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Preparation and characterization nanoparticles of cytarabine: exemplification, stockpiling and in-vitro discharge

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Abstract

This work presents results of the preparation and characterization of nanoparticles for entrapping cytarabine, a chemotherapeutic agent. The particle size analysis indicated a uniform particle size. The study of the release of drug from nanoparticles exhibited a prolonged release profile as studied over a period of 16 hours. The drug release was constant from the 10th to the 16th hours, which showed that the formulation was successful for long-term treatment. The drug entrapment efficiency of the nanoparticles having the same ratio of polymer and drug was about 90.2%. The physical stability of the nanoparticles was good as studied over a period of 4 weeks. These results are promising for producing nanoparticles by entrapping cytarabine, which can be useful for cancer therapy.

Keywords: Cytarabine, nanoparticles, *in-vitro* release, sodium tripolyphosphate.

INTRODUCTION

The use of biodegradable polymers as drug carriers has long been of interest in controlled- release technology because of the ability of these polymers to be reabsorbed by the body. The field of biodegradable polymers is progressing rapidly, so that researchers now have substantial number of degradable polymers with a range of degradation rates. Micro and nanoparticulate systems formulated with these polymers have shown wide applicability of oral delivery, subcutaneous injection and sustained delivery of lipophillic and some hydrophilic drugs (Barichello et al., 1999). One way of achieving the transport of drug directly by a delivery device is by means of liposomes which can deliver drugs to the desired location in the body and reach a high local concentration. More recently solid submicronic drug carrier of a polymeric nature in the nanometer size range (nanoparticles) has been proposed as biological target. Nanoparticles can be employed as carriers for targeting of drugs, thereby reducing toxicity as in case of PLA nanoparticles containing primaquine and also for increasing the bioavailability of drug as in case of nanoparticles containing drug cyclosporine. Since its introduction into clinical practice cytarabine has an important role in the treatment of acute lymphocytic and granulocytic leukemias and various types of tumors (Tricot et al., 1984; Roberts et al., 1985; Winter et al., 1985). Cytarabine is a S phase specific drug. Prolonged exposure of cells to cytotoxic concentration is critical to achieve maximum cytotoxic activity. Activity of cytarabine is decreased by its rapid deamination to the biologically inactive metabolite uracil arabinoside. The rapid deamination is the reason for ongoing search for effective formulation and derivatives of cytarabine that cannot be deaminated and exhibit better pharmacokinetic parameters. Protection of cytarabine from fast degradation and elimination has been investigated by encapsulating the drug into pharmaceutical carriers. The narrow therapeutic index, high volume of distribution, low lipophillicity and poor tissue specificity requires cytarabine to be delivered as nanoparticles (Allen et al., 1992; Zou et al., 1994). Due to this, various problems result when administered orally so the alternative route of administration is the parenteral route. The purpose of this investigation was to develop and characterize cytarabine encapsulated nanoparticles and to study the *in-vitro* release and its storage.

MATERIALS AND METHODS

Cytarabine (10 g) was obtained from Biochem. Labs, Mumbai, Chitosan was obtained from Central Institute of Fisheries Technology, Cochin. All other reagents were of analytical grade. UV spectrophotometer Model 1700-E (SHIMADZU, Japan). Sonicator (Vibra Cell), Zetasizer (Malvern HAS 3000),

Table 1. Encapsulation efficiency of chitosan nanoparticles using sodium tripolyphosphate as cross-linking agent.

S. No.	Batch code	Ratio polymer: Drug	Encapsulation efficiency (%)
1.	Cy-A	1:1	90.2 ± 0.7
2.	Cy-B	2:1	87.4 ± 1.2
3.	Cy-C	3:1	86.7 ± 0.7
4.	Cy-D	4:1	85.2 ± 0.5

Four Batches of formulation (Cy-A,Cy-B,Cy-C,Cy-D)containing polymer(Chitosan):Drug (Cytarabine)in the ratio of 1:1,2:1,3:1,4:1. Encapsulation efficiency denotes the % of drug encapsulated within the Chitosan nanoparticles using tripolyphosphate as cross linking agent. Cy-A showed better encapsulation Efficiency.

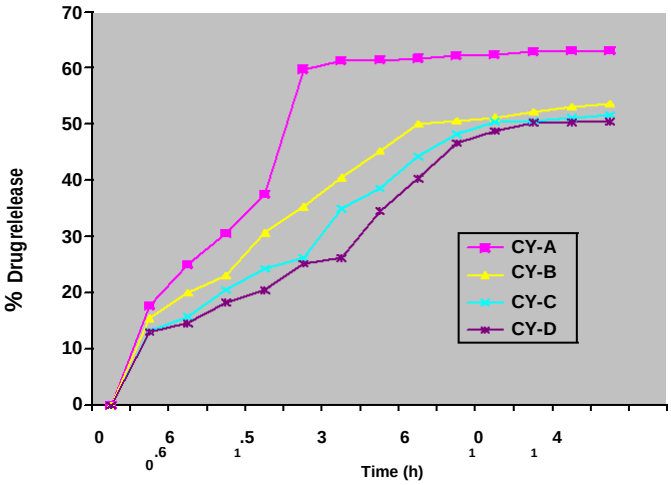

Figure 1. *In-vitro* release of chitosan nanoparticles using sodium tripolyphosphate as cross-linking agent. Figure 1 shows the plot between time in hours and % drug release. Figure showed that CY-A exhibited controlled drug release from the third hour.

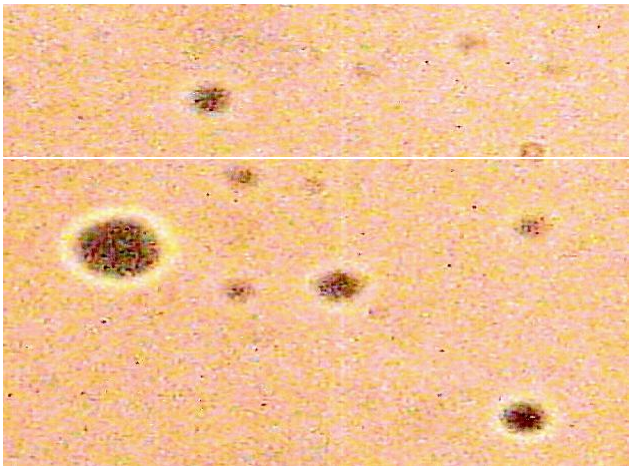

Figure 2. SEM of Cytarabine containing nanoparticles. Figure 2 shows the Scanning Electron Microscopy of the nanoparticles. The nanoparticles show a spherical shape.

Transmission Electron Microscope (TEM), Phillips 1011, Scanning Electron Microscope (HITACHI S 450).

Preparation of nanoparticles

Cytarabine nanoparticles were prepared via a modification of the ionic cross-linking technique (Yong-Zhuo et al., 2005) . Cytarabine was dispersed in deionized water and added to an acetic acid solution containing Chitosan. Sodium tripolyphosphate (6% w/v) was then added to the above solution while stirring. After 30 min, the cross -linked solution containing cytarabine nanoparticles was obtained. Then it was filtered through 0.45 m filter membrane after sonicated by ultrasound probe (400 w).

Morphological characterization

The surface morphology of nanoparticle was observed by transmission electron microscopy.

Encapsulation efficiency analysis

An aliquot of the above suspension was added to the sample reservoir and centrifuged for 10 min at 12,000 rpm. The supernatant filtrate was assayed to determine the concentration of the un-encapsulated drug. Another aliquot of the suspension was added to methanol (1:10, v/v) and sonicated for 10 min in a bath sonicator to release the encapsulated molecules, which were quantitated to determine the total drug concentration. Encapsulation efficiency (%) was calculated by the following formulae.

Encapsulation efficiency % = [1- (Un-encapsulated drug/ total drug)] x 100.

***In-vitro* release of cytarabine from nanoparticles**

After separation of the free drug, the nanoparticle preparation was transferred to a dialysis tube and subjected to dialysis with the dialysis tube immersed in a phosphate buffer saline pH 7.4 (100 ml). At different time intervals, samples were withdrawn from the receptor compartment and the drug content was determined spectrophotometrically at 272 nm. An equal volume of phosphate buffer saline replaced the samples that were withdrawn.

Stability studies of cytarabine nanoparticles

The formulated nanoparticles were separated into three portions. One portion was kept at room temperature, the second at 45^{o}C and the third at 4^{o}C for 1 month. At weekly intervals, these were then evaluated for their drug content and release characteristics.

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Table 2. *In vitro* release of chitosan nanoparticles using sodium tripolyphosphate as cross-linking agent.

S. No.	Time (h)	Cy-A(%)	Cy-B(%)	Cy-C(%)	Cy-D(%)
1	0	0	0	0	0
2	0.5	17.6	15.5	13.2	13.0
3	0.66	25.0	20.0	15.8	14.6
4	1	30.5	23.0	20.8	18.3
5	1.5	37.5	30.7	24.3	20.5
6	2	59.8	35.3	26.2	25.2
7	3	61.3	40.5	35.0	26.2
8	4	61.4	45.2	38.6	34.5
9	6	61.7	50.1	44.3	40.3
10	8	62.2	50.6	48.2	46.7
11	10	62.3	51.2	50.5	48.8
12	12	62.9	52.2	50.6	50.3
13	14	63.0	53.1	51.2	50.4
14	16	63.1	53.6	51.6	50.5

The values of Cy-A, Cy-B, Cy-C, Cy-D are the % drug released at different time intervals. Table 2 showed that Cy-A showed controlled drug release from the third

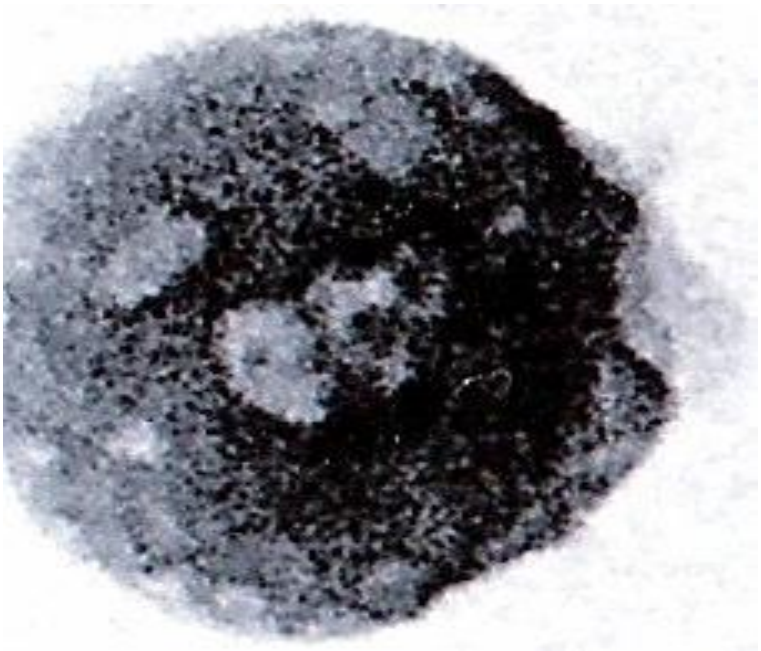

Figure 3. TEM of Cytarabine containing nanoparticles. Figure 3 showed the surface morphology of nanoparticles.

RESULTS

The preparation method of nanoparticles produced well-formed nanoparticle with good morphological characteristics (Figure 2). Transmission Electron Micro-scopy characterized the particle size and shape (Figure 3). Table 1 showed the encapsulation efficiency of nanopartticles. The electrostatic interaction is advantageous for nanoparticle formation (Yang et al., 2005). Zeta potential has been used for characterizing colloidal drug delivery system (data not shown). The particle size of the formulation CY–A was good and it was showing excellent controlled release property (Table 2) and (Figure 1). Drug release showed that ionic reaction of Chitosan- sodium tripolyphosphate is dependent on the pH of tripolyphosphate solution. The ionization degree of tripolyphosphate

Table 3. Stability studies of Nanoparticles (Cy-A).

Time	4°C	% of drug remaining	
		Room temp.	45°C
Initial	100	100	100
1st Week	98.9	98	98
2nd Week	98	98	96
3rd Week	98	98	90
4th Week	97.6	97.9	80

Stability Studies of the batch (Cy-A) when stored at conditions 4oC, room temperature and 45oC for one month and % of drug remaining was calculated. 4oC and room temperature is the suitable storage condition for the formulation.

is dependent on the pH value of solution.

The *in vitro* release profile of cytarabine from Chitosan nanoparticles showed that cytarabine release was quite rapid followed by a very slow drug release (Table 2 and Figure 1). The initial release of drug is associated with those drug molecules dispersing close to the nanoparticle surface, which easily diffuse in the initial incubation time. Since the size of cytarabine molecule is small, cytarabine molecule, diffuse easily through the surface or pores of nanoparticles. Therefore dissolution process suggests the penetration of release medium into the particles due to the hydrophilic nature of Chitosan and followed by dissolving the entrapped cytarabine. The stability analysis data (Table 3) showed that nanoparticle can be stored at room temperature and at $4^{o}C$.

DISCUSSION

The study demonstrated that nanoparticles of cytarabine can be prepared by modification of ionic cross linking method using sodium tripolyphosphate as cross linking agent. Chitosan molecular weight, polymeric composition and polymer/drug ratio in the nanoparticles did not influence the particle size characteristics (Genta et al., 1998**)**. Because chitosan and the drug is oppositely charged, their electrostatic interaction may be advantageous for the formation of Chitosan nanoparticles that exhibit high encapsulation efficiencies (Yang et al., 2005). The optimal formula for high encapsulation efficiency of the drug was found to be polymer: drug (1:1) (Table 1) and the particle size of the nanoparticle was found to be good (Data not shown). The release study (Table 2 and Figure 1) also showed that the formulated nanoparticle showed a controlled release in the acidic pH. In original tripolyphosphate solution (pH 8.6) tripolyphosphate was dissolved into OH^- and ($HP_3O_{10}^{4-}$ and $P_3O_{10}^{5-}$). However, in low pH, only $P_3O_{10}^{5-}$ anions exists. Moreover chitosan is a weak polybase and as pH of solution decreased the ionization of amine group of chitosan increased (Mi et al., 1990). Tripolyphosphate – chitosan nanoparticles prepared in original tripoly-phosphate solution are dominated by deprotonation and slightly ionic cross-linking, but chitosan nanoparticle prepared in acidic tripolyphosphate solution are completely ionic cross-linking dominated (Mi et al., 1990; Mi et al., 1996; Shu and Zhu, 2000; Shu and Zhu 2001; Shu and Zhu 2001, Lee ST et al 2001). Stability studies predict that the suitable storage condition for the nanoparticles to be 4^{oC} and room temperature (Table 3). Thus desirable goals can be achieved by a systematic formulation approach in the shortest possible time with a reduced number of experiments thereby reducing the cost of development of the formulations.

REFERENCES

Allen TM, Mehra T, Hansen C, Chin YC (1992). Stealth liposomes: an improved sustained release system for 1- -D arabinofuranosyl cytosine. Cancer Res. 52: 2431-2439.

Ida Genta, Pola Perugini, FrancePavanetta (1998). Different molecular weight chitosan micro spheres, Influence on drug loading and drug release. Drug Development and Industrial Pharmacy. 24:779-84..

Jose Mario Barichello, Mariko Morishita, Kozo Takayama(1999). Encapsulation of hydrophilic and lipophillic drugs in PLGA nanoparticles by nanoprecipitation method. Drug Development and Industrial Pharmacy. 25:471-6..

Lee ST, Mi FL, ShenYJ (2001). Equilibrium and kinetic studies of copper (II) ion uptake by chitosan –tripolyphosphate chelating resin. Polymer. 42:1879-92.

MI FL, Shyu SS, Kuan CY, Lee ST, Lu KT, Jang SF (1990). Chitosan polyelectrolyte complexation for the preparation of gel beads and controlled release of anti- cancer drug.Enzymatic hydrolysis of polymer. J. Appl. Polym. Sci. 74: 1868-1879.

Mi FL, Shyu SS, Lea ST, Wong TB (1996). Kinetics study of chitosan-tripolyphosphate complex reaction and acid-resistive properties of the chitosan-tripolyphosphate gel beads prepared by liquid curing method. J. Polym. Phys. 37: 1551-1564.

Roberts JD, Ershlew WB. Tindle (1985). Hollow dose cytosine arabinoside in the treatment of myelogenous leukemia Cancer. 56: 1001-1005.

Shu XZ, Zhu KJ (2001). Chitosan/gelatin micro spheres prepared by modified emulsification and ionotropic gelation. J. Microencapsulation. 18:237-245.

Shu XZ, Zhu KJ (2000). A novel approach to prepare tripolyphosphate /chitosan complex beads for controlled drug delivery. Int. J. Pharm. 201:51-58.

Shu XZ, Zhu KJ (2001). Novel pH sensitive citrate cross-linked chitosan film for drug-controlled release. Int. J. Pharm. 212: 19-28.

Tricot G, DeBock R, Dekker (1984). Low dose cytosine arabinoside (Ara C) in myelodysplastic syndromes. Br. J. Haematol. 58: 231-240.

Winter JN, VariakojisD, Gaynor ER (1985). Low dose cytosine arabinoside (Ara-C) therapy in myelodysplastic syndromes and acute leukemia. J. Cancer. 56: 443-449.

Yang Wu, Wuli Y, Chang CW, Jian-hua H, Shoukuan Fu (2005). Chitosan nanoparticles as novel delivery system for Ammonium glycyrrhizinate. Pharmaceutical Nanotechnology. Inter. J. Pharm. 295:235-245.

Yong-Zhuo H, JIan-Qing C, Wen-Quan L, Shinsaku N (2005). Preparation and characterization of liposomes encapsulating chitosan nanoparticles. Biol. Pharm. Bull. 28(2): 387-390.Zou Y, Ling YH, Vant NT, Priabe W. Perz Solar R (1994). Antitumour activity of free and liposome–entrapped Kanamycin,alipophillic anthracyclin antibiotic with non cross resistance properties .Cancer Res. 54: 1479-1484.