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CHITOSAN-BASED NANOSPHERES AS DRUG DELIVERY SYSTEM FOR CYTARABINE

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ABSTRACT

Nanoparticle made up of biodegradable carrier such as chitosan have advantage of providing stearic interference in the systemic circulation. Cytarabine nanospheres were prepared by ionic gelation method with an objective of improving its intracellular targeting and thereby targeting the cancer cells. The average particle size determined through SEM was found to be 466.45 ± 5.32 nm. The average drug loading was found to be 62% for the batch loaded with 1.50 mg/ml of drug when compared to other batches. The cumulative percentage of drug release from all the drug loaded batches at 18 hours was found to be in the range of 86.73 % to 93.50%.Among the four batches of nanoparticles formulated the batch containing 1.50mg of drug/ml of polymer showed the highest of about 93.50% of release. Release of drug from the matrix was by non-Fickian analomous diffusion mechanism. *Invivo* bio distribution studies showed that the Cytarabine in the form of nanoparticles was having a greater bio distribution when compared to free drug in different organs like liver, spleen, lungs and kidney.

KEY WORDS

Cytarabine, Chitosan, Ionic Gelation, Bio-Distribution.

INTRODUCTION

In recent years, there has been a considerable interest in the development of novel drug delivery system in order to modify and control the pharmacokinetic behavior of the drug [1, 2]. By incorporating in to carrier system, it is possible to alter the therapeutic index and duration of activity of drugs. Nanoparticles are colloidal drug delivery system that offers site specific as well as controlled drug delivery[3]. The major limitation of current anticancer, anti-parasitic and anti-infectious drugs are their toxicity and lack of specificity, to overcome such problems an endocytosable carrier such as microsphere, liposomes and nanoparticles can be used to make the treatment more effective [4, 5].

Nanoparticles are capable of delivering drugs to the lysosomes of phagocytes of the mononuclear phagocyte system after intravenous administration. The macrophage that is in blood and lymph serve as site for proliferation of certain microbes during infection process. Using antibiotics in mixtures with polymers or modifying antibiotics by attaching them to polymer prolong their biological

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action in the human body, decreases toxicity, improves solubility and increases resistance to the action of enzyme as the major limitations of current anticancer, ant parasitic and ant infectious agents are their toxicity and lack of specificity. The ideal dosage form in cancer chemotherapy is the one that provides a specific delivery of anticancer agent to the tumor site in sufficient amount for a long period of time with no interaction with normal tissue [6, 7, 8]. Nanoparticles containing cytotoxic agents could be useful for the treatment of certain cancer that often shows resistance to uptake of free drug. Nanoparticles bound antitumor drugs have demonstrated a prolonged survival of the tumor bearing animals [9, 10].

On the contrast, the Cytarabine administration through daily IP injection results in peaks, generally of high plasma concentration of Cytarabine which could cause toxicity due to shorter half-life, hence different system or devices has been developed to maintain suitable levels of Cytarabine, for an optimum period of time, in these fluids [11].

The activity of Cytarabine is decreased by its rapid deamination with cytidine deaminase to the biologically inactive metabolite uracil arabinoside. Protection of Cytarabine from fast degradation and elimination has been investigated by encapsulating the drug into pharmaceutically acceptable carriers. Because it can increase the bioavailability of the drug and it can reduce the toxic effects which is produced by the pure drug when it is given in the nanoparticle form to the body. Chitosan which is a natural polymer widely used in pharmaceutical area has a good biocompatibility, biodegradability, bio adhesion, film forming activity, antimicrobial effect and shows a good safety profile has made it best polymer for drug delivery system [12]. Hence in the present study, an attempt has been made to formulate, optimize and to check the suitability and potentiality of natural carrier such as chitosan for the selected anticancer drug.

MATERIALS AND METHOD

Cytarabine was obtained as a gift sample from Naproad neon, Mumbai. Chitosan, Glacial acetic acid and Sodium Tri-poly phosphate was purchased from Sigma chemicals, Mumbai. Acetone, acetonitrile and Water are of HPLC grade. Potassium dihydrogen phosphate, Sodium hydroxide and Cedar wood oil are of lab grade.

Formulation of Cytarabine nanospheres

Preparation of chitosan gel

200 mg of chitosan was dispersed in 50 ml of 5% glacial acetic acid solution and stirred for 4 hours continuously to obtain 0.4% chitosan gel solution. Then it was stabilized for overnight to obtain clear chitosan gel.

Ionic gelation method

To 3ml of 0.4% chitosan gel, Cytarabine 0.50mg/ml was added and stirred and then 1.2ml (0.5%w/v) of Tripolyphosphate solution which is the cross linking agent was added. Chitosan nanoparticles formed spontaneously upon addition of aqueous Tripolyphosphate solution to chitosan solution under high speed rate of 3000 rpm using high speed stirrer for 1hr. The resulting chitosan particle suspension were subsequently centrifuged four times for 15 minutes cycles at 10,000 rpm and washed with distilled water and freeze dried [13]. This batch was named as Chito-1.

Similarly other four batches of drug-loaded nanospheres were prepared by following the same procedure. But the amount of drug added were 1.00mg/ml (Chito-2), 1.50mg/ml (Chito-3), 2.00mg/ml (Chito-4) and 2.50mg/ml (Chito-5). Here only the concentration of drug was varied and the

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concentrations of chitosan gel were kept constant [14]. (Figure 1)

Estimation of amount of drug incorporated to chitosan nanospheres

A known quantity of drug loaded nanospheres (33mg) from each batch was dispersed in 100 ml of normal saline and sonicated for half an hour. The solution was centrifuged at 10000rpm for 15mins, and the absorbance was determined using UV spectrophotometer at 254nm with plain chitosan nanospheres as reagent blank [15].

Determination of particle size Scanning Electron Microscopy analysis (SEM)

An aqueous dispersion of the nanospheres was finely spread over a gold-coated stab and was dried by keeping in a desiccator. The dried film of the nanospheres was given a 25 nm thick gold layer and was observed by SEM. The sizes of minimum of 50 particles were checked for their size distribution to determine the average particle size and size range [16]. (Figure 1)

Study on invitro drug release

A quantity of nanospheres equivalent to 20.79mg of drug was taken in a 250ml conical flask and to it 100ml of pH 7.4 phosphate buffer was added. Then the flask was kept in a shaker cum incubator and shaker was adjusted to 40-50 horizontal stokes / min at 37˚C. 2ml of drug release medium was withdrawn at various time intervals of 0.5, 1, 2, 4, 6, 8, 12, 15 and 18h while replacing it with fresh 2ml of normal saline. The samples were centrifuged and filtered. From the filtrate 1ml of the sample was withdrawn and diluted to 10 ml with normal saline and the drug content was analyzed by UV Spectrophotometer at 254nm [17]. The in vitro release of drug from all the batches of nanospheres is depicted in Figure 2.

Determination of kinetics of drug release

In order to predict and correlate the release behavior of the drug from the polymer matrix it is necessary to fit *invitro* release data in to a suitable model. Hence the dissolution data were fitted according to the well-known exponential equation, which is often used to describe the drug release behavior from polymeric system.

The equation, which is used to describe drug release mechanism, is:

$$m_t / m_\infty = k\, t^n$$

Where m_t / m_∞ is the fraction release of the drug 't' is the release time 'k' is the constant. Which indicates the properties of the macromolecular polymer system, and 'n' is the release exponent indicative of the mechanism of release. The 'n' value was used for the analysis of drug release mechanism from the drug-loaded nanospheres [18].

Invivo bio distribution studies

Invivo bio distribution studies were carried out using Wister rats weighing 100 to 150 g and were divided into 3 groups containing 3 animals each. The three groups namely group I treated with free drug, group II treated with drug loaded nanospheres and group III treated with solvent control. On the first day the mice of group I were treated with free drug of 3.6mg/200g of rats through intravenous route. The similar concentration of Cytarabine nanospheres was administered to group II and phosphate buffer pH 7.4 as solvent control for group III. After18h of injection, animals were sacrificed, then blood was taken and plasma was separated out, and also different organs like liver, lung, kidney and spleen were extracted out and homogenized in pH 7.4 phosphate buffer saline followed by centrifugation. Supernatant of the homogenized tissue were analyzed by HPLC to estimate the bio distribution of the drug administered

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in pure and nanospheres bound form [19]. The above mentioned study was approved by Institutional animal ethics committee (IAEC) baring the proposal number JSSCP/IAEC/M.PHARM/PH.CEUTICS/09/2006-07.

RESULTS AND DISCUSSIONS

Cytarabine nanoparticles were prepared by ionotropic gelation method with chitosan and 0.50%w/v Tripolyphosphate solution was used as a cross-linking agent. The chitosan molecules has abundant NH_3 group which can react with negatively charged phosphoric ions of TPP to form cross-linked chitosan nanoparticles [20, 21]. During the process of cross-linking and hardening process water was extruded from the particles, which may help in sustaining the release of drug. The particle size of the nanoparticles was determined using Scanning Electron Microscopy (SEM) and the average particle size for drug loaded nanoparticles was found to be 466.45 ± 5.32 nm [23].

To determine the carrier capacity of chitosan with respect to Cytarabine, various batches of nanoparticles containing 0.50, 1, 1.50,2 and 2.50 mg of drug were prepared. The concentration of polymer was kept constant. Table 6 shows the percentage drug loading of various drug-loaded batches. From the results obtained it was observed that as the concentration of drug increases, loading of the drug increases but after certain concentration the loading efficacy decreases due to saturation of the polymer matrix. The batch containing 1.50 mg/ml of drug showed a good drug loading when compared to other batches [24].

To evaluate the release of drug from all the drug loaded batches, an *in vitro* release study was undertaken by centrifugal ultra-filtration method. Figure 2 shows the cumulative percentage release of drug-loaded batches at various time intervals. From the graph it can be interpreted that all the drug-loaded batches show a biphasic pattern of initial burst effect and the remaining amount of drug was found to be released slowly in a sustained manner. The cumulative percentage of drug release from all the drug loaded batches at 18 hours was found to be in the range of 86.73 % to 93.50%. Whereas among the four batches of nanoparticles formulated the batch containing 1.50mg of drug/ml of polymer showed the highest of about 93.50% of release when compared to other batches. So this batch as with highest drug loading and a good invitro release was selected for the further in vivo bio distribution studies [25].

To study the release kinetics of the formulated Cytarabine nanoparticles the dissolution data's were fitted to a well-known exponential equation. The 'n' value used for the chitosan nanoparticles were determined from log % drug release vs. log time plots. Table 7 shows the values of n and r for drug loaded batches, and n value was found to be more than 0.5 so it was found that the mechanism by which drug is being released is a non-Fickian analomous diffusion mechanism, that is drug release during dissolution test is controlled by all diffusion, erosion and swelling mechanism.

The batch of nanoparticles with a better drug loading capacity and higher *invitro* release was subjected for invivo bio distribution study. A simple invivo study was carried out, where three different groups of animals were treated with free drug, drug loaded nanoparticles and solvent controls. Then the organs were extracted, homogenized, centrifuged and the drug content in the organ was determined by HPLC. The results shows that the Cytarabine in the form of nanoparticles was having more bio distribution when compared to free drug in different organs like liver, spleen, lungs and kidney. And also Cytarabine nanoparticles were found to be distributed more in the lungs and liver and next to the organs of spleen and

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kidney. This is due to the intravenously injected nanoparticles which have the priority too large to pass efficiently across epithelial and endothelial cell layers.

Table 1.
The amount of Cytarabine loaded in chitosan nanospheres

S.NO	Amount of drug per ml of polymer(mg/ml)	Drug loading (mg)
Chito A	0.50 mg	0.22
Chito B	1 mg	0.52
Chito C	1.50 mg	0.93
Chito D	2 mg	1.02
Chito E	2.50 mg	1.23

Table 2.
Invitro release Kinetics

Batch No	Drug concentration (mg/ml)	Korsmeyer and Peppas	
		Slope value (n)	Correlation Coefficient (r^2)
A	0.50	0.643	0.938
B	1.00	0.626	0.935
C	1.50	0.699	0.953
D	2.00	0.608	0.949

Table 3.
Bio distribution of free drug Cytarabine and Cytarabine loaded nanoparticles

S.No.	Organs	Amount of drug distributed (ng)	
		Free drug	Drug loaded Nanoparticle
1	Liver	36.2	67.02
2	Spleen	45.42	55.01
3	Kidney	55.84	48.03
4	Lungs	27.27	60.85

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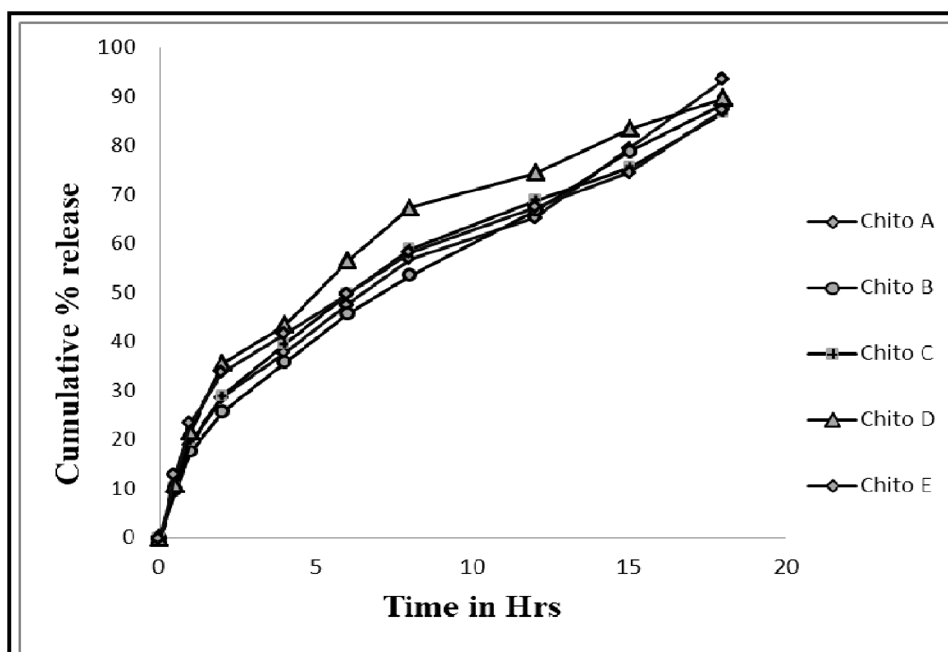

Fig 1. *Invitro release profile of all drug loaded batches*

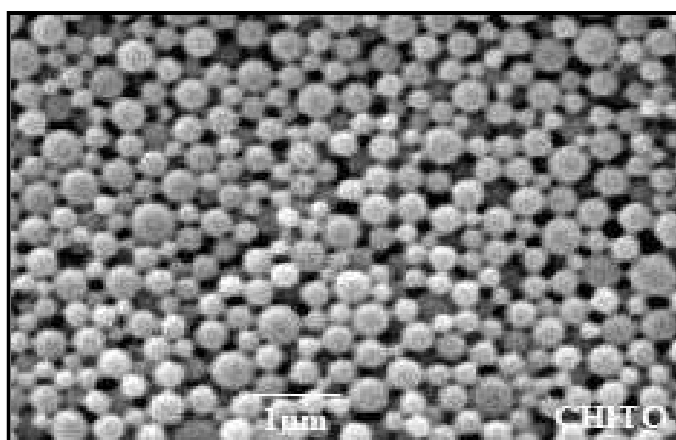

Fig 2. *SEM of Drug Loaded nanoparticles prepared by Ionic Gelation Method (Batch-C)*

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CONCLUSION

The formulated Cytarabine nanoparticles with chitosan as a carrier was found to be a suitable and potential natural carrier in terms of their particle size, drug loading capacity, invitro release characteristics and invivo bio distribution study. Hence it may be used as an alternative and cheaper carrier in site specific delivery of anticancer drug. In turn it may be useful in reducing the total cost of the therapy. And also the toxicity and side effects of the drug may be considerably reduced after linkage to nanoparticles because of the modified tissue distribution of the agent.

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