Egypt. J. Chem. **Vol. 66** No. 1 pp. 497 - 507 (2023)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

In Vitro Release of Curcumin as an Anticancer Drug from Gelatin Nanoparticles

Salwa A Ahmed [a], Wael S Mohamed [b], Sabrnal H El Hamouly [c], Nabila A Maziad [d], Mahmmoud S Abd Elmonem [a*]

[a]*Egyptian Drug Authority (EDA), Giza, Egypt.*
[b]*Department of Polymers and Pigments, National Research Centre, Giza, Egypt.*
[c]*Polymer Laboratory, Department of Chemistry, Faculty of Science, Menoufia University, Shibin el Kom, Egypt.*
[d]*Department of Polymer Chemistry, National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt.*

Abstract

This study focuses on combine nanoscale structure with biomolecules to create new biological and nanotechnology applications. Gelatin nanoparticles was prepared by nanoprecipitaton method with various parameters and utilized the optimum as a carrier for curcumin as anticancer drug, the obtained nanoparticles had a homogenous well-defined morphology that confirmed using transmission electronic microscopy and FT-IR, as well a curcumin was loaded on gelatin nanoparticles by emulsification linkage technique in the presence of SLES as emulsifier, and the EE of the drug was controlled by the polymer concentration, the emulsifier concentration, and the drug to polymer ratio. The results showed that GNps exhibit high Encapsulation Efficiency (EE) which reached to 82%. Additionally, studying the curcumin release profile at different pH values (1.2 and 7.4) at 37 °C for 72 h showed that the amount of drug released at acidic pH 1.2 is higher than that of pH 7.4 and also the release rate had slow and sustained.

Keywords: Anti-cancer drug, Curcumin, Gelatin, Nanoparticles, Emulsification linkage

Introduction

An ideal drug delivery system should involve targeted delivery with optimum controlled release to improve the efficacy of the proposed drug [1]. Recently, nanoparticles technology has achieved a special role to improve the effectiveness of less soluble and low bioavailability drug molecules, due to the particle size property, Long shelf life and the ability to entrap more drugs, The pharmacological and pharmacoeconomic utility of a drug is increased when it is placed into a nanoparticle and the synthesis of polymeric nanoparticles for targeted delivery and regulated drug release has the ability to improve drug therapeutic index [2]. In particular, biopolymers are polymers derived from living organisms and are divided into three categories, protein as (silk, collagen, gelatin), protein-mimicked polypeptides and polysaccharides as (chitosan, alginate, starch) , which considerd an excellent candidate for drug carrier due to their versatile properties, such as biodegradability, biocompatibility, and low toxicity. When using biopolymer-based nanoparticles as drug delivery carriers it is critical to manage the particle size, surface morphology, and loaded molecule release rate. So a variety of substances and preparation processes have been investigated in order to obtain a nano-carrier for therapeutic uses [3]. Although various biodegradable nanoparticles of natural polymers are largely used as drug carriers for targeted drug delivery technology but gelatin nanoparticles is the most controlled carrier system for drug delivery [4]. Recently, GNps (gelatin

*Corresponding author e-mail: salwa_ch1987@yahoo.com; (Salwa A Ahmed).
Receive Date: 28 March 2022, Revise Date: 16 April 2022, Accept Date: 24 April 2022.
DOI: 10.21608/ejchem.2022.129597.5738

nanoparticles) are extremely effective at delivering and controlling the release of medications, proteins, and peptides. The delivery approach based on GNps is biocompatible and biodegradable in the body, with no hazardous breakdown products. They're nontoxic, biodegradable, bioactive, and low-cost. Gelatin is a polyampholyte that contains cationic, anionic, and hydrophilic groups as well as a hydrophilic group. Mechanical features of gelatin NPs, including as swelling behaviour and thermal properties, are known to be influenced by the degree of cross linking between anionic and cationic groups [5, 6]. Several techniques have been used to synthesize GNps, including coacervation, desolvation, water-in-oil (w/o) emulsion, and precipitation [7].

Curcumin is a natural nontoxic yellow pigment, bioactive agent of the perennial herb curcuma longa (known as turmeric). Curcumin has been extensively studied as a cancer therapeutic or preventive agent, and it has been shown to have a wide range of bioactivities, including antioxidant, anti-inflammatory, antibacterial, antimicrobial, and wound healing properties, as well as the ability to prevent cancer in a variety of tissues, including the skin, oral cavity, forestomach, mammary gland, stomach, oesophagus, intestine, colon, liver and lung [8- 10]. On the other hand Curcumin usefulness is limited due to its poor water solubility, instability at physiological pH, and low bioavailability [11, 12].

Thus, it has been suggested that loading this drug in nanoformulations to improve its pharmacokinetics and biopharmaceutical value and overcome these problematic limitations caused by using the free drug or low molecular weight prodrugs [13]. Polymeric nanoparticles, which are usually produced from biodegradable and biocompatible polymers like gelatin and chitosan, are one of these nanoformulations [14, 15].

This research focuses on the synthesis of gelatin nanoparticles in a variety of parameters using the nanoprecipitation method, as well as the evaluation of their efficiency in loading curcumin as an anticancer drug and showing their effect on drug entrapment and *in vitro* drug release.

Experimental

Materials

Gelatin powder was purchased from EL. NASR Pharmaceutical Chemicals co., Curcumin was purchased from Sigma-Aldrich, Pluronic F-127 (Poloxamer 407) obtained from BASF Chemicals Company, Glutaraldehyde (25% solution) obtained from S d fine-chem limited, Texapon N70 (sodium lauryl ether sulfate (SLES)) was provided from BASF chemicals company, Potassium dihydrogen phosphate (KH_2PO_4) was provided from Gen-Lab. Sodium hydroxide (NaOH) 99%, Potassium chloride (KCl) and Hydrochloric acid (HCl) were given from Modern Lab, Ethanol (99%) was provided from Fisher Chemical and all were used as received. All experiments were performed with double distilled water.

Methods

Gelatin Nanoparticle preparation by Nanoprecipitation

Gelatin (1.25 gm) was dissolved in 100 ml distilled water at 60°c. 200 ml ethanol solution and Pluronic F-127 (2%, w/v) was added drop-wise to gelatin solution under continuous stirring with emulsifier/ gelatin mass ratio 32:1, after 15 min glutaraldehyde solution (5% w/v) was added. Kept stirring the solution for 4 hours and frozen for 1 day then redispersion in 5 ml distilled water and 10 ml ethanol solution was added drop by drop for 10 min at 60°C then the solution was centrifugation at 8000 r.p.m for 30 min and dryed at 40°C [16].

Effect of Emulsifier Concentration

The same procedure was used with different emulsifier concentrations (1, 2, 3, and 4% (w/v)), and the effect of emulsifier concentration on particle size and morphology was examined using a transmission electronic microscope (TEM).

Effect of Gelatin concentration in the solvent

The mass ratios of emulsifier to gelatin (32 to 1) and also glutaraldehyde to gelatin (0.12 to 1) were remained the same as in the control solution, as was the non-solvent volume (200ml ethanol). In the solvent phase, different gelatin ratios (0.5, 1, 2, and 3% w/v) were used. A transmission electronic microscope (TEM) was used to study the effect of gelatin content on particle size and morphology.

Effect of crosslinker (glutaraldehyde) ratio

The mass ratio of glutaraldehyde to gelatin (0.12:1) and the factor of crosslinker ratio was studied by varied glutaraldehyde concentration (0.06, 0.12, 0.18 and 0.24), The rest of the procedure followed the standard control recipe. The effect of cross linker ratio on particle size and morphology was examined using a transmission electronic microscope (TEM).

Effect of Non-solvent volume

The mass ratios of emulsifier to gelatin and glutaraldehyde to gelatin were maintained in the control formulation. In contrast, the amount of gelatin in the solvent phase was maintained constant 1.25 gm. To investigate the impact of non solvent volume, the volumes of non-solvent were adjusted as (150, 200 and 250 ml) and investigate the particle size and morphology by using transmission electronic microscope (TEM).

Curcumin drug Loaded GNps

Curcumin-loaded gelatin nanoparticles (C-GNp) were synthesized using an emulsification linkage stabilized by SLES as an emulsifier. To form an aqueous phase, curcumin was distributed in a pre-swelled gelatin solution (from 10 to 20%) and stirred at 500 rpm for 5 minutes. The aqueous phase was then dropped into a solution containing SLES dissolved in distilled water and stirred at 800 rpm at 55°C. After 30 minutes of emulsification, the system was immersed in a 4°C ice water bath and agitated for 30 minutes at 600 rpm. The gelatin droplets were then crosslinked with a 25% Glutaraldehyde solution. After 30 minutes, dehydrate the gelatin particles by adding isopropyl alcohol to the system. The mixture was filtrated and washed using isopropyl alcohol. After that, it was dried at 40°C to remove any remaining organic solvent [17]. The main parameters of the preparation evaluated by the following table1.

Table 1. Optimal parameter and levels of the Preparation

Level	Factors		
	Gelatin concentration	Weight ratio of drug and polymer	Emulsifier concentration
1	10%	10:200	1 %
2	15%	15:200	1.5 %
3	20%	20:200	2 %

Characterizations

Particle Size and Morphology Analysis

Transmission electronic microscopy (TEM) was used to examine the particle size of the nanoparticle, TEM images was performed using a JEM-2100 electron microscope operating at 60 Kv. Before taking the images, diluted the sample in water at least ten times, and the well distributed diluted sample was placed onto a copper grid 200 mesh covering with a carbon membrane, then allowed to dry at room temperature then a drop of phosphotungstic acid (1%) was deposited over the dried sample as a stain. The average particle size was determined from ten different particles.

Fourier Transform Infrared spectroscopy (FTIR)

The FTIR spectra of free drug, gelatin nanoparticle free from drug and loaded with drug were identified using Thermo Nicolet FTIR spectrophotometer in the region of (4000 - 400 cm^{-1}) using KBr pellets.

Drug Entrapment Efficiency (EE)

The drug entrapment efficiency (EE) was determined by the determination of the drug content in polymers which carried out by an indirect method [18, 19], by measuring the free drug (unloaded drug). The unloaded drug was collected from the nanoparticles stable dispersion by dissolving through ethanol then the free drug was measured in the clear supernatant, after separation of nanoparticles using a combined ultracentrifugation process at 8000 rpm for 20min, Then the drug concentration in the solution was evaluated using Shimadzu UV visible spectrophotometer with double beam to measure the absorbance at 420 nm for curcumin, and obtained the standard calibration curve of the free drug using ethanol solutions experimentally.

The entrapment efficiency EE was calculated as the ratio of the weight of the drug entrapped in the polymeric nanoparticles to that added initially [20-23].

EE=

(The weight of the drug entrapped in the polymeric nanoparticles)/

(*The weight of the drug initially used*) %

In vitro release studies

The *in vitro* release behavior of curcumin drug from GNps was evaluated in a buffer solution with pH 1.2 which corresponds to the acidic medium of cancer cells or gastric fluid and pH 7.4 which refers the pH of the a blood buffer solution or intestinal fluid at 37 ±0.5 ^{0} c, using a dialysis bag technique [24].

250ml of 0.1M KH_2PO_4 with 195.5ml of 0.1M NaOH was mixed to prepare a buffer solution of PH 7.4 (simulated intestinal fluid). While the buffer solution of pH 1.2 (simulated gastric fluid) was prepared by mixing 250ml of 0.2M HCL with 147ml of 0.2M KCL. Before the experiments, the dialysis bags (molecular weight cut-off 12 kDa, Sigma) were equilibrated in the buffer solution for a few hours.

Sigma-Aldrich commercially obtained curcumin was used in the release studies as free curcumin, 0.5 g of curcumin drug-loaded GNps and the equivalent amount of curcumin free were accurately weighed and then placed into 3 ml of dissolution media (buffer saline) containing tween 80 (1% v/v), introduced in the dialysis bag and inserted into the receptor compartment containing 50 ml of dissolution media BS, and maintained under stirring (100 rpm) at constant temperature of 37°C ± 0.5°C. The receptor compartment was closed to prevent dissolution medium evaporation losses [25]. At predetermined time intervals, 3ml of dissolution medium was withdrawn and replaced with the same fresh release media volume to maintain sink conditions. The concentration of the released curcumin was estimated using spectrophotometer at 420nm and then calculated using a curcumin standard curve [26].

The following equation was used to calculate the percentage of curcumin released:

$$\textbf{Release (\%)} = \frac{\textbf{Released drug}}{\textbf{Total drug}} \ \%$$

Results and Discussion

Gelatin nanoparticles used as delivery carrier for anticancer drugs. The main goal of that encapsulation is to achieve the good entrapment efficiency and narrow size distribution with efficient delivery of bioactive compounds through control a sustained release at the targeted sites.

Gelatin nanoparticles (GNps) were constructed by nanoprecipitation technique with different parameters studied to determine the optimum conditions were the emulsifier concentration, gelatin concentration in the solvent phase, crosslinker (glutaraldehyde) ratio and the non-solvent volume was also studied. To avoid the aggregation and help in better dispersing and stabilization of polymer matrix, it was noted that the emulsifier should be present in the non-solvent phase. Moreover the addition of the emulsifier may enhance the interlayer spacing between polymer matrixes, which allowing for tolerating more drug molecules.

Furthermore, the increased surface area of the nanoparticles caused by the emulsifier greater dispersion effect assisted the formation of smaller nanoparticles and so improving the drug loading capacity [27].

On the other hand the crosslinker concentration enhancing the drug loading efficiency by formation of complex polymer network structure with precipitate and solidify the nanoparticles from water, increase the nanoparticles mechanical and thermal stability in physiological settings and limit the pace of deterioration in vivo [28].

During cross-linking, a 32:1 mass ratio of emulsifier to gelatin was utilized to be suitable for producing stable nanoparticles without inter-particle agglomeration induced by charge neutralization. After drying the yield of GNps was determined gravimetrically, and studied the effect of above discussed parameters on the particle size to examine the size and shape of the dried nanoparticles and obtain the suitable partical size utilized for loading the drug.

Then, the curcumin drug was loaded by emulsification technique by incorporating the drug solution with required volume into GNps which fully dependent on the content of gelatin nanoparticles, ratio of drug and the emulsifier concentration, and the properties of drug-loaded polymeric nanoparticles were examined.

Formaldehyde and glutaraldehyde were the most often utilized cross-linking chemicals. Glutaraldehyde was chosen as cross-linking agent due to its ability to solidify at neutral settings, whereas formaldehyde only could solidify entirely at basic conditions, in which curcumin degradation was too easy [29].

Moreover and as the result of the poorly dissolved of curcumin drug in water used common approaches in the composition of dissolution medium for hydrophobic drugs as increasing the volume of the aqueous sink or separating the dissolved drug, solubilization of the drug by anionic or non-ionic emulsifier (in post micellar concentration), or changing the pH to improve the solubility of non-soluble drug molecules [30].Therefore to improve the solubility, loading and the release kinetics of curcumin, texapon N70 (sodium lauryl ether sulfate SLES) was utilized as an anionic emulsifier and solubilizing agent, thus the efficiency of drug loading was observed to improve as the emulsifier content was increased [31].

Partical Size and Morphology Analysis

Generally, the morphological analyses of gelatin nanoparticles were carried out using TEM before and after drug loading.

By comparing the morphologies of the polymer nanoparticles in different nanoprecipitation parameters the TEM images revealed spherical nanoparticles with almost different size and showed both ridged and smooth surfaces for the polymer.

According to TEM observations find that the entrapment of drugs may cause a great morphological alteration, and the higher drug loading is associated with morphological transformation, and vice versa [32].

Figure 1 shows TEM images of the produced nanoparticles in the effect of varied emulsifier concentrations, noticeable that the average particle size decreases from 142 to 38 nm with increases the emulsifier concentration from 1% to 4%, respectively, where in the case of 1% had visible agglomerates. As a result, lowering the emulsifier content was less effective in stabilizing of the particles, resulting in the formation of large flocculates. So as the emulsifier concentration increases the particle division obtains more defined and clear, indicating that inter-particle aggregation is decreased [33]. On the other hand the yield of the mass was decreased gradually as 15.6, 12.2, 10.9, 8.7 % respectively, by increasing the emulsifier concentration which could be related to the smaller particle size that could not be easily retrieved by the centrifuge [34].

TEM images represented in **figure 2** shows that as the gelatin concentration increasing from 0.4g/ml to 2.5g/ml leads the particle size increased from 70 to 235 nm and the morphologies of the nanoparticles had a different shape from each other, that may be due to increased the viscosity of the polymeric phase [35], while the morphology with the 2.5g/ml gelatin showed increasing in the particle size with low inter-particle aggregation degree during the crosslinking process compared with 0.4g/ml gelatin which showed decreasing in the particle size with still clear division between particles. Otherwise, the yield of the mass was increasing gradually with gelatin concentration increase from 9.2 to 18.7 %.

Figure 3 showing the system tend to agglomerate gradually by increasing the crosslinker ratio with decreasing in the particle size from 48 to 28 nm and these increasing showing particles becomes indefinite and clearness. When the mass yield was decreasing gradually with crosslinker increasing from 15.6 to 10.8 %.

On the other hand from **figure 4** observed that average particle size was decrease when the non-solvent volume increases from 150 ml to 250 ml and the morphology of the nanoparticles takes on a sphere-like shape with incipient aggregation. Moreover find that the mass yield was decreased from 20.5 to 9.5 as the non solvent volume increased.

Previously find that samples within the parameter of different emulsifier concentration have more definite and homogenous particles with small particle size especially sample with 4% emulsifier concentration which enables it optimun condition to entrap more drugs.

In **figure 5** TEM revealed that curcumin loaded on the smaller gelatin naoparticles which have 4% emulsifier concentration (C-GNps) were discrete and spherical in shape with size distribution ranges from 8 to 48 nm, approximately. The changes in TEM size before and after drug loading indicates the presence of curcumin in GNps.

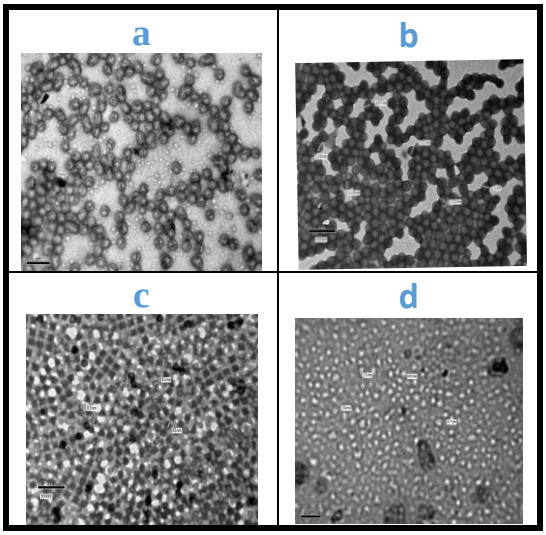


Fig.1 Typical TEM micrographs of gelatin nanoparticles with the effect of different emulsifier concentration as (a) 1%, (b) 2%, (c) 3%, (d) 4%

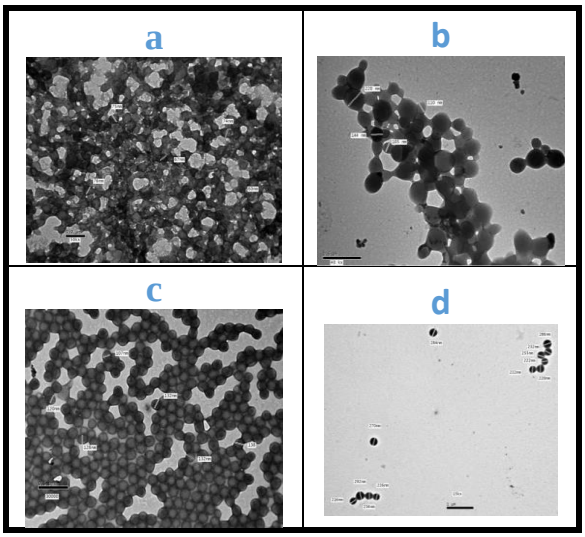


Fig.2 Typical TEM micrographs of gelatin nanoparticles with the effect of different Gelatin ratios as (a) (0.4g/ml), (b) (0.6g/ml), (c) (1.25g/ml), (d) (2.5g/ml)

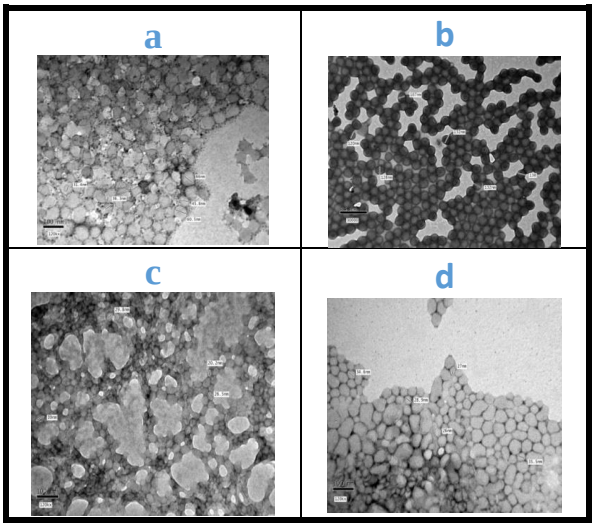


Fig.3 Typical TEM micrographs of gelatin nanoparticles with the effect of different cross linker ratios as (a) 0.06g, (b) 0.12g, (c) 0.18g, (d) 0.24g

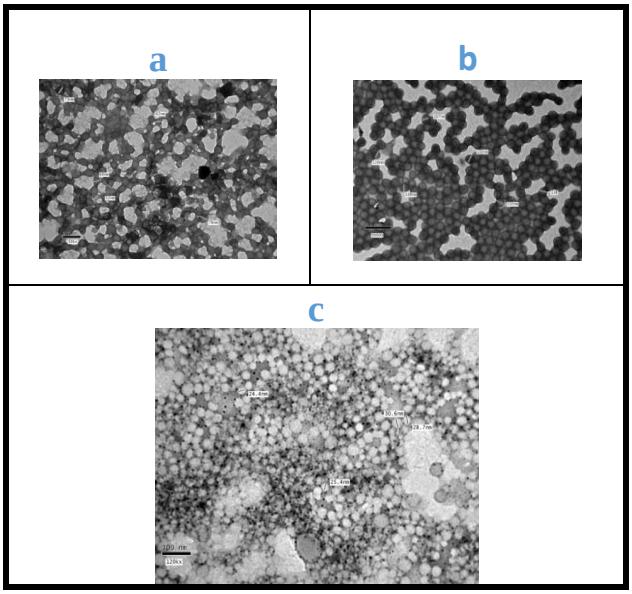


Fig.4 Typical TEM micrographs of gelatin nanoparticles with the effect of different non solvent volume as (a) 150ml , (b) 200ml, (c) 250ml

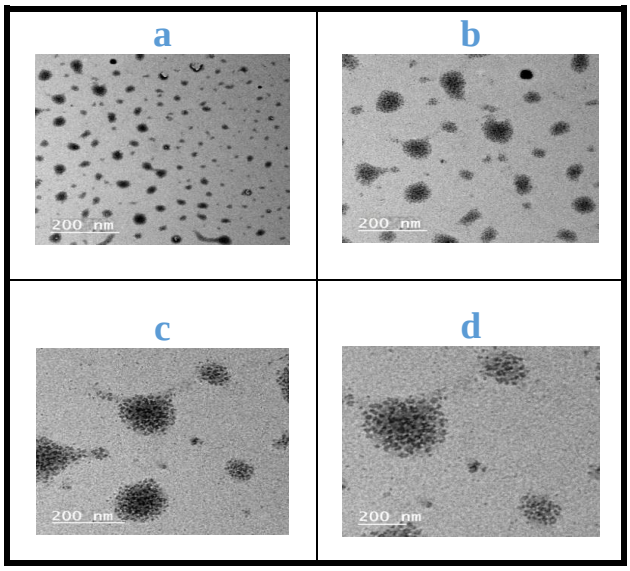


Fig.5 Typical TEM micrographs of curcumin loading gelatin nanoparticles which has 4% emulsifier concentration (C-GNps)

Entrapment Efficiency

Drug entrapment efficiency for the curcumin drug in gelatin nanoparticles was measured using the indirect technique as shown in the experimental procedure. The EE of the drug is controlled by the polymer concentration, the emulsifier concentration, and the drug to polymer ratio.

Overall, drug EE results revealed high values, indicating that the approach of emulsification technique of curcumin drug in gelatin nanoparticles under the presence of SLES as an emulsifier is a viable method for incorporating hydrophobic drugs to produce drug-loaded polymeric nanoparticles having high drug EE values.

It was previously established that the absorption of the drug from emulsion formulation influenced by particle size [36].Therefore as a result of the small particle size of the polymeric nanoparticles created via nanoprecipitation process, the nanoparticles may have been more vulnerable to drug percolation, resulting in high EE values [37].

Also EE was chosen as the index because of the medicinal and pharmacological benefits of high EE, the optimization of formulation and preparation techniques can help to improve EE. Based on a previous report, the affecting parameters on EE in the formation of C-GNPs, such as stirring speed, emulsification temperature, and the time of emulsification, were studied [38]. So stirring speed was set to 800 rpm, emulsification temperature was set to 55±1°C, and emulsification time was set to 30 minutes.

Table 2 provides the EE values of curcumin on GNps and their relation with the concentrations of gelatin, drug, and emulsifier. It is showed that the entrapment decreases with increases the gelatin content; three batches of C-GNps were made based on the obtained conditions, with an average EE of 43.5%. EE was influenced significantly by the consistency of the gelatin solution. So when the concentration of gelatin was increased to 20%, the solution became so viscous that dispersion of gelatin droplets into the dispersion media were difficult, causing nanoparticles adhesion or enlarged particle size, hence the drug could not be effectively entrapped in the nanoparticles, as previously reported [39,40]. In addition, the weight ratio of the drug to the polymer had an effect on the EE, so when the weight ratio of drug to polymer was altered between (10:200) and (20:200), the EE increased from 40% to 57%, respectively. But when the drug was added in excess of 10% of the polymer, however, portion of a drug was unable to be encapsulated throughout the nanoparticles. These results could suggested by the amount of drug used surpassed the polymer nanoparticles loading capacity. [41].

An optimum experimental condition was a combination of the set factor's ideal levels. The best formulation combination with a drug to polymer weight ratio of 20:200 (100 mg to1000 mg), a gelatin concentration of 10% (w/v) (1 g in 10 ml), and an emulsifier concentration of 2% (w/v) with a higher EE 82 % for the optimized formulation.

Table.2 Curcumin entrapment efficiency on Gelain nanoparticles

Factor effecting on EE	Levels	Entrapment efficiency %
(A) Gelatin concentration	10%	54%
	15%	40%
	20%	36%
(B) Weight ratio of drug and polymer	10:200	40%
	15:200	46%
	20:200	57%
(C) Emulsifier concentration	1%	54%
	1.5 %	80%
	2 %	82%

FTIR Spectra

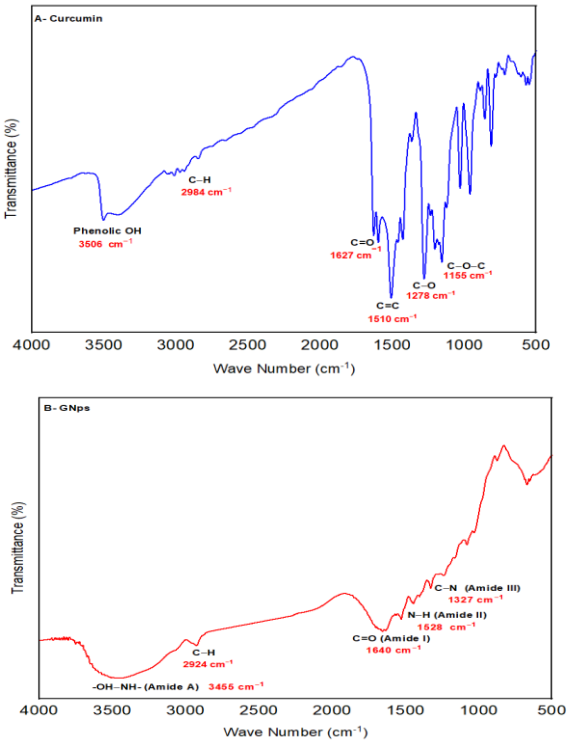
Fourier transform infrared (FTIR) spectroscopy was used to identify the characteristics of curcumin, gelatin nanoparticles (GNps) and curcumin-loaded gelatin nanoparticles (C-GNps). This characterization carried out to identify the functional group of both GNps and curcumin and to validate the entrapment of curcumin in the polymeric nanoparticles.

In **figure.6(A)** the FTIR spectra of curcumin, showed an intense broad band at 3506 cm^{-1} due to phenolic O–H stretching vibration, the appeared band as a little shoulder at 2984 cm^{-1} is assigned to the vibration of C–H stretching of aliphatic group, the observed band at 1627 cm^{-1} is attributed to a stretching vibration of C=O group and C=C exhibits at 1510 cm^{-1}, where a stretching vibrations band of aromatic ring observed at 1598 cm^{-1}, and 1425 cm^{-1} corresponds to the bending vibration of olefinic C–H bound to benzene ring, curcumin has also bending vibration of the two hydroxy groups of the phenolic and enolic group at 1373 cm^{-1}, 1243 cm^{-1}, respectively, 1278 cm^{-1} aromatic C–O stretching vibrations and at 1155 cm^{-1} C–O–C stretching vibrations have also observed. Finally the bands at 956 & 719 cm^{-1} which corresponds to the out of plane bending of C–H for the aromatic ring [42].

According to its FTIR analysis, curcumin includes three reactive functional groups associated to its numerous biological effects, one diketone moiety and two phenolic moiety groups; it has a lot of chemical reactions that are important. C=O groups contribute as hydrogen in biological activity.C-4 acts as a hydrogen donor and acceptor. These functional groups will be contributed to the curcumin's properties [43].

On other hand, FTIR spectra of GNps in **figure.6(B)** shows the characterization absorption band at 3455 cm^{-1} which corresponds to the stretching vibration of N–H group of amide A and –OH bonds due to physically adsorbed water [44]. Moreover the absorption band at 2924 cm^{-1} assigned to C–H stretching vibration of CH_2 group. The bands at 1640 cm^{-1}, 1528 cm^{-1} attributed to Amide I and Amide II, respectively, the amide-I band, which appeared at 1640 cm^{-1} is the most significant peak for IR analysis of gelatin among all the absorption bands which corresponds to a strong C=O stretching, where amide-II shows at 1528 cm^{-1} for N–H bending and also absorption bands at 1445, 1327, 1237 cm^{-1} are assign to C–H bending, C–N stretching, and N–H bending of amide III, respectively., According to their observations, the amide and carboxylic groups are active sites that can coordinate with GNps surface and allow the Nps to conjugate with gelatin biopolymer [45].

Curcumin encapsulated in GNps shows in **figure.6(C)** had shifted its C=O band from 1627 to 1636 cm^{-1}, C=C band from 1510 to 1516 cm^{-1} and C–O–C stretching band from 1155 to 1159 cm^{-1}, which indicated an interaction between curcumin and GNps; where the disappearance of the 3506 cm^{-1} peak in the CGNps spectrum indicated an interaction of curcumin's phenolic –OH with GNps , most likely through hydrogen bonding, and appearing N–H stretching of amine group at 3468 cm^{-1}. When curcumin was encapsulated in GNps, the major absorption peaks weakened and shifted, indicating that the curcumin molecules were located inside the GNps and therefore their spectrum "signature" was "hidden" [46].



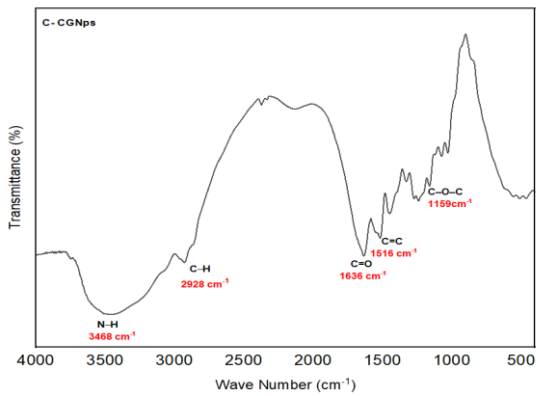


Fig.6 FT-IR spectra of, A- Curcumin, B- GNps and C- C-GNps

In Vitro Release Studies

Studies of the anticancer drugs release behaviour to the desired site are critical for developing an effective cancer targeted drug system. As a result, the curcumin drug release characteristics were investigated in buffer solutions with two pH values in the presence of tween to improve the release efficiency and complete the solubility of the released curcumin [47].

Figures.7, 8 show the percent of curcumin released as a function of time from gelatin nanoparticles loaded with curcumin (C-GNps), compared to free, which reveal that gelatin nanoparticles has controlled sustained slow of curcumin release as significantly prolonged compared with the drug release of curcumin free was extremely rapid.

When comparing the *in vitro* release patterns of free curcumin and curcumin encapsulated into GNps in the first 6 hours, free curcumin released was very rapid with approximately 98 % and 96 % of the curcumin released, whereas only 17% and 13% of curcumin was released from GNps in the media of PH (1.2) and PH (7.4), respectively, following that, a sustained release pattern of encapsulated curcumin from C-GNps was observed.

The presence of a small dispersed amount of curcumin on the surface of the nanoparticles caused drug to be released at first [48], as well as the prolonged release seen after that when compared to free curcumin may be due to curcumin slow diffusion through GNps [49].

Figures. 7, 8 represented the release of the drug from GNps at different pH values which indicated an initial burst at first 30 minutes however the release was faster in acidic media, followed by a sustained release pattern of the drug. According to the studies, it was reported that the drug release rate is pH dependent and closely correlated with the swelling of Nps in the two fluids [50].

Additionally, in the simulative gastric fluid for acidic pH 1.2 the release performance which shows in **figure.7** was more visible and higher drug release percent than that in alkaline pH. (7.4) may be also due to the repulsive forces between drug molecules and GNps in the acidic solution in combination with the swelling effect force drug release into the solution.

Following that C-GNps showed a sustained drug release property, with a 78% accumulated drug release percentage after 72 hr in the dissolution media, on the other hand the dissolution media of pH 7.4 in **figure.8** showed a release percentage 60% after 72 hr.

The *in vitro* release of drug-loaded gelatin nanopartices was primarily based on three processes: drug diffusion through the medium, polymer decomposition, and polymer swelling or disolution. The release profile can also be divided into four segments: (a) the initial burst phase, when the drug surface was dropped into the release medium; (b) the induction phase, when the drug was released at a gradually decreasing fast rate; (c) the slow release phase, when the drug was released at a sustained slow rate; and (d) the final release phase (not shown), when the particle decomposes to release the residual drug [51].

In vitro drug release studies revealed that the carrier GNps is pH sensitive indicating that it could be useful for cancer-targeted drug delivery in which the acidic media of cancer cells aids active release of the drug from GNps, increasing drug bioavailability in cancer cells, and resulting in high therapeutic efficacy when compared with the normal cells [52].

The non-specific distribution of drugs in tissues and rapid burst release, which leads to toxicity, is one of the fundamental problems of conventional anticancer therapy. So the encapsulation of drugs in Nps, results in prolonged drug release and improved pharmacokinetic [53].

These considerations led to GNps had a sustained releasing property of curcumin which takes around 6 days to approach the total breakdown using the current technique which confirms the applicability of this nanoformulation for the delivery of curcumin in cancer treatment.

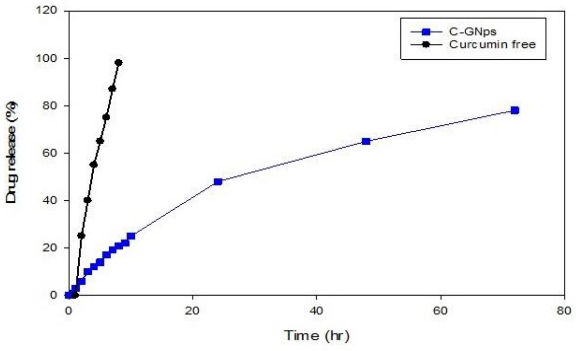


Fig.7 Release profiles of curcumin and C-GNps at PH 1.2

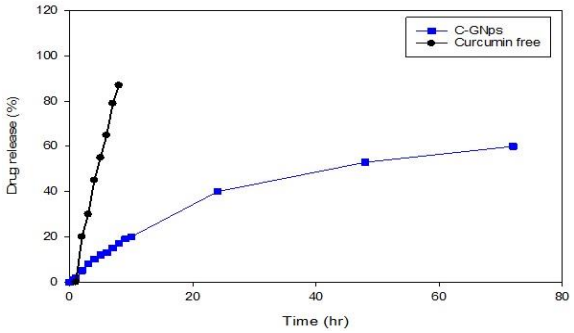


Fig.8 Release profiles of curcumin and C-GNps at PH 7.4

Conclusion

In the current study, GNps was prepared by nanoprecipitaton method with various parameters and utilized the optimum which has 4% emulsifier concentration as a carrier for curcumin as anticancer drug, after that C-GNps was synthesized successfully using an emulsification linkage process with SLES as an emulsifier resulting in high drug entrapment efficiency. The properties of the curcumin-entrapped on GNps as well as the EE values were influenced by the content of gelatin nanoparticles, ratio of drug to polymer and emulsifier concentration. The resulting nanoparticles were spherical and had a symmetrical size distribution. The curcumin incorporated in the polymeric nanoparticles was investigated by FTIR and TEM. The *in vitro* release studies showed that curcumin exhibited a slow and sustained release property from its loaded polymeric nanoparticles as compared to the free drug which shows rapid release. Based on these results, can be concluded that, in the presence of SLES as an emulsifier, GNps could be a promising carrier for delivering curcumin as cancer treatment.

References

1- Debbage P., Targeted drugs and nanomedicine: present and future. *Curr. Pharm Des*., 15:153–172 (**2009)**.

2- Patra J. K., Das G., Fraceto L. F., Campos E .V. R., del Pilar R. T., Acosta Torres L. S., Diaz Torres L. A., Grillo R., Swamy M. K., Sharma S., Nano based drug delivery systems: recent developments and future prospects., *J Nanobiotechnol*., 33:16-71**(2018)**.

3- Xing M., Yanli Z., Biomedical applications of supramolecular systems based on host-guest interactions., *Chem Rev*.,115:7794–7839 (**2015)**.

4- Mokhtari P. N., Arsalani N., Marjan M., Hamishehkar H., Development of biocompatible fluorescent gelatin nanocarriers for cell imaging and anticancer drug targeting., *J Mater Sci.*, 53:10679–10691 **(2018)**.

5- Fathollahipour S., Ghaee A., Mehrizi A., Koosha M., Controlled Antibiotic Delivery by Gelatin Nanospheres: Optimization, Characterization and Antibacterial Evaluation., *Journal of nanostructure*., 6:285-292 (**2016)**.

6- Felekoria M., Khakbiza M. N., Ezafatib N., Mohammadia J., Eslaminejadc M., Comparative analysis and properties evaluation of gelatin microspheres crosslinked with glutaraldehyde and 3-glycidoxypropyltrimethoxysilane as drug delivery systems for the antibiotic vancomycin., *International Journal of Pharmaceutics*., 557:208-220 **(2019)**.

7- Akhter K. F., Zhu J., Zhang J., Nanoencapsulation of protein drug for controlled release., *J Physic Chem Biophysic*; doi:10.4172/2161-0398.S11-001 **(2012)**.

8- Kumar S.S.D., Mahadevan S., Vijayaraghavan R., Mandal A.B., Macfarlane D.R., Curcumin loaded poly(2-hydroxyethyl methacrylate) nanoparticles from gelled ionic liquid—*in vitro* cytotoxicity and anticancer activity in SKOV-3 cells., *Eur J Pharm Sci*; 51:34–44 **(2014a)**.

9- Samrot A., Burman U., Philip S., Chandrsekaran K., Synthesis of curcumin loaded polymeric nanoarticles from crapshell derived chitosan for drug delivery, *Informatics in medicine unlocked*.,10: 159-182 **(2018)**.

10- Krausz AE, Adler BL, Cabral V, et al. Curcumin-encapsulated nanoparticles as innovative antimicrobial and wound healing agent., Nanomed Nanotechnol Biol Med.,11:195-206 **(2015)**.

11- El-Abd M., Agwa., M. M., Synthesis and characterization of microbial poly3-hydroxybutyric acid nanocarrier for curcumin as an antibreast cancer agent., Egyptian Pharmaceutical Journal., 19:266–273 **(2020)**.

12- Anand P., Sundaram C., Jhurani S., Curcumin and cancer:An"old-age" disease with AN "Age-old" solution., *CancerLett.*, 267:133-164 **(2008)**.

13- Trapani A., Denora N., Iacobellis G., Sitterberg J., Bakowsky U., Kissel T., Methotrexate-Loaded Chitosan- and Glycolchitosan-Based Nanoparticles: A Promising Strategy for the Administration of the Anticancer Drug to Brain Tumors., *AAPS Pharm. Sci. Tech*., 12:1302-1311 **(2011)**.

14- Trapani A., Sitterberg J., Bakowsky U., Kissel T., The potential of glycol chitosan nanoparticles as carrier for low water soluble drugs., *Int.J. Pharm*., 375: 97-106 **(2009)**.

15- Naksuriya O., Okonogi S., Schiffelers R., Hennink W., Curcumin nanoformulation:A review of pharmaceutical properties and preclinical studies and clinical data related to cancer treatment, *Biomaterials*., 35: 3365-3383 **(2014)**.

16- Lee E., Khan S., Lim K., Gelatin nanoparticle preparation by nanoprecipitation., *Jornal of biomaterials science*., 22: 753-771 **(2011)**.

17- Cao F., Ding B., Sun M., Guo C., Zhan L., Zhai G., Lung targeted delivery system of curcumin loaded gelatin microspheres., *Drug delivery*., 18: 545-554 **(2011)**.

18- Moustafa A.B., Sobh R.A., Rabie A.M., Nasr H.E., Ayoub M.M.H. Synthesis and *in vitro* release of guest drugs-loaded copolymer nanospheres MMA/HEMA via differential microemulsion polymerization., *J App Polym Sci*.,129:853–865 **(2013)**.

19- Sobh R.A., Nasr H.E., Mohamed W.S., Formulation and *in vitro* characterization of anticancer drugs encapsulated chitosan/multi-walled carbon nanotube nanocomposites., *J App phar Sci*., 8: 32-40 **(2019)**.

20- Sobh R., Mohamed A., Moustafa A., Nasr H., Encapsulation of curcumin and curcumin derivative in polymeric nanospheres, *Polymer-plastic technology and engineering*.,54:1457-1467 **(2015)**.

21- Chu H., Liu N., Wang X., Jiao Z., Chen Z., Morphology and *in vitro* release kinetics of drug loaded micelles based on well defined PMPC-B-PBMA copolymer., *Int.J.Pharm*., 371:190-196 **(2009)**.

22- Valot P., Baba M., Nedelec J., Sintes Zydowicz N., Effects of process parameters on the properties of biocompatible ibuprofen-loaded microcapsules., *Int. J. Pharm*.,369:53-63 **(2009)**.

23- Thompson C., Hansford D., Higgins S., Rostron C., Hutcheon G., Evaluation of ibuprofen- loaded microspheres prepared from novel copolyesters, *D.L. Int. J. Pharm*., 329:53-61 **(2007)**.

24- Joshi G., Kevadiya B., Patel H., Bajaj H., Jasra R., Montmorillonite as a drug delivery system intercalation and *in vitro* release of timololmaleate, *Int. J. Pharm*., 374: 53-57 **(2009)**.

25- Liu L., Sun L., Wu Q., Guo W., Li L., Chen Y., et al., Curcumin loaded polymeric micelles inhibit breast tumor growth and spontaneous pulmonary metastasis., *Int J Pharm*., 443:175–82 **(2013)**.

26- Khodashenas B., Ardjmand M ·, Baei M. S., Rad A. S ·, Khiyavi A. A., Gelatin–Gold Nanoparticles as an Ideal Candidate for Curcumin Drug Delivery: Experimental and DFT Studies., Journal of Inorganic and Organometallic Polymers and Materials., 29:2186–2196 **(2019)**.

27- Gogoi P., Dutta A., Anand Ramteke A., Maji T. K., Preparation, characterization and cytotoxic applications of curcumin-(±) α-lipoic acid coloaded phosphorylated chitosan nanoparticles in MDA MB 231 breast cancer cell line., polymers and advanced technologies., 31:2827-2841 **(2020)**.

28- Tabata Y., Nagano A., Ikada Y., Biodegradation of hydrogel carrier incorporating fibroblast growth factor., Tissue Eng., 5: 127–138 **(1999)**.

29- Esposito E., Cortesi R., Nastruzzi C., Gelatin microsphere: influence of preparation parameters and thermal treatment on chemico-physical and biopharmaceutical properties., *Biomaterials*., 17: 2009–2020 **(1996)**.

30- Rahman S. M., Telny T. C., Ravi T. K., Kuppusamy S., Role of surfactant and pH in dissolution of curcumin., *Indian journal of pharmaceutical sciences*.,71(2):139-142 **(2009)**.

31- Alhmoud H., Ibrahim N.E., El-Hallous E. Surfactants Solubility, Concentration and the other Formulations effects on the drug release rate from a controlled release matrix., *Afr J Pharm Pharmaco*.,8(13): 364–371 **(2014)**.

32- Zhang J.X., Li S.H., Li X.D., Li X.H., Zhu K., Morphology modulation of polymeric assemblies by guest drug molecules: TEM study and compatibility evaluation., *J. Polymer*., 50: 1778-1789 **(2009)**;.

33- Qian C.; McClements D.J., Formation of nanoemulsions stabilized by model food-grade emulsifiers using high-pressure homogenization: Factors affecting particle size., *Food Hydrocolloids*., 25: 1000-1008 **(2011)**.

34- Bilati U., Allemann E., Doelker E., Eur. J. Development of a nanoprecipitation method intended for the entrapment of hydrophilic drugs into nanoparticles., *Pharm. Sci*., 24:67-75 **(2005)**.

35- Saxena A., Sachin K., Bohidar H. B., Verma A. K., Effect of molecular weight heterogeneity on drug encapsulation efficiency of gelatin nano-particles., Colloid Surfaces B: *Biointerfaces*., 45: 42-48 **(2005)**.

36- Rozner S., Shalev D. E., Shames A. I., Ottaviani M. F, Aserin A, Garti N. Do food microemulsions and dietary mixed micelles., *Colloids Surf. B.*, 77:22-30 **(2010)**.

37- Özer F., Beşkardeş M.O., Zareie H., Pişkin E., Nanoparticles carrying hydroxyl groups produced by microemulsion., *J. Appl. Polym. Sci.*, 82: 237-240 **(2001)**.

38- Wang S.N., Deng Y.H., Zheng S.H., et al., Studies on preparation of ciprofloxacin liposomes and its tissue distribution in mice., *Chin Pharm J.*,38: 274–277 **(2003)**.

39- Raouf G., Cecilia S., Johan C., Effect of preparative parameters on the characteristics of poly (d,l-lactideco-glocolide) microspheres made by the double emulsion method., *Int. J. Pharm*., 4: 205–216 **(1996)**.

40- Ghaderi R., Struesson C., Carlfors J., Effect of preparative parameters on the characteristics of poly (d,l-lactideco-glocolide) microspheres made by the double emulsion method. *Int. J. Pharm*., 141: 205–216 **(1996)**.

41- Jain A, Gupta Y, Jain SK. Potential of calcium pectinate beads for target specific drug release to colon., *J Drug Target*., 15: 285–294 **(2007)**.

42- Sindhu K., Rajaram A., Sreeram K.J., Rajaram R., Curcumin conjugated gold nanoparticle synthesis and its biocompatibility., *RSC Adv*., 4 (4):1808-1818 **(2014).**

43- Gunathilake T. M. S. U., Ching Y. C., Uyama H., Hai N. D., Chuah C., Enhanced curcumin loaded nanocellulose: a possible inhalable nanotherapeutic to treat COVID-19., *Cellulose*., 29:1821–1840 **(2022)**;.

44- Nhari R. M. H. R., Ismail A., Che Man Y. B., Analytical methods for gelatin differentiation from bovine and porcine origins and food products. *Journal of Food Science*., 77:42–46 **(2012)**.

45- Paramera, E.I.; Konteles, S.J.; Karathanos, V.T., Microencapsulation of curcumin in cells of Saccharomyces cerevisiae., *Food Chem*., 125: 892–902 **(2011)**.

46- Muvaffak A., Gurhan I., Hasirci N., Prolonged cytotoxic effect of colchicine released from biodegradable microspheres., *J Biomed Mater Res B Appl Biomater.*, 71: 295–304 **(2004)**.

47- Ratanajiajaroen P., Watthanaphanit A., Tamura H., Tokura, S., Rujiravanit R., Release characteristic and stability of curcumin incorporated in chitin non-woven fibrous sheet using Tween 20 as an emulsifier., *Eur. Polym. J*., 48: 512-523 **(2012)**.

48- Sun L.,Wu Q., Guo W., Li L., Chen Y., Li Y., Gond C., Qian Z., Wei Y., Curcumin loaded polymeric micelles inhibit breast tumor growth and spontaneous pulmonary metastasis., *Int. J. Pharm*., 443:175-182 .,**(2013)**.

49- Ma X., Wang H., Jin S., Wu Y., Liang X.J., Construction of paclitaxel-loaded poly (2-hydroxyethyl methacrylate)-g-poly (lactide)- 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine copolymer nanoparticle delivery system and evaluation of its anticancer activity., *Int. J. Nanomed*., 7:1313-1328 **(2012)**.

50- Zhang X., Meng L., Lu Q., Fei Z., Dyson P. J. ,Targeted delivery and controlled release of doxorubicin to cancer cells using modified single wall carbon nanotubes. *Biomaterials.*, 30:6041–6047 **(2009)**.

51- Budhian A., Siegel S.J., Winey K.I., Controlling the *in vitro* release profiles for a system of haloperidol-loaded PLGA nanoparticlesInt., *J. Pharm*., 346: 151-159 **(2008)**.

52- Mu L., Feng S.S., Fabrication, characterization and in vitro release of paclitaxel (Taxol) loaded poly (lactic-co-glycolic acid) microspheres prepared by spray drying technique with lipid/ cholesterol emulsifiers., *J. Control Release*., 76: 239–254 **(2001)**.

53- Muddineti O.S., Kumari P., Ajjarapu S., Lakhani P.M., Bahl R., Ghosh B., Biswas S., Xanthan gum stabilized PEGylated gold nanoparticles for improved delivery of curcumin in cancer., *Nanotechnology.*, 27:325101–325112 **(2016)**.