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Formulation and Evaluation of Herbal Extract of *Allivum sativum* (Garlic) Loaded Chitosan Nanoparticle

Gupta Durgesh Kumari [1]*, Kesharwani Shivangi, Sharma N.K., Gupta Mahesh K.

Oriental College of Pharmacy & Research, Oriental University, Indore, India

ABSTRACT

The present study aim to get novel approach of herbal chitosan nanoparticle to get improved efficacy of the drug with a less side effect. The present study include formulation and evaluation of chitosan loaded nanoparticle of *Allivum sativum* by using ionic gelation method. In present study five different formulations were prepared by using the different ratio of polymer and TPP. The prepared nanoparticle was evaluated for its particle size, zeta potential, scanning electron microscopy (SEM), production yield, encapsulation efficiency (EE), stability and *in-vitro* drug release. The optimized *Allivum sativum* extract loaded chitosan nanoparticle was found with production yield of 73.59%. They were found round shape and with smooth surface. The *in-vitro* release profile sustained up to 90.65 %. Thus results have found F1 formulation was more stable. It showed highest *in-vitro* drug release. So it was found that F1 was best formulation among the all batches prepared. This formulation can be further use to treat disease like cancer.

Keywords: Chitosan nanoparticle, SEM, *Allivum sativum*, Anti-cancer activity, Chemotherapy

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***Address for Correspondence:**

Durgesh Kumari Gupta, Oriental College of Pharmacy & Research, Oriental University, Indore, India

INTRODUCTION

In India, various herbal medicines have been used for centuries to treat various health problems. It involves that plants or mixture of plant extract to treat illness and health promote [1, 2]. Over the past decades, in developing countries due to low economic rate and majority of populations and high cost of Western medicine, because of that herbal medicine has an uninterrupted history of continuous usage by maximum population in the developing country, in developing country due to their cultural and spiritual points herbal medicines are more acceptable [3, 4]. Nanoparticles (NPs) are the particulate dispersions or solid particles drug carrier [5]. The nanoparticles are generally contain various polymeric Nano-sphere, liposomes, hydrogel, solid nanoparticle etc. They have been generally used as a potential drug carrier for alternative of conventional drug release. In current year silver and gold nanoparticles have been attracted significant interest. However, in previous report of silver nanoparticle shows toxic effects and instability of silver nanoparticle [6]. Chitosan is linear polysaccharide compound; it contains distributed β-(1-4) linked D-glucosamine and N-acetyl-D-glucosamine units. And it is obtained from deacetylation of chitin which is found in exoskeleton of crustaceans [7].Chitosan nanoparticle are biodegradable, biocompatible, cationic and relatively non-toxic in nature.so they are widely used in drug delivery system [8]. Chitosan shows many advantages in spread nanoparticles including, biodegradability, biocompatibility, and low immunogenicity. Chitosan also show a very low or no toxicity, in this manner it has drawn expanding consideration for pharmaceutical and biomedical applications [9]. The garlic has been used in thousands of years to treat various types of disease. Botanically is known as *Allium sativum* and is a member of alliaceae or liliaceae family. The complete part of the Garlic is called either head or knob. But individual part is known as the garlic clove. It is formed by crushing or cutting of the garlic clove. It is used in various filled such as Anti-cancer, anti-viral, anti-oxidant, and anti-inflammatory, Anti-biotic, Beneficial for heart, Anti-acne, Immunity booster, Anti-diabetic, anti-obesity, Anti-arthritic [10, 11, 12]. The garlic are used in various form like extract of garlic, garlic powder, macerate garlic oil, essential garlic oil. Garlic contains 0.1% volatile oil. The chief constituents of the oil are di allyl di sulphide (60%), dially tri sulphide (20%), ally propyl disulphie (6%), a small quantity of diethyl di-sulphide and probably di ally poly sulphide. and it is also contain some amount of ajoene, allicin, allin, allixinetc [13, 14]. *Allivum sativum* inhibits the genesis and as well as growth of the cancer by enhancing activity of the

natural killer cells and the macrophages. In previous study allium sativum increase the count of the suppressor T cells and makes the lymphocytes more cytotoxic to cancerous cell. It inhibits the growth by inducing differentiation and apoptosis and scavenging carcinogen- induced free radicals [15].

MATERIALS AND METHODS:

Materials:

Chitosan (CS) was and TPP was procured from Shreya health care, India. Fresh garlic bulbs were purchased from local producers in India. And other material and solvent used was of analytical grade. Distilled water was used to prepare all dilute solutions.

Sample preparation:

Garlic extract preparation: Approx. 6 gm. garlic was chopped (not crusted) into approx. ¼ piece, added in 50 ml of distilled water, and allowed to stand at room temperature for 24 hours. The resulting solution was decant to collect a pale white transparent garlic extract solution, and allowed solid garlic pieces were removed. The garlic extract concentration was determined to be 22.8 ± 0.5 mg/ml by measuring the remaining solid weight after evaporating the 2.0 ml of liquid extract in a vacuum oven at 40^{0}C, averaging three measurement. Crushing the garlic extract soaking at an elevated temperature was found to increase the extraction efficiency resulting in larger mass concentration of extract. The variation in the preparation of garlic extracts preparation directly affects the preparation of nanoparticle, that is larger quantities of garlic extract employed to generate poly disperse population of nanoparticle. It is also the extract composition is significantly dependent on the extraction methods, for example, chopping, crushing and temperature of the extraction.

Preparation of garlic extract loaded chitosan nanoparticle:

The chitosan nanoparticle was prepared according to the ionic gelation method using TPP as cross linker with slight modification. After complete dissolution of chitosan in acetic acid garlic extract (5 %) was added to this solution with magnetic stirring. Then TPP (0.5%) was added in drop wise through syringe at a uniform rate. In this method glacial acetic acid 1.6 % dissolved in distilled water and further and chitosan used in various concentration % (0.25, 0.5, 1:1, 1:5:1, and 2:1) [16, 17]. It was further stirred for 2 hours followed by centrifugation for 10 min at 10000 rpm. Supernatant was discarded and pellet was re-suspended in phosphate buffer saline (PBS). The nanoparticle was collected by centrifugation at 10,000 rpm for 10 min and then washed three with distilled water [16]. Stirring until the Opalescent color was observed and stirring continued for 30 min. and the pellet obtained was washed thrice with distilled water. The prepared nanoparticle were lyophilized and stored at 4 ^{0}C until further use.

Standard curve of extract of *Allivum sativum*:

In the study of drug release 100mg of drug was dissolved in the 100 ml of distilled water the concentration of the solution was 1000µg/ml and note it and then1 ml sample was taken and make up the volume with 10ml in the volumetric flask and the concentration of the above solution was find to be 100µg/ml. sample was taken such as 1ml, 2ml, 3ml, 4ml and 5ml and make it volume up to 10ml in volumetric flask. And the concentration of above sample was found to be 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, and 50µg/ml. and the absorbance of the sample were check through the UV-visible spectrophotometer in triplicate.

Table 1: Formula of different formulations of garlic extract loaded chitosan nanoparticle:

S.no.	Name of ingredient	F1	F2	F3	F4	F5
1.	Garlic extract %	5	5	5	5	5
2.	Chitosan	0.25	0.5	1:1	1:5:1	2:1
3.	Acetic acid	1.6	1.6	1.6	1.6	1.6
4.	TPP	0.5	0.5	0.5	0.5	0.5

Characterization of chitosan nanoparticle:

Characterization of the formulated chitosan nanoparticle:

The nanoparticle prepared by ionic gelation method, in this method chitosan dissolved in glacial acetic acid was entrapped by drop wise addition of tri polyphosphate (TPP). We have replace ascorbic acid with glacial acetic acid. Ratio of chitosan to TPP was varied from 0.25, 0.5, and 1:1, 1:5:1, and 2:1 % with acetic acid (1.6%) and TPP (0.5%) were used. TPP added carefully because it forms larger particle size. The formation of large size particle may be due to aggregation of the nanoparticle due to cross linking with each other.

Zeta potential studies:

The zeta potential (surface charge) of chitosan nanoparticle were analysed by zeta sizer. To determine the zeta potential of the prepared nanoparticle sample were diluted with the water (0.1ml) and it placed in the electrophoretic cell where an electrical field of 15.5 V/cm was applied. Each sample was measurement carried out in triplicate.

Morphological studies by scanning- electron microscopy:

The morphology of the nanoparticle was evaluated by scanning electron microscopy. In preliminary step, 100µl of the chitosan nanoparticle formulation were added in to a 10mm glass slide and dried in a vacuum desiccator at overnight in room temperature till SEM analysis was performed. For the analysis of nanoparticle were fixed on adequate support and coated with gold using gold sputter module in a higher vacuum evaporator. Observation was taken under different magnification performed at 15kv [18, 19, and 20].

Drug encapsulation efficiency:

The drug encapsulation efficiency of chitosan nanoparticle were determined by the ultra-centrifugation method. Briefly, acetic acid, and TPP with the chitosan nanoparticle were separated from the chitosan nanoparticle by using the ultra-centrifugation at 12,000 rpm for 30 min. The pellets obtained were re-dissolved in distilled water and the supernatant was also scanned in this parameter with the UV-visible spectrophotometer 350nm. The drug encapsulation

efficiency was determined by using the relation in this equation [19].

$$\% \text{ Drug encapsulation efficiency} = \frac{\text{experimental drug content}}{\text{Theoretical drug content}} \times 100$$

Production yield of nanoparticle: The yield of nanoparticle was determined by comparing the whole of nanoparticle formed against the combined weight of the copolymer and drug [19].

$$\% \text{ Yield calculation} = \frac{\text{Amount of drug}}{\text{Amount of drug + polymer}} \times 100$$

***In-vitro* release study of drug release:**

In the *in-vitro* release study of prepared chitosan nanoparticle were investigated in phosphate buffer saline (PBS) (PH 7.4) at 37 ^{0}C. Chitosan nanoparticles were placed in a dialysis bag and dialyzed against 50 ml of PBS with constant shaking for 1 hours. Aliquots were removed periodically. The volume of removed sample in PBS being replaced by fresh volume of PBS. The amount drug release was determined by the monitoring the absorbance with UV-visible spectrophotometer at 350nm [19].

Stability of Nanoparticle:

The prepared nanoparticle stability studies determined by storing optimized formulation in stability chamber for 8 weeks. The samples were analyzed after a time period such as 0, 1, 2, and 8weeks for their physical appearance (According to ICH Q1A) [18].

RESULT AND DISCUSSION:

Morphological studies by scanning electron microscopy: The surface morphology of the chitosan nanoparticle was investigated by scanning electron microscopy. Study provided a better understanding of the morphological characteristics of the nanoparticle. There were large number of nanoparticle with an approximately spherical shape and they will separate from each other. SEM image of freeze dried chitosan nanoparticle at higher cross linking time, little forwarded but spherical nanoparticle with small size are obtained in F1.

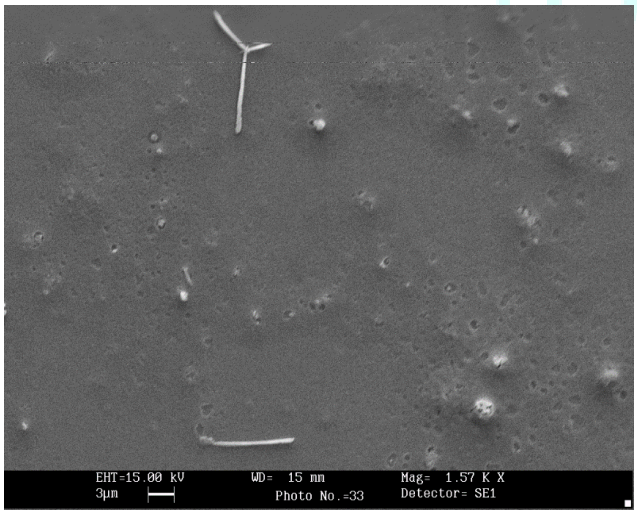

Fig1: SEM image of F1 formulation

Encapsulation efficiency (% EE): Entrapment efficiency of garlic extract loaded chitosan nanoparticle were recorded in the range between '50% to 80%' for 'F1 to F5' formulation. It is obtained at 350 nm. It revealed lowest entrapment efficiency of drug that is F4 formulation 60.84%, while F1 displayed 75.54% highest drug encapsulation efficiency and F3 and F5 formulation are also show the better formulation.

Table 2: % encapsulation efficiency

Formulations	Encapsulation efficiency (%)
F1	75.54
F2	62.87
F3	71.23
F4	60.84
F5	71.65

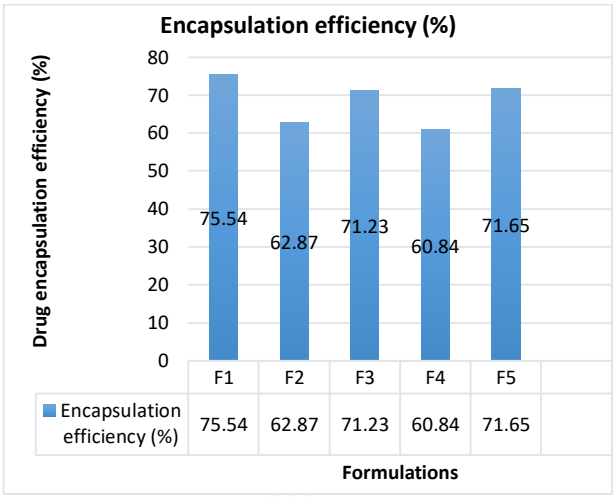

Fig 2: graphical representation of encapsulation efficiency

***In vitro* drug release studies:**

I*n vitro* drug release studies of garlic loaded chitosan nanoparticle of all formulation were noted. The maximum drug release revealed that 90.65 % of drug release from formulation F1. It was found that on increasing the concentration of the polymer, drug release from the nanoparticle decreased. The high stirring speed and high concentration of polymer make stiff nanoparticle. The more hardness of the nanoparticle decrease the drug release rate from nanoparticle, thus F1 formulation exhibited the sustained release of drug from nanoparticle in comparison of others.

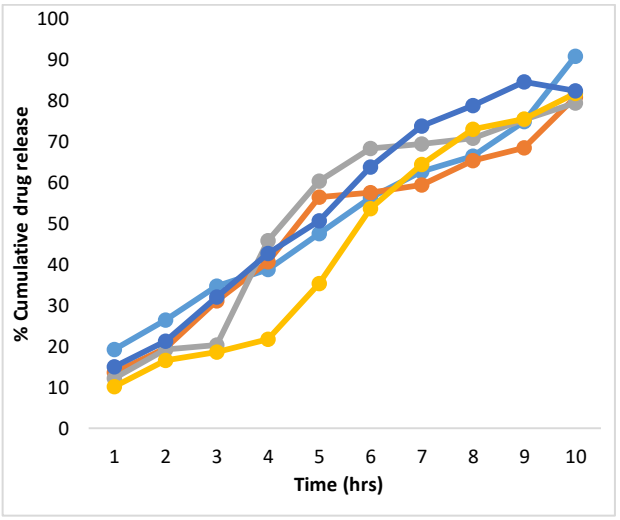

Fig 3: % Cumulative drug release of all formulation

Production yield of nanoparticle: It was recorded that as concentration of polymer increase production yield decrease, since increase in polymeric concentration make solution

viscous this was difficult to pour and get sticked on the wall of the beaker. The production yield of garlic extract loaded chitosan nanoparticle was found to be maximum for F1 (73.59%) and it having lowest amount of polymer. the lowest % yield was of formulation F4 (51.46%) Stirring rate also affect the production yield of nanoparticle.

Table 3: production yield of all formulation

Formulation no.	Practical mass (mg)	Theoretical mass (mg)	Production yield (%)
F1	73.59	100	73.59
F2	63.12	100	63.12
F3	61.24	100	73.59
F4	51.46	100	51.46
F5	54.03	100	54.03

Stability studies:

The stability of the chitosan nanoparticle was also used for the determined by measuring its absorption spectra after 8 Weeks. There is no significant changes occur during the storage condition, the chitosan nanoparticle did not agglomerate it indicate they were more stable during the period.

CONCLUSION:

In present study, an attempt was made to develop formulation of garlic extract loaded chitosan nanoparticle for many type of drug delivery like anti-cancer. In conclusion, prepared nanoparticle were having lower size range, good practical yield, smooth surface and satisfactory release rate. Prepared nanoparticles can be used to deliver variety of drugs including various drug extracts.

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