1:Effect of Alginate concentration**

| S.No. | Conc.Of Alginate (mg) | BufferSolution(pH6.0) (ml) | Particles ize(nm) | % Entrapment Efficiency | Polydispersity Index | Zeta Potential(mV) |
|---|---|---|---|---|---|---|
| ACHNP1 | 5 | 10 | 135.16 ±1.58 | 82.24±3.42 | 0.075±0.081 | +42.13 |
| ACHNP2 | **10** | **10** | **139.54 ±1.41** | **85.24±3.15** | **0.085±0.074** | **+35.17** |
| ACHNP3 | 15 | 10 | 134.29 ±1.53 | 86.14±2.34 | 0.070±0.069 | +48.81 |
| n=3,Mean±S.D. | | | | | | |

**Table 2: In vitro percent cumulative drug release from ACHNP formulations in phosphate buffer (pH 7.4)**

| Time( inhrs) | %cumulative Drug release from Alginate chitosan nanoparticles |
|---|---|
| 0.25 | 0.89 ± 0.32 |
| 0.50 | 1.88 ± 0.42 |
| 1 | 6.14 ± 0.38 |
| 2 | 18.24 ± 0.28 |
| 4 | 30.85 ± 0.42 |
| 8 | 43.42 ± 3.25 |
| 12 | 50.42 ± 2.14 |
| 24 | 54.13 ± 3.42 |
| 48 | 59.42 ± 2.17 |
| 72 | 63.85 ± 1.62 |
| 84 | 68.74 ± 1.90 |
| 96 | 72.87 ± 2.54 |

Fig. 1: Particle Size of Alginate Chitosan Nanoparticles

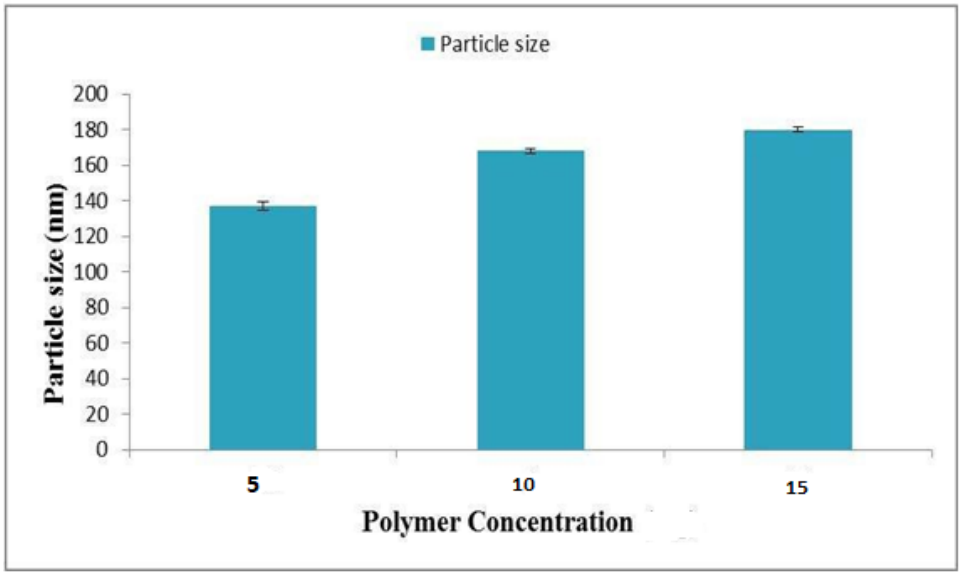


Fig 2: Entrapment efficiency of Alginate chitosan Nanoparticles

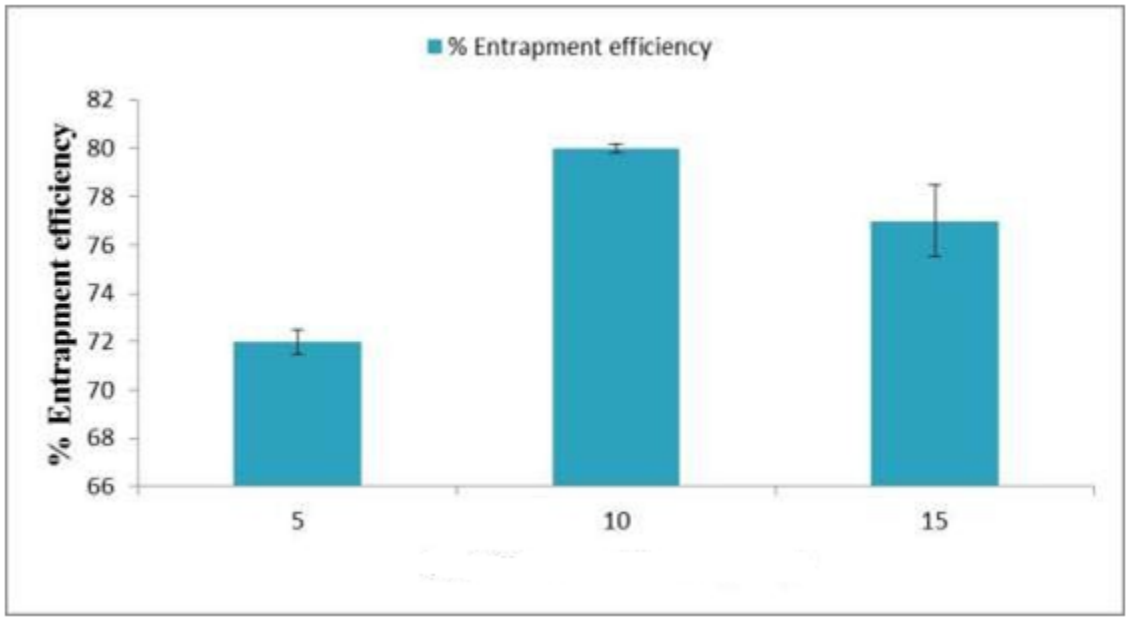



Fig 3: Polydispersity Index of Alginate chitosan nanoparticle

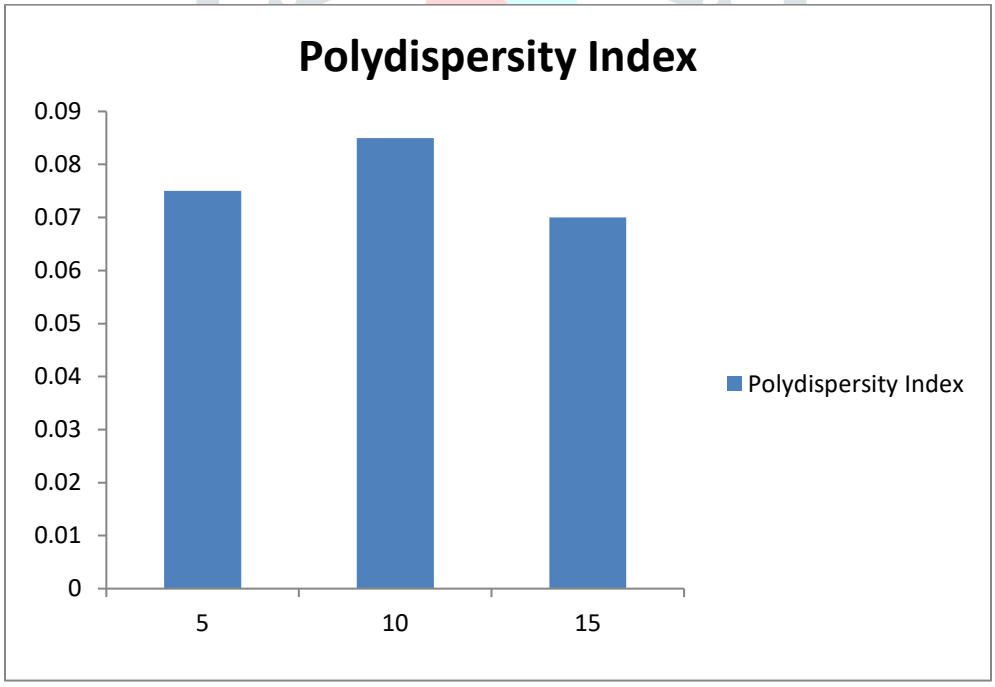


Fig 4: Zeta Potential of Alginate Chitosan Nanoparticle

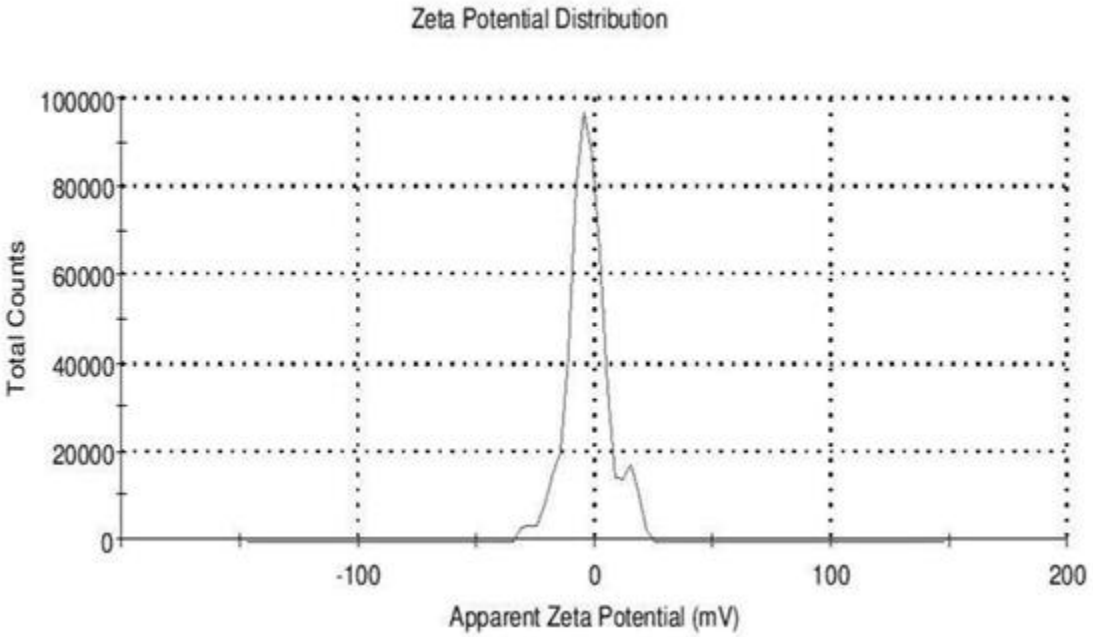



Fig 5: In-vitro release of Alginate Chitosan Nanoparticles

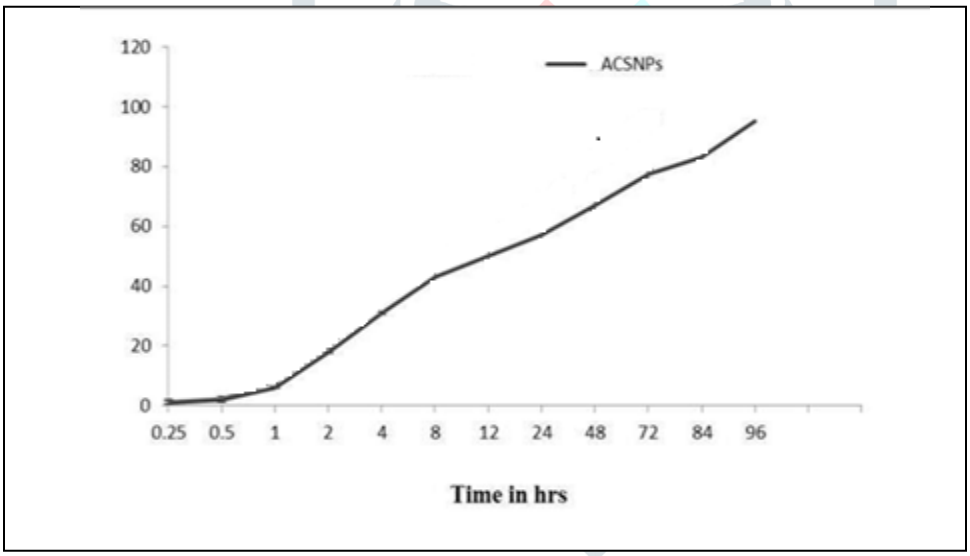
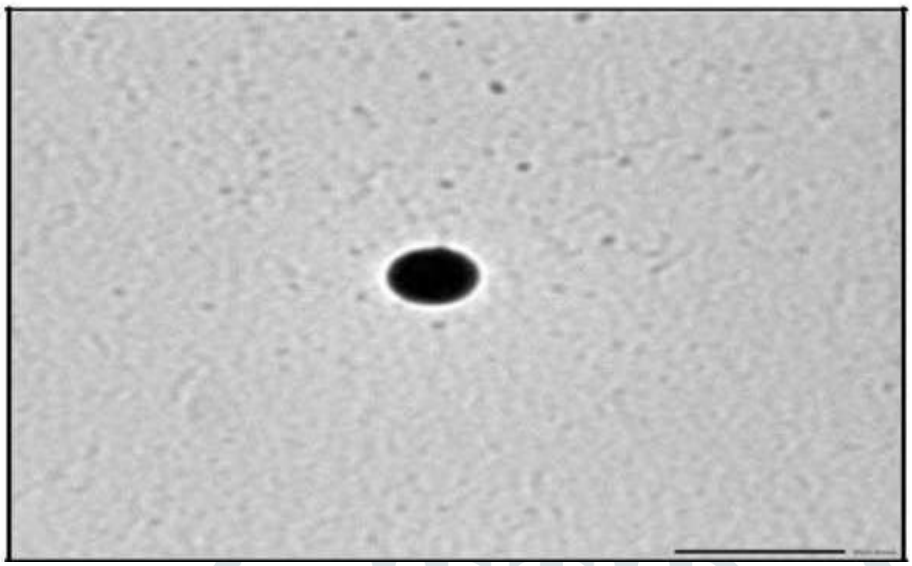


Fig.6: SEM image of ACHNP



For fabrication of Alginate anchored chitosan nanoparticles, we selected ionic interaction method. As chitosan has positive charge and Alginate negative charge for good coating of A on surface of chitosan nanoparticle. Concentration of A is need to optimized initially 5 mg of Alginate solution was added on surface of chitosan. With increasing concentration of hyaluronic acid (5 to 10 mg) the particle size of Alginate Chitosan NPs was increased (135.16 ± 1.58 to 139.54 ± 1.41 nm) and entrapment efficiency was increased (82.24 ± 3.42 to 85.24 ± 3.15). When the concentration of Alginate (10to15mg) was increased, the particle size of NPs was decreased (139.54± 1.41 to 134 ± 1.53) and entrapment efficiency of Alginate-Chitosan NPs was increased (85.24 ± 3.15 to 86.14 ± 2.34). When the concentration of A was increased (10 to 15mg) little or no effect on particle size and entrapment efficiency observed.

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