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Synthesis and Characterization of Aqueous Extract of *Allivum Sativum* (Garlic) Loaded Chitosan Nanoparticles

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ABSTRACT

Background and objective: The present work aims to the done modified method of herbal chitosan nanoparticle to improve the garlic nanoparticles extraction by decreasing the side effects of the aqueous extract.

Methods: Garlic loaded chitosan nanoparticles (GCNPs) were prepared by combination of emulsification-solvent evaporationwith sonication method and slightly modifications into three forms, garlic (GNPs)), chitosan (CNPs), and garlic-chitosan (GCNPs). Three types of NPs were characterized by FESEM for particle size (PS) and zeta potential (ZP), FTIR, drug loading (DL%), encapsulation efficiency (EE%), and in vitro extract release. **Results:**GCNPS particle size was PS, 151.9±12.7 nm, PDI 0.152, and have ZP, -23.2.5. The higher DL% content of EV was detected as 7.32±1.1% and EE% was 87± 2.6% in formulation B. The results suggested that the GNPs dissolution was 80% within 4h but the release profiles of GCNPs form within 4h were 34, while GNPs and CNPs were 23, 26%, respectively. **Conclusion:**GCNPs were synthesized and characterized in specific properties for next labs animal experiments.

Keywords: Garlic, Chitosan, Nanoparticles, particle size, FTIR analysis

INTRODUCTION

In the world, plants or mixtures of plant extract were used to treat many illnesses and public health development (1). Over the past decades, in developing countries due to low economic rate and the majority of populations and high cost of western medicine, that herbal medicine including garlic, 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 (2). Fresh or crushed garlic yields the sulfur-containing compounds allicin, ajoene, diallyl polysulfides, vinyldithiins, and S-allylcysteine; as well as enzymes, saponins, flavonoids, and Maillard reaction products, which are not sulfur-containing compounds (3). The phytochemicals responsible for the sharp flavor of *Allivum sativum* are produced when the plant's cells are damaged. When a cell is broken by chopping, chewing, or crushing, enzymes stored in cell vacuoles trigger the breakdown of several sulfur-containing compounds stored in the cell fluids (cytosol) (4). *Kodali et al; 2015 were reported that Allivum sativum* inhibits the genesis and as well as growth of the cancer by enhancing activity of the natural killer cells and the macrophages (5). In previous study *allivum sativum* increase the count of the suppressor T cells and makes the lymphocytes

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more cytotoxic to cancerous cell. It inhibits the growth by inducing differentiation and apoptosis and scavenging carcinogen- induced free radicals (6). Chitosan, b-(1-4) linked 2-amino-2-deoxy-b-D-glucopyranose, is an N-deacetylated derivative of chitin obtained by transforming the acetamide groups into primary amino groups (7). It was demonstrated that chitosan had a great potential for a wide range of uses due to its versatile biological, chemical and physical properties (8). The application of chitosan includes a variety of areas such as pharmaceutical and medical applications, paper production, textile wastewater treatment, biotechnology, cosmetics, food processing and agriculture (9). But the biocompatibility, biodegradability, nontoxicity and antimicrobial activity of chitosan attract the attention of researchers in the recent years and garnered considerable interests in the field of biomedical engineering (10).A wide range of polymers and various polymer combinations are suitable for the encapsulation of nanostructures and the chitosan is commonly used as a wall material polymer because it does not show toxicity and it biodegradability, and stability NPs, and for these reasons, chitosan NPs have great importance in controlled drug delivery systems (11). The aim of this study to synthesis and characterization of garlic loaded chitosan nanoparticles (GCNPs) for next animal administration experiments.

MATERIALS AND METHODS

MATERIALS

Chitosan and garlic powder were purchased from Beijing (China). Other solvents such as acetic acid, TPP, and PBS were bought fromBDH (England).

Preparation of garlic loaded chitosan nanoparticlesGCNPs

Five mg of dry garlic powder were dissolved in 100 ml D.W and mixed well, the mixture allowed over night with covering status.After 24 hours the extract was decanted and filtered to remove any solid particles to obtain of the pure extracted garlic solution. 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 was used in various concentration % (0.25, 0.5, 1:1, 1:5:1, and 2:1) (11). 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) and sonication for 30 seconds. The liquid obtains from the above step were keeps in cooling status for further characterization and next lab animal experiments(Jasim *et al*.,2019 a, .Jasim *et al*.,2021).

Characterization of the GCNPs loaded NPs

Particle size (PS), Zeta potential (ZP), and Polydispersity Index (PDI)

The suspension GCNPs were mixing well and sonication. Three types of NPs solutions were analyzed bydynamic light scattering (DLS) technique for determination of ZP, PS, and PDI.

Encapsulation Efficiency EE%

EE% of the preparedGCNPs were quantified by measuring the absorbance at 356 nm by using spectrophotometer.One ml of garlic loaded NPs samples were used for spectrophotometric measurement. NPs solution of various formulations GNPs, CNPs, and GCNPs)were prepared, and the absorbance at 222 nm was measured at different concentrations.EE% were calculated from following equations (12):

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EE %= Wt. of G for preparing formulation - Wt. of G in supernatant/ Wt. of G for preparing formulation × 100

TEM analysis

The surface morphology of EV loaded NPs was observed using field emission transmission electron microscope FETEM. The NPs samples were sputter-coated with platinum (4–5 nm) at a current intensity of 40 mA for 40 s. The images were captured keeping the accelerating voltage between 1–5 kV.

FTIR analysis

Garlic loaded chitosan NPs were scanned over a wave number range of 400–4000 cm^{-1} in an inert atmosphere in an FTIR spectrophotometer (without garlic). GCNPs were scanned over a wave number range of 4000–400 cm^{-1} in an inert atmosphere in an FTIR spectrophotometer (with garlic).

Long term stability study

In closed glass vials, 1 ml of samples of garlic loaded NPs formulations (GNPs, CNPs, and GCNPs) were stored for 30 days at 4 C°, room temperature (RT) (25-26 C°), 37C° , and 50 C° away from direct sunlight exposure by covering with appropriate piece of aluminum foil. The formulations were assayed periodically, at the time points of 0, 10, 20, 30,40, 50, and 60 days, for particle size (PS) and drug EE% (Jasim *et al.*,2019 B).

Statistical Analysis

statistical analysis were performed by using SPSS version 22 and data were expressed as (mean ± SD). The normality of the distribution of all variables was assessed by the student′s ANOVA test and person correlation analyses that have been used to determine the significant difference between the groups. P-values less than (0.05) was considered significant.

RESULTS AND DISCUSSION

Particle size (PS), Zeta potential (ZP), and Polydispersity Index (PDI)

Table 1, showing the mean ±SD of the Zeta Potential (ZP), particle size (PS) , andPolydispersity Index (PDI) of GNPs, CNPs, and GCNPs formulations. GCNPs showing optimal PS (151.9) nm, higher PDI (0.262) and larger negative charges as ZP (-61.3 mV) rather than the other types GNPs and CNPS:

Table (1): PS (nm), ZP(mV), and PDI of study formulations

Formulation type	PS (nm) mean±SD n=3	PDI mean±SD n=3	ZP(mV) mean±SD n=3
GNPs	230±11.8	0.213±0.014	-27.9±2.9
CNPs	191.9±14.7	0.262±0.091	-29.8±2.7
GCNPs	151.9±12.2	0.160±0.023	-61.3±3.1

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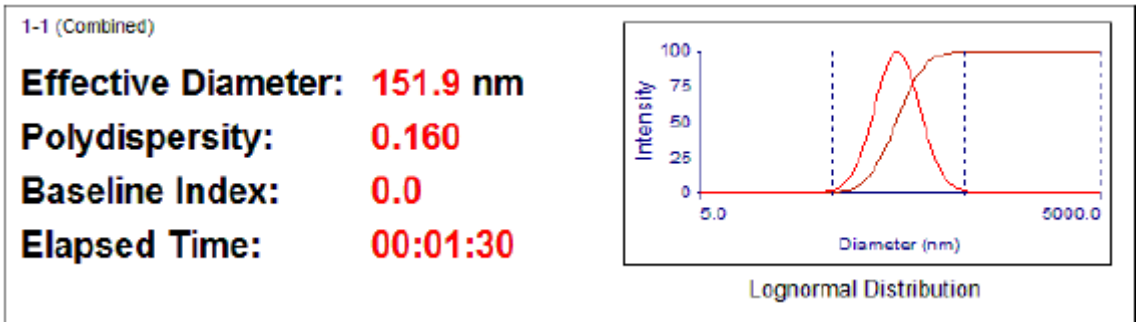

Fig.1: particle size PS (nm) and PDI analysis of GCNPs

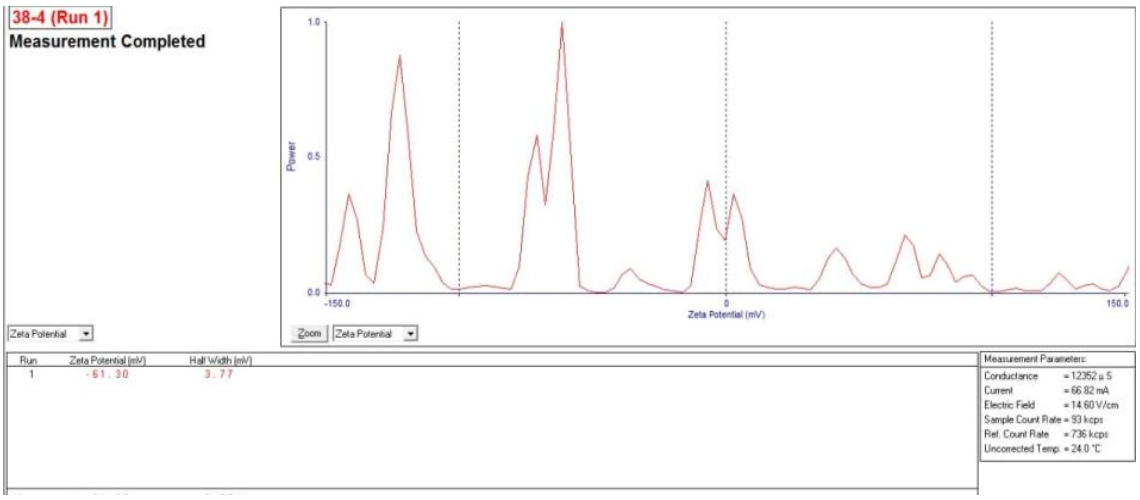

Fig.2: Zeta potential ZP (mV) analysis of GCNPs

Absorbance spectrum and Encapsulation Efficiency(EE%):

Absorbance spectrum and the higher EE% content of garlic NPs in GCNPs formulation was detected was 86± 3.9% and less than this results showing in other types of nanoparticles 83,67% for CNPs and GNPs respectively, as shown in table2 and figure 3:

Table (2): EE%of study formulations

Formulation type	EE (%) mean±SD n=3
GNPs	67±4.5
CNPs	83±3.1
GCNPs	86±3.9

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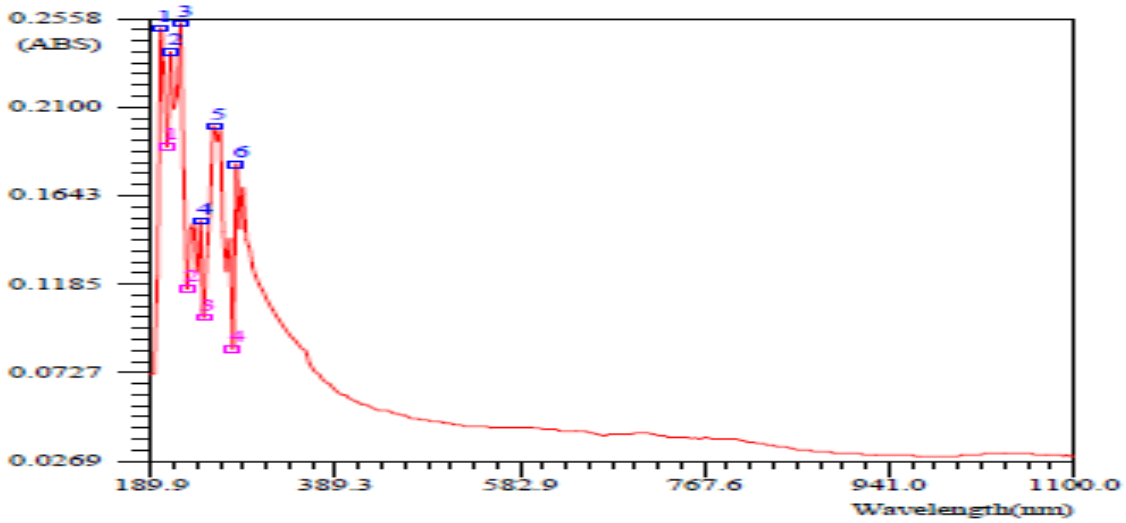
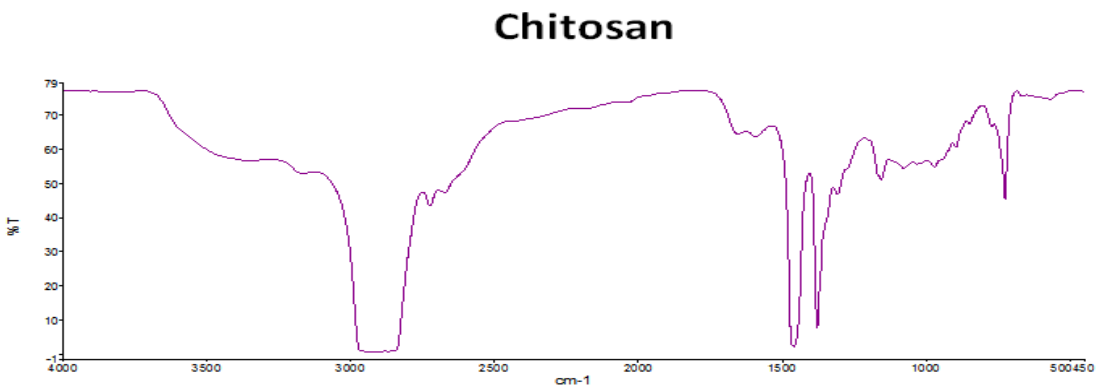


Fig.3: Absorbance spectrum of GCNPs

GNPs, CNPs, and GCNPs FTIR spectrum study

The effects of garlic encapsulation on the chemical group of the formed components and the interaction between the components were studied by FTIR. The FTIR spectra of chitosan, garlic, GNPs, CNPs, and GCNPs absorption peaks, as shown in figure4:The structure of the chitosan, CNPs, GNPs, and GCNPs were confirmed by FTIR, where the spectrum ofthe free chitosan shows more broad absorptionbands at 3422 cm^{-1}. This broad band might be corresponded to hydroxyl groups (OH) stretching vibrations of water molecules, OHs and NH_2stretching vibrations of free amino groups. The twobands observed at 2923.56 and 2859.92 cm−1 correspondto asymmetric stretching of CH_3 and CH_2 inboth free chitosan, CNPs, and GCNPs. The observed band at2221 cm^{-1} is attributed to C–N group of C–NH_2. In addition, the stretching band of C–O in chitosanspectrum was observed at 1038.48 cm^{-1} in all types of NPs unless GNPs.



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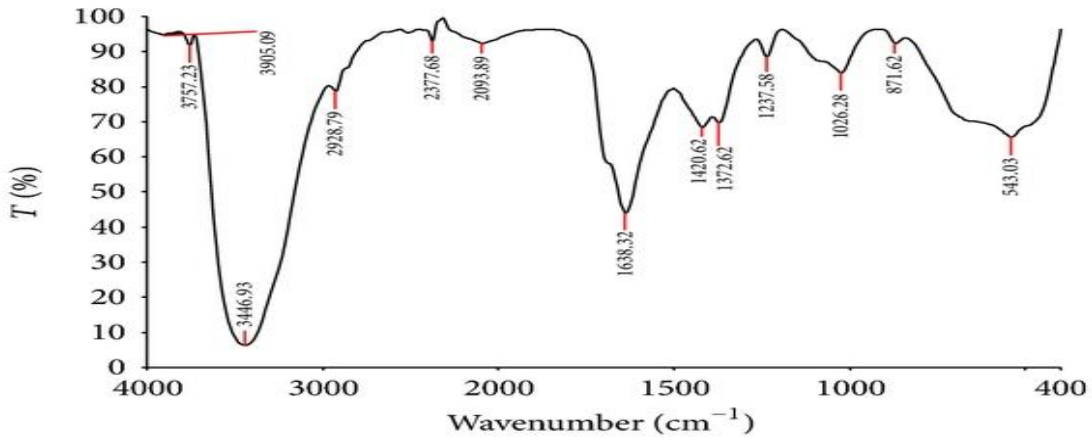

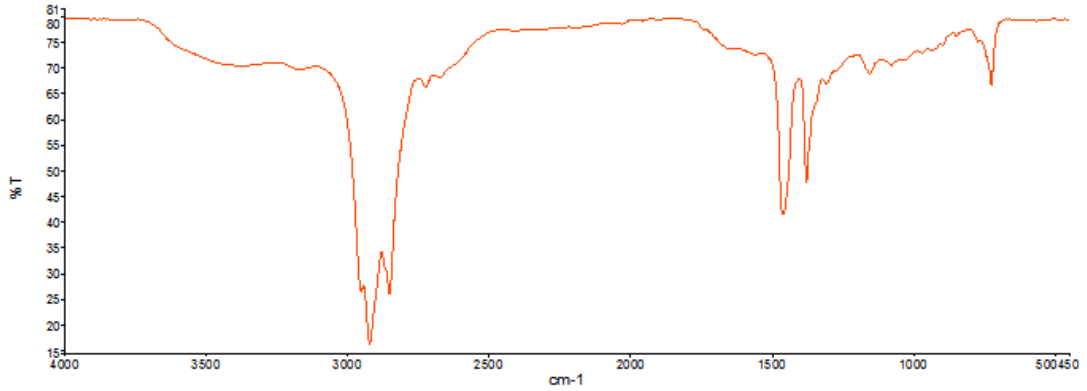

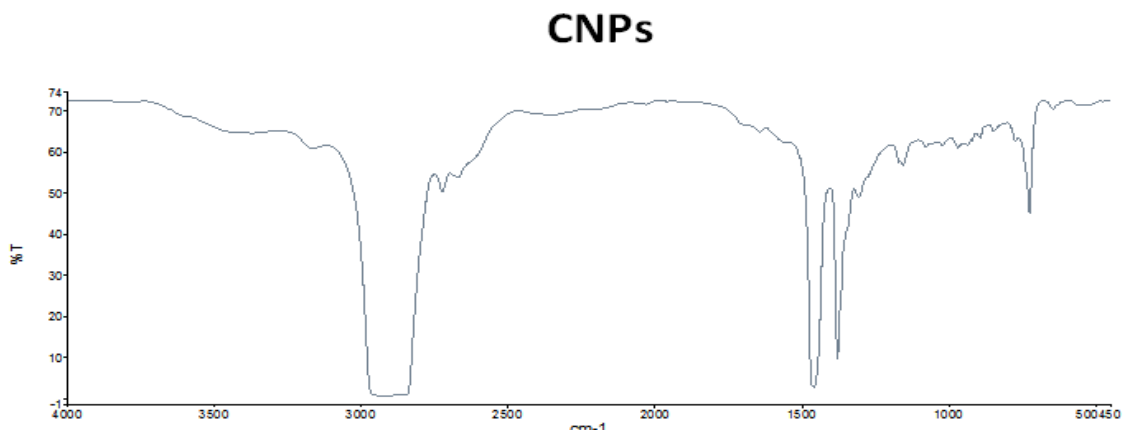

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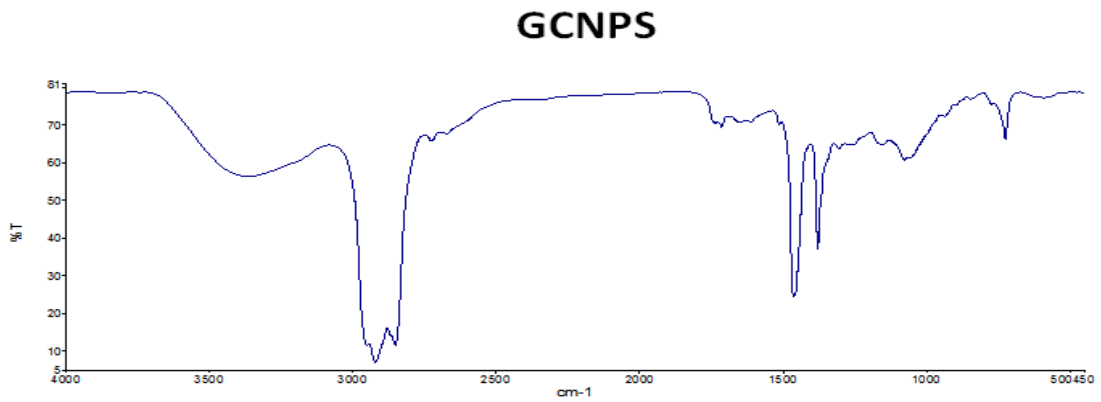
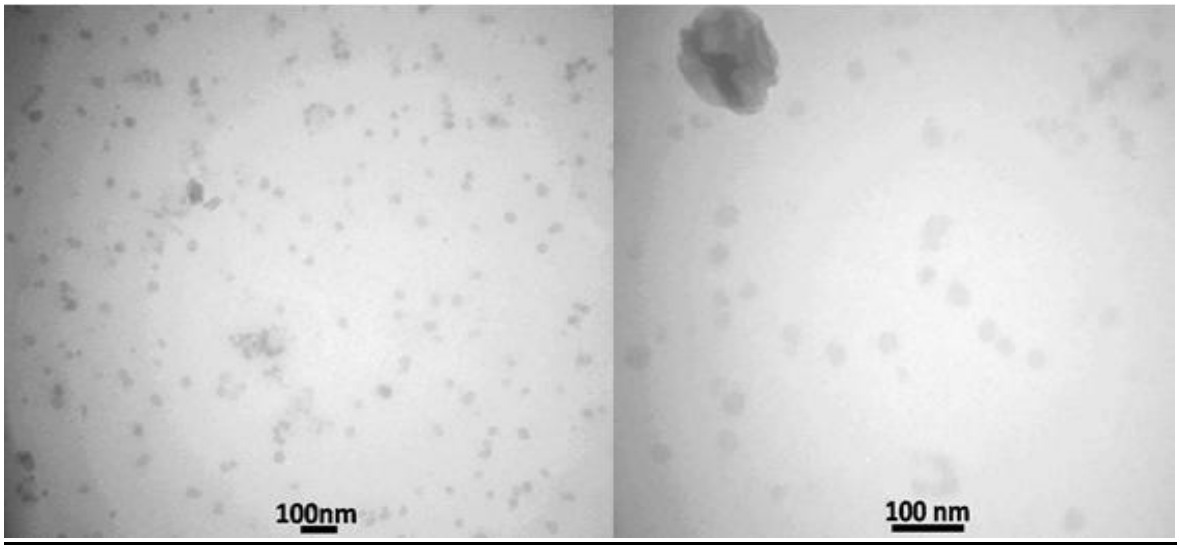


Fig.4: FTIR spectrum of study formulations. GCNPS (garlic-chitosan nanoparticles)

Transmission Electron Microscopy (TEM)

Figure 5, showing the TEM images that revealed that GCNPs were approximately spherical in shape.



Figure(5): TEM images of GCNPs

XRD analysis

The structural nature (crystal and phase purity) of the prepared GCNPs was studied using the X-ray diffraction XRD patterns. Figure 6 , represents the XRD) analysis of GCNPs .The decreasing of crystallinity could be due to that CNPs and GCNPs are composed of a dense network structure of interpenetrating counter ions of TPP, where the polymer chains crosslink with each other by TPP. Thus, the XRD pattern of GCNPs is characteristic of an amorphous polymer.

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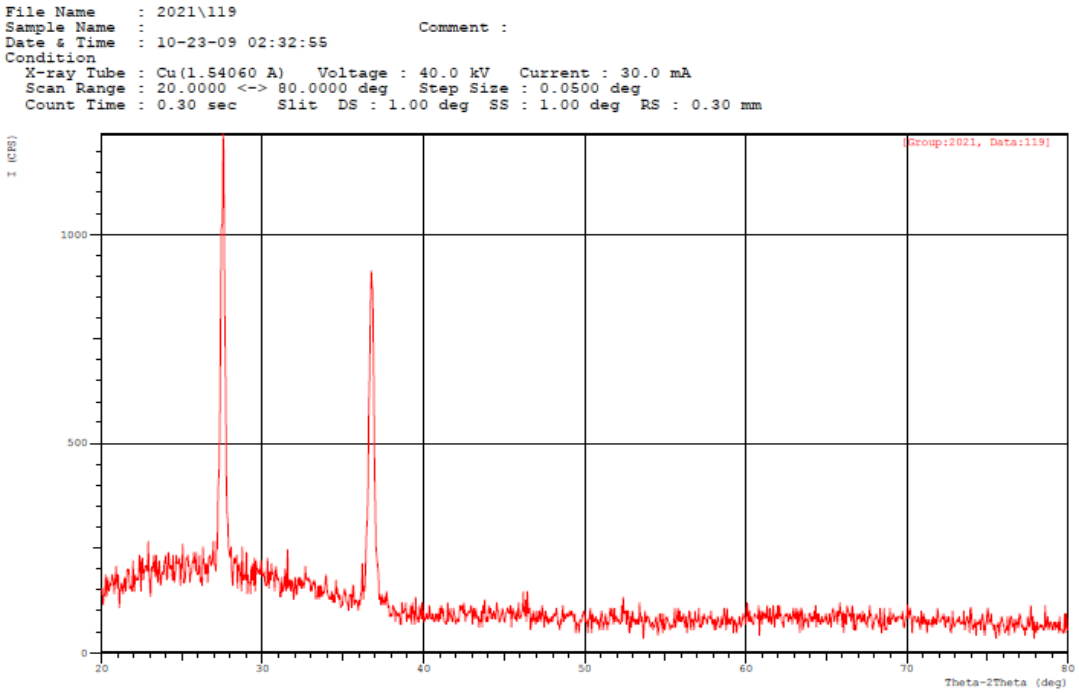

Fig.6: XRD analysis of GCNPs

Long term stability study

Figure 7 , represents the influence of long term storage on stability of GNPs, CNPs, and GCNPs in different storage time (0,30,60, and 100 days) at room temperature, 25-26 Cº and this range of temperature was the best degree in analysis.

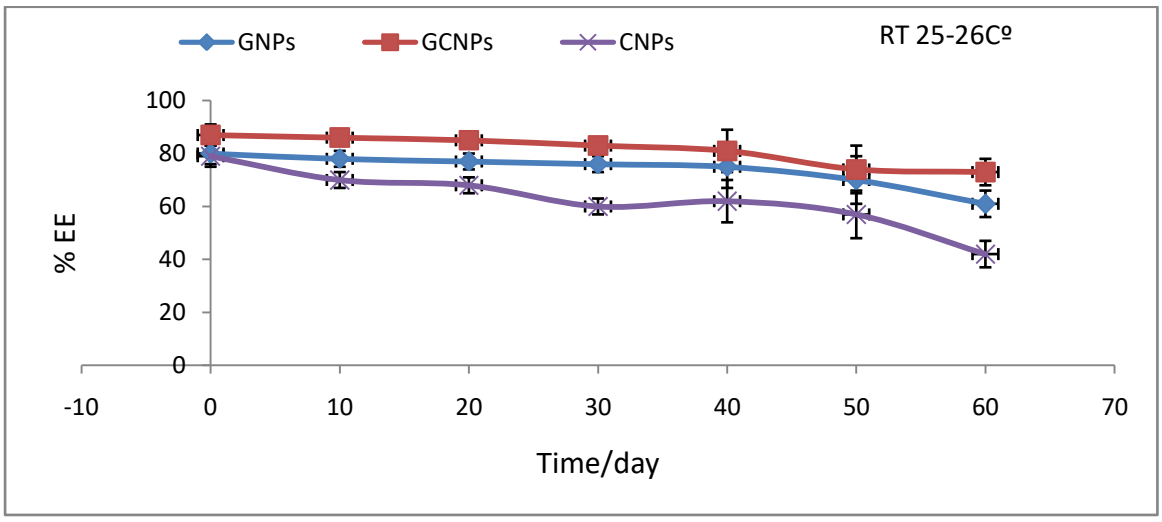

Fig.7: Effects of storage time on EE% of NPs

To the best of the present study knowledge, this is the first study reporting that GCNPs may be useful to improve the garlic side effects by resultant compounds were responsible for the sharp or hot taste and the strong smell and the treatment role of garlic in a huge spectrum of new diseases. The particle size analysis PS showing that statically differences (p-value < 0.05) of mean±SD of GCNPs ,151.9±12.2 and the mean±SD of the other nanoparticles were 191.9±14.7 nm and 230±11.8 for GNPs and CNPs, respectively. The particle size of the study nanoparticles was determined using the DLS technique, with triplicate

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measurements as showing in table 1 and figures 1, the GCNPS have a PS 151.9 nm,PDI 0.160, and ZP -61.3Mv. The PDI for all the prepared formulations CNPs, GNPs, and GCNPs were in the ranged between 0.16-0.26, thus complying with the accepted limits less than 0.4 (12).The effects of garlic encapsulation on the chemical group of the formed components and the interaction between the components were studied by FTIR. The FTIR analysis of three types of prepared nanoparticlesCNPs, GNPs, and GCNPs were showing no chemical bonding between function groups but only chemical interactions as described by Budama-Kilinc et al; 2017 (13).The results of present study showing that the Absorbance spectrum and the higher EE% content of garlic NPs in GCNPs formulation was detected was 86± 3.9% and less than this results showing in other types of nanoparticles 83,67% for CNPs and GNPs respectively. During the encapsulation process, garlic and chitosan polymers are likely to interact with each other. Based on the weak interactions, such as hydrogen bonding and other chemical interactions and this agree with the results were reported by Shukla et al; 2013 (14). The present study suggesting the influence of high long term storage on stability of GCNPs EE% of 81% in two month time storage at room temperature, 25-26 Cº. The prepared GCNPs were analyses and characterized to using in next laboratory animal experiments. A number of studies have reported that suggesting of chitosan as a polymer for preparation of nanoparticles in different treatment methods (15-17).

Conclusion:

Chitosan was high benefit for synthesis of garlic nanoparticle in optimal Nano particle size and other properties for future biomedical applications

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Nil

Conflict of interest

No potential conflict of interest relevant to this paper was reported.

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