

Amino Acid Combined Chitosan Nanoparticles for Controlled Release of Doxorubicin Hydrochloride

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THERAPY with amino acids (TAAI) has large interest recently for tumor treatment. Complementing to our previous studies in biopolymers and amino acids for medical applications this study was addressed to design a unique therapeutic regime consisting of a cocktail from therapy with amino acids (TAAI) and polymer therapy for cancer disease. This regime is depending on synthesized Doxorubicin loaded chitosan - glutamic acid (Cs-Ga-DOX) nanoparticles via ionic gelation path- way. The encapsulation efficiency was 69%. The average size was 20-37 nm with spherical, homogenous structure, and positive zeta potential.

FTIR of Cs-Ga confirmed the formation of amide linkage at 1644 cm⁻¹. The in -vitro release of DOX was examined for both pH 5.5 and 7.4 to be biphasic with mutual burst release followed by sustained release for 168 h to reach 58% at pH 5.5 and 25% at pH 7.4.

This result suggested that Cs-Ga nanoparticles presented a promising mixture of glutamic amino acid and chitosan as pH-responsive nano-carrier for anticancer drugs.

Keywords: Therapy with amino acids (TAAI), Chitosan- glutamic nanoparticles, Smart drug delivery systems, Doxorubicin hydrochloride, In-vitro controlled release study.

Introduction

In Egypt and other African countries; several types of diseases are widespread especially chronic diseases and all cancer types. Majority of world's new cancer cases (> 60%) occur in Africa, Asia, Central and South America. In addition, 70 % of the world's cancer deaths also occur in these regions [1].

Despite of their relative efficacy, chemotherapeutic drugs present major drawbacks, such as drug resistance and nonselective cytotoxicity leading to severe side effects [2,3]. Among the common chemotherapeutic drugs, doxorubicin is the first line treatment used for a wide range of cancers that is administered systemically and exhibits rapid systemic elimination, short plasma circulation time and non-specific bio distribution profile.

Small amounts of DOX reach the target site associated with low efficiency, in addition to its cardiotoxicity and nephrotoxicity associated with

unformulated doxorubicin have led researchers to develop new and innovative strategies to entrap this drug in different nano-carriers [4,5]. One of their solutions is polymer therapeutics which is an expression recently widespread recently in pharmaceutical field and used in nanomedicine. Polymeric backbone to which drug can be conjugated directly or via a cross linker is designed to facilitate the release of the drug from the conjugate at the target site and to be one type of the drug delivery systems (DDS).

Intelligent drug delivery systems [6,7] designed by using smart natural or synthetic polymers in nano-scale, have high surface area than the micro and macro forms which increase its chemical reactivity. One of those smart natural polymers is chitosan, which has received a great attention in biomedical applications due to its well-documented biocompatibility, low toxicity [8-11], and degradability by human enzymes [12] in addition to its pH-sensitivity which enables to design a smart polymeric drug delivery system administrated by injection intravenous (IV) [13-

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Primary amine groups in chitosan structure facilitate interaction with numerous drugs, offering controlled drug release [17,18], when particles degrade in an acidic environment simulating the tumor cells or around inflammation sites, this allows site-specific or targeted drug release. Because of their small size, nanoparticles enter the cells and interact with subcellular structures easier and this improves cellular uptake followed by increasing the inhibition rate % against tumor cells [18].

In spite of all useful properties of chitosan and nano chitosan that can be exploited in the field of drug delivery, its low solubility in aqueous media and low stability in nanoscale [19] compromises its applications, so we have to modify chitosan with other materials to offer additional desirable properties, including P glycoprotein 1 (P-gb) inhibition, enhanced mucoadhesiveness and increased stability and solubility of chitosan nanoparticles.

Nowadays, therapy with amino acid imbalance, called TAAI, or with amino acids as a dose of nutrients has been widely used to treat tumors due to the ability of some amino acids to minimize tumor cells [20,21]. Some amino acids improved the anti-cancer therapeutic effect via conjugation with it [22,23]. Due to the variation of chemical structures and properties of amino acid side chains, they are used in many other applications and as chelating agents for several elements.

Anyhow, there are a few papers which refer to the antitumor effect and the benefits of amino acids in biomedical applications but most of them were about the poly amino acids derivatives for gene delivery applications ([24]. The poly L-Ga derivatives with paclitaxel (PGTXL) are water-soluble which have activity against some cancer types [25]. Using poly L-Ga derivatives is complicated and has some toxicity. So, we still need a simple biocompatible carrier in nanoscale to enhance the antitumor effect.

Based on the above, in this paper we try to combine therapy with amino acid (TAAI) and polymer therapy in one regime depending on direct modification of chitosan nanoparticles with glutamic amino acid without cross linker to

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limit the drawbacks of chitosan and add new free carbocyclic group to its structure which will make it useful for a wide range of medical applications. We used the synthesized chitosan / glutamic nanoparticles as a carrier for DOX nanoparticles, the optimum conditions that affect drug loading, encapsulation efficiency and the in-vitro release profile for DOX release were investigated. As far as our knowledge, this is the first time to synthesize this composite in nanoscale and use it in cancer treatment.

Experimental

Materials

Chitosan powder (Cs) with degree of deacetylation (DD) 87%, L- Glutamic acid and Sodium tripolyphosphate (TPP) purity 85% and Doxorubicin hydrochloride (DOX) purity 99% were purchased from Sigma-Aldrich. All other chemicals of pure grade were purchased from local and international companies and used without further purification.

Methods

Synthesis of chitosan-glutamic acid (Cs-Ga) adduct

Chitosan- glutamic adduct was prepared according to our previous publications [8,9] as follows: Chitosan and l-glutamic amino acid were mixed in equimolar ratios and condensation reaction takes place using Dean–Stark apparatus in presence of xylene until the theoretical amount of water was separated. Chitosan amide product was separated by filtration, washing several times with methanol, hot distilled water, ethanol and then dried in an electric oven at 50°C and weighed.

Synthesis of chitosan-glutamic nanoparticles (Cs-Ga NPs)

Cs-Ga NPs were obtained through ionic gelation pathway by using TPP with Cs-Ga adduct as follows:

Cs-Ga (3 mg/ml) was dissolved in acetic acid solution (1% w/v) until the solution is clear then, TPP solution was added to Cs-Ga solution with ratios; 1:3, 1:2.5, 1:2 & 1:1 (w/w %) with continuous stirring at ambient temperature for 6h. The production of Cs-Ga /TPP nanoparticles started via the TPP initiated ionic gelation mechanism. These nanoparticles were separated, washed several times then supernatant layer was removed and the precipitate re-suspended in water

followed by freeze drying for further use.

Synthesis of Doxorubicin-HCl loaded chitosan-Ga nanoparticles

Cs-Ga was dissolved in acetic acid (1% w/v) with concentration 3 mg/ml with chitosan: TPP (w/w %) ratio DOX (10, 15, and 20%) was dissolved in distilled water and mixed with TPP solution then added to Cs-Ga NPs solution dropwise and stirred at pH (4.5) at 37°C for 6h. DOX loaded Cs-Ga nanoparticles were purified and the precipitate was re-dispersed in water, and then freeze dried.

To highlight the effect of glutamic acid on chitosan nanoparticles, we synthesized native chitosan nanoparticles and loaded them with DOX to compare between the results obtained from both.

Synthesis of chitosan nanoparticles

Chitosan nanoparticles were prepared through ionic gelation pathway using TPP with chitosan [26,27] as follows: Chitosan with concentration 3 mg/ml was dissolved in 1% acetic acid solution. TPP solution was added to chitosan solution with ratio 2.5:1 (w/w %) and magnetically stirred for 6 hr at room temperature. Cs-TPP nanoparticles were formed progressively by the ionic gelation mechanism initiated by TPP. These nanoparticles were centrifuged at 5000 rpm for 20 min then, the precipitate resuspended in water and freeze dried.

Loading Doxorubicin-HCl on chitosan nanoparticles

Chitosan was dissolved in acetic acid (1% w/v), with concentration 3 mg/ml. TPP solution (w/w %) added dropwisely to chitosan solution with ratio 2.5:1 and DOX solution (10, 15, and 20%) and stirred together in acidic medium (4.5) for 6 hr at room temperature. DOX loaded chitosan nanoparticles were separated then freeze dried.

Determination of loading efficiency

The loading efficiency for both modified & native Cs NPs was determined indirectly by measuring the absorbance values of unloaded drug in the supernatant with a UV spectrophotometer at 481 nm (Equation 1)

$$\text{DOX loading efficiency (\%)} = (\text{Total DOX-Free DOX}) / (\text{Total DOX}) * 100 \quad (1)$$

In vitro drug release

In vitro release profiles of DOX from both NPs were tested according to the method reported by Jin et al. [28] as follows: 2 ml of a suspended NPs was sealed in a dialysis bag (MWCO 12,000 to 14,000 Da), and dialyzed against 50 mL of 0.1 M buffer solution of the desired pHs (pH 5.0: acetate buffer solution and 7.4: phosphate buffer solution) at 37°C under continuous shaking at 100 rpm.

Determination of optimum criteria for Cs -Ga Nps production

The optimum criteria for selecting Cs -Ga Nps reproduced from the various formulations of Cs-Ga: TPP (3:1, 2.5:1, 2:1, 1:1 & 1:2) (v/v) respectively, were determined from the formulation which gave the best nanosize (the smallest nanosize) determined by DLS analysis.

Investigation of Cs-Ga NPs pH sensitivity

Investigating pH sensitivity of Cs-Ga NPs was carried out according to the method reported by Aydin & Pulat [29] a follows: Cs-Ga NPs were soaked in buffer solutions (3-7.4) at ambient temperature for 1 and 3 hr. Investigating the pH response of DOX loaded Cs-Ga NPs was done according to changing in particles size using Zetasizer Nano S after the incubation period.

Solubility test for chitosan-glutamic nanoparticles

Solubility of Cs-Ga nanoparticles was investigated in different solvents (water, acetic acid and DMSO) with concentration 1% under continuous stirring for 4 hr.

Determination of DOX loading efficiency %

Loading efficiencies of prepared Cs nanoparticles and Cs-Ga nanoparticles were determined by indirect method as reported in our previous work [30] : nanoparticles were centrifuged at 5000 rpm for 30 min, followed by measuring supernatants DOX solutions via UV spectrophotometer at 481 nm. Calculations were done using the calibration curve, and loading efficiency was calculated according to the following equation:

$$\text{DOX loading efficiency (\%)} = (\text{Total DOX-Free DOX}) / (\text{Total DOX}) * 100$$

Instruments and measurements

i. Fourier Transform Infrared (FTIR) Spectral Studies:

FTIR spectra of Cs, Cs-Ga, Cs-Ga NPs and DOX loaded Cs-Ga NPs were recorded by FT-IR spectrophotometer on Bruker Vector 22 Germany in the range 400 to 4000 cm^{-1} at resolution of 4 cm^{-1} .

ii. Particle size determination and zeta potential:

The particle size distribution of the Cs nanoparticles and its derivative was determined by Dynamic light scattering (DLS) and zeta potential in deionized water solution (pH 6.3, ionic strength 0) using a Nicomp 380 ZLS particle seizer (PSS, USA).

Transmission Electron Microscopy (TEM): To determine particle size and shape of Cs, Cs-Ga NPs and DOX loaded NPs, transmission electron microscopy (TEM) was used and they were negatively stained with 1.0% (w/v) phosphotungstic acid.

iii. Scanning Electron Microscopy (SEM)

The morphological characteristics of both Cs-Ga and Cs-Ga-DOX NPs were examined by scanning electron microscope (SEM). The samples were coated with gold and imaging at accelerating voltage 30kV.

Results and Discussions

FTIR spectra studies

The IR spectral data for (Cs-Ga) and (Cs-Ga-DOX-TPP) are shown in Fig. 1(a & b). Structures of these compounds were confirmed by the presence of characteristic absorption bands for Cs-Ga and DOX encapsulated nanoparticles as follows: One can see from Fig. 1a for Cs-Ga an absorption band at 1644 cm^{-1} characteristic for the amide linkage -CONH which proves the reaction between one of the carboxylic groups of glutamic acid and NH_2 of chitosan through condensation reaction. From the other hand, the absorption bands at 1700–1600 cm^{-1} , 1500–1550 cm^{-1} and 2870–2970 cm^{-1} are assigned for N–H bending, C–N stretching and aliphatic C–H respectively. Figure 1, also illustrates that the band which appears at 1421 cm^{-1} can confirm that the other carboxylic group of glutamic acid is still free and not reacted. Also, CO stretching band of acid was present at 1312 cm^{-1} and 1515 cm^{-1} for (N–H stretch band of amino group).

From the spectrum given in Fig. 1b, the

characteristic absorption bands for chitosan-glutamic DOX encapsulated nanoparticles are as follows: there are characteristic band at 3420 cm^{-1} which corresponds to (O–H stretching overlapped with N–H stretch) which is shifted from 3438 cm^{-1} in Cs-Ga and became wider with increase in intensity indicating an enhancement of hydrogen bonding between TPP and NH_2 of Cs-Ga derivative nanoparticles, and at 1410 cm^{-1} which indicates reaction between DOX and COO^- of glutamic acid. There is also an absorption band at 1151 cm^{-1} represents $\text{P}=\text{O}$ which indicates crosslinking reaction between chitosan and TPP; also, the absorption band 1650 cm^{-1} of chitosan – glutamic encapsulated with DOX is assigned to the amide linkage; 1457 cm^{-1} (amide II band; N–H bending) for L-glutamic acid as shown in Fig. 1b and at 1515 cm^{-1} (N–H stretch band of amino group).

Therefore, we can conclude from the FTIR results that one of the COOH groups of L-glutamic acid has been successfully bonded to NH_2 group of chitosan to form (CONH) amide linkage at 1644 cm^{-1} through condensation reaction and the band at 1550 cm^{-1} refers to the free COOH group of Cs-Ga

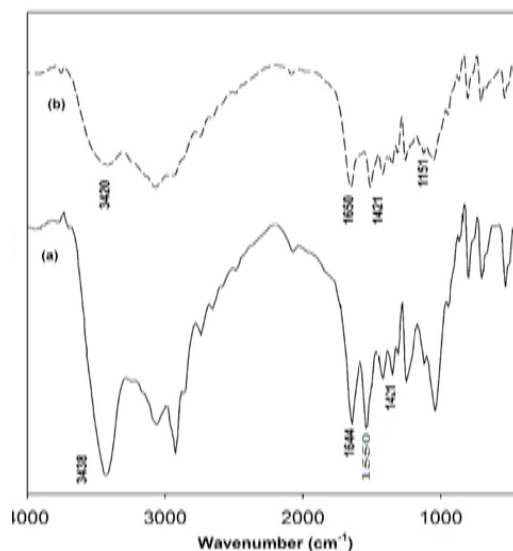


Fig. a (a) FTIR of chitosan-glutamic particle, (b) FTIR spectra of chitosan-glutamic nanoparticles loaded with DOX.

NPs which reacted with DOX during loading process.

Reaction mechanism

Formation of Cs–Ga adduct occurred through condensation reaction between chitosan and glutamic acid using Dean–Stark apparatus with equimolar ratio in the presence of xylene until the theoretical amount of water was separated as shown in Scheme 1.

Cs–Ga adduct was separated, washed several times with methanol, hot distilled water, ethanol and then dried in an electric oven at 50 °C.

Chitosan is quickly gelling when interacts with polyanions via formation of inter and intra molecular cross linkages mediated by polyamines [27]. Synthesis of chitosan–glutamic nanoparticles is depending on electrostatic interaction between remaining positively charged quaternary ammonium of chitosan and negatively charged sodium tripolyphosphate (TPP) at 37°C immediately to produce ionically cross linked Cs–Ga Nps.

The optimum conditions for preparation of the chitosan–glutamic nanoparticles

Size of the prepared nanoparticles is considered an important feature which affects the biological performance of nanoparticles.

For this reason, we have studied the optimum Cs–Ga / TPP ratio which gives the smallest size of Cs–Ga NPs, the results shown in Table 1 illustrate the effect of Cs–Ga: TPP ratio on particle size and the ability of formation of nano-sized particles by visual observation, since when we added TPP to Cs–Ga solution, its appearance was converted from a clear to opalescent solution which indicated a change in its physical properties

to form nanoparticles. In this study; our visual observation is a clear solution and opalescent suspension (Table 1).

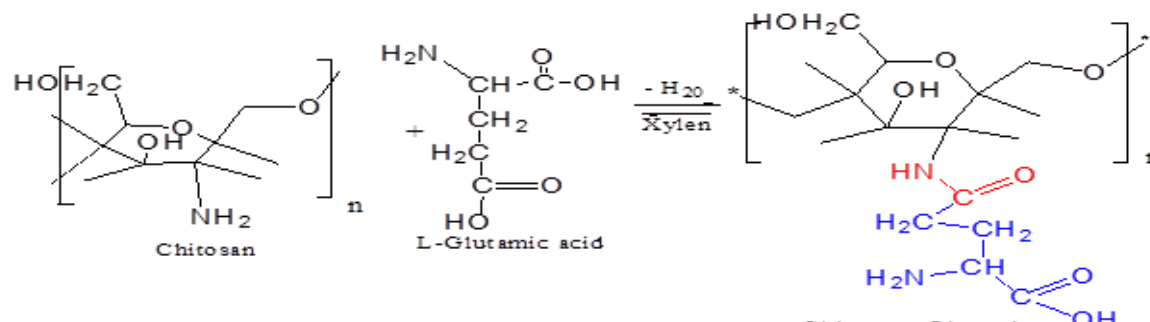
Formation of clear solution may be due to formation of nano complexes not nanoparticles. From all mentioned remarks, the best Cs–Ga: TPP ratio is 2:1 which gave the smallest size of particles 90 nm and from zeta potential results we found the best sample size has positive zeta potential with (+46) which means that we have very good stable nano particles which can be used in several applications.

TEM and SEM analysis

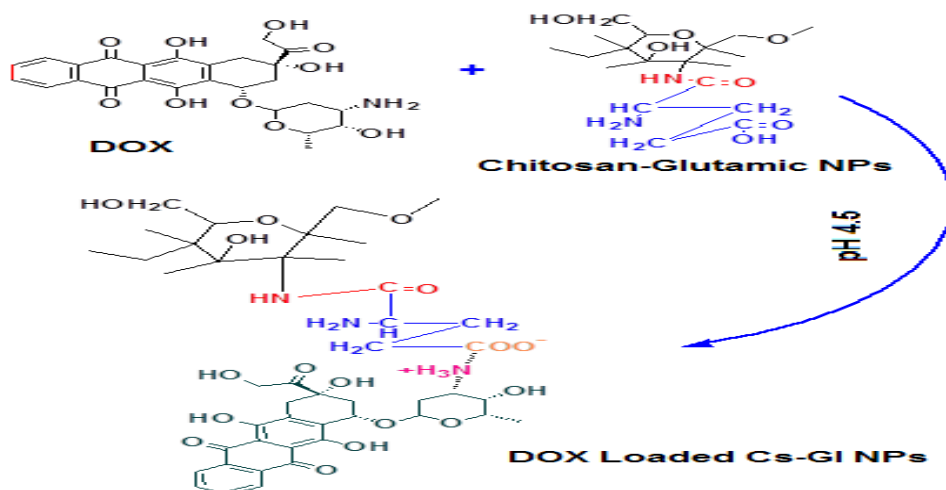
TEM micrographs of prepared Cs–Ga NPs confirmed the DLs results (taking into consideration the difference between TEM & DLS mechanisms as DLS determines the size of particles in liquid state and the swelling rate of chitosan directly affects the particles size, but TEM investigates the size of particles in solid state so we considered that it gives the accurate size of prepared NPs and showed that the formed nanoparticles were spherical in shape with a diameter ranging between 20 nm and 37 nm as illustrated in Fig.2 (1).

SEM images (Fig.2-2-a) of Cs–Ga NPs showed that the prepared Cs–Ga NPs have spherical shape with good ionic crosslinking to each other; in case of Cs–Ga–DOX nanoparticles SEM image (Fig. 2-2-b), also the shape of Cs–Ga NPs didn't change after loading DOX.

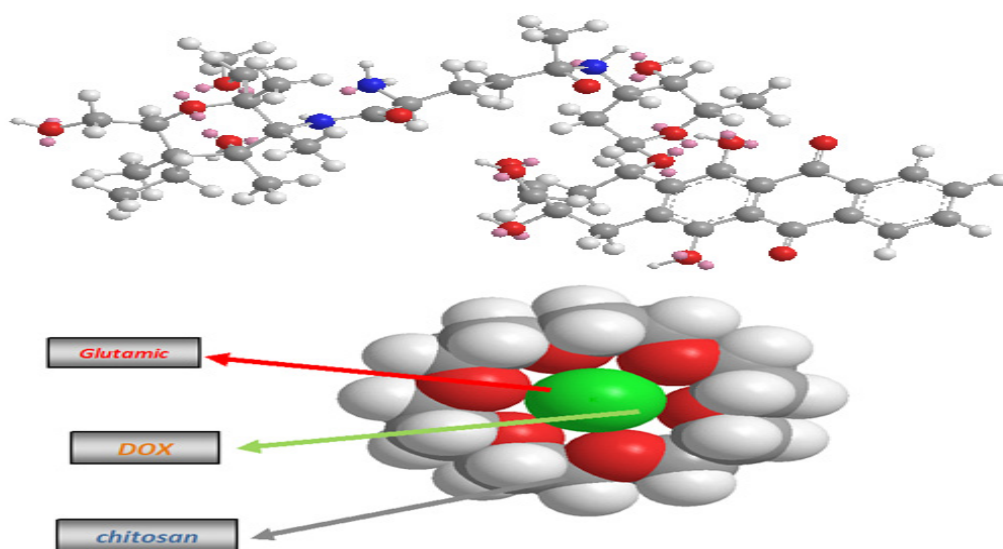
The results obtained from TEM, DLS and SEM measurements suggest successful preparation of spherical Cs–Ga nanoparticles that ranged from (20 – 37) nm and 90 nm respectively which can be considered optimum for prolonged circulation and for accumulation in tumor tissue plus enhancing the drug



Scheme 1. Preparation of Cs–GA acid adduct.



Scheme 2. Preparation of Cs-Ga acid nanoparticles conjugated with DOX



Scheme 3. 3D structure of DOX conjugated with chitosan-glutamic nanoparticle.

TABLE 1. Particle size distribution of Cs-Ga NPs and polydispersity determined by DLs after 48h from its preparation.

Sample	CS/TPP(w/w) ratio	Nanoparticle diameter (nm)	Polydispersity index (PDI)	Physical appearance
Cs-Ga NPs	1:1	303.5	0.51	Clear solution
	2:1	90.6	0.294	Opalescent suspension
	3:1	133.2	0.396	Opalescent suspension
	4:1	173.4	0.716	Aggregates
	6:1	288.6	0.48	Aggregates

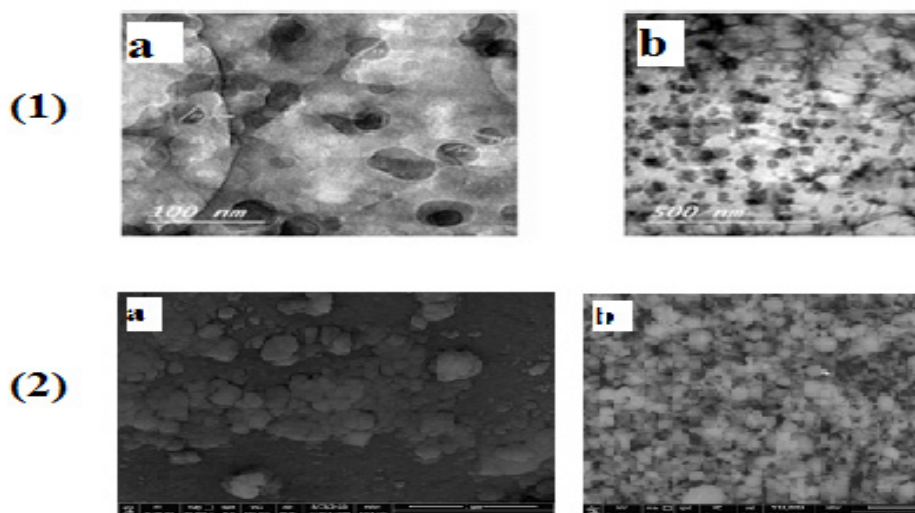


Fig. 2. (1) TEM images of (a) Cs-Ga NPs (b) Cs-Ga-Dox NPs, (2) SEM images of (a) Cs-Ga NPs (b) Cs-Ga-DOX NPs.

diffusion in tissues.

pH sensitivity of Cs-GA nanoparticle

Effect of glutamic acid on chitosan pH sensitivity was investigated by imposed Cs-Ga in phosphate buffers with different pH values (pH: 3 - 7.4). The data in Fig. 3 present the average size of chitosan nanoparticles by DLS after 1, 2 and 3 incubation hours. From Fig.3 particle size rapidly increased by increasing pH from 3 to 5.5 but, with increasing pH from 5 up to 7.4 a slight increase in particle size occurred, this is due to the nature of chitosan in basic or slightly basic media since the NH_2 groups of chitosan are not protonated in basic media so Cs-Ga NPs not only maintains its stability in crosslinked form with TPP but also, swelled in slow rate which leading to this slight increase in particles size. This high increasing of particle size in acidic media (3-6) may be due to formation of NH_3^+ ion of the residual NH_2 on the chitosan chain in acidic buffer media (pH 3-6), leading to an increase in the electric density and repulsion force between crosslinked chitosan chains. These results are in accordance with previous results for native chitosan nanoparticles reported by Uhrich and Singh, et al. [17,31]

These results summarize that, the synthesized Cs-Ga NPs are pH- sensitive,

also indicate that surface density of NH_3^+ ion and the degree of protonation are reversibly responsive with increasing in pH value.

From all over mentioned results, the modification of chitosan with glutamic acid has no effect on pH susceptibility of native chitosan nanoparticles.

So, Cs -Ga nanoparticles are expected to be a smart carrier for DOX with effective and accelerated drug release in the tumor environment with pH value (around 5).

Solubility test for Cs- Ga nanoparticles

One of the biggest limitation of chitosan in biomedical applications is its insolubility in aqueous media (water) [32,9] So, solubility of Cs-Ga derivative in water, acetic acid and DMSO solvents with different concentrations was examined to show the modification effect of chitosan with glutamic acid. The results are presented in Table 2. These results confirm that the Cs-Ga adduct is water soluble at concentration of 8% (w/v) and 5% (w/v) for acidic media and soluble in DMSO at 8% (w/v). Increasing the solubility was due to presence of more hydrophilic Ga (COOH) group in the new polymer adduct. From all mentioned results, Cs-Ga adduct with its improved solubility features can be considered very promising in the

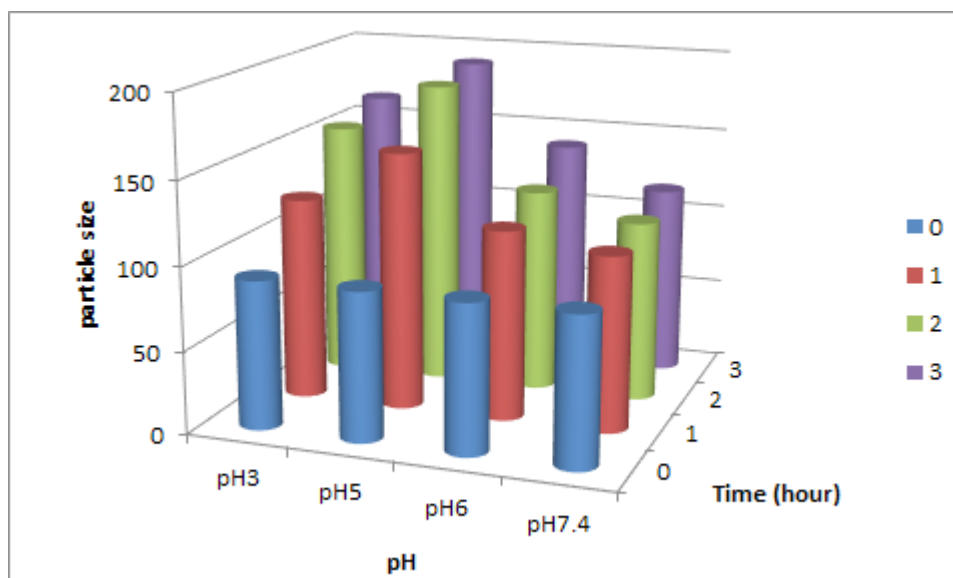


Fig.3. pH Sensitivity of Cs-Ga NPs according to particle size changes in different times.

TABLE2. Solubility of Cs –Ga NPs toward water, acetic acid and DEMSO.

Concentration (w/v) %	Water	Acetic acid (1%)	DMSO
3	Insoluble	Soluble (immediate)	Slightly soluble
4	Insoluble	Soluble	Slightly soluble
5	Slightly Soluble	Low viscosity gel	Soluble
6	Slightly Soluble	Low viscosity gel	Soluble
7	Soluble	viscous gel	Soluble (immediate)
8	Soluble (immediate)	viscous gel	Soluble (immediate)

environmental and biomedical applications [33,34].

Drug loading efficiency

Loading efficiency of both [Cs/DOX and Cs-Ga/DOX] drug nanoparticle concentrations 10, 15 and 20% mg/ml were calculated by Equation 1, from the results presented in Table 3, the loading efficiency % increases with increasing DOX

TABLE 3. Loading efficiency % of DOX with different percentages on Cs & Cs-Ga NPs.

Nanoparticles	Drug	
	concentration% (DOX: Cs w %)	L.E %
Chitosan-DOX	10%	20%
	15%	25%
	20%	30%
Chitosan-Ga-DOX	10%	50%
	15%	57%
	20%	69%

concentration to reach the highest concentration 69% for Cs-Ga /DOX and 30% for Cs-DOX.

This enhancement in the loading % of DOX on Cs-Ga as carrier for the drug is due to the modification with Ga acid which adds free COOH group to the chemical structure of chitosan-glutamic adduct which is attached to DOX nanoparticles and increased loading % in addition to the original way for loading by entrapment of some DOX through chitosan matrix in case of pure chitosan-DOX nanoparticles.

In vitro drug release

From the *in vitro* release profiles of DOX-loaded Cs and Cs-Ga nanoparticles in Fig. 4, the Cs-Ga-DOX NPs at the physiological pH 7.4 release behavior was biphasic with 9% in the first hour then reached 12% after 12 hr. In the second phase a controlled release took place to reach ≈23% after 168 hr. In acidic medium (pH 5.5), the release was also biphasic with 8.5% release

in the first hour with no much increase after 12 hr to reach 12%. In the second phase, a controlled release was obtained to reach 65% after 168 hr. In case of Cs-DOX NPs, release depended on DOX diffusion from chitosan matrix as it reached 17% at the first hour and no much increase after 12 hr to reach 20% then sustained release occurred through the degradation and swelling of chitosan nanoparticles in acidic media to reach 80% after 168 hr. From the obtained results we confirm that DOX is loaded in the Cs-Ga nano-carrier through two different mechanisms, first one is through adsorption on the surface of nanocarriers and this appears in the initial burst release in the first

hour till 12 hr in both pH 7.4 and pH 5.5 media. The second mechanism started through sustained controlled release of DOX from nano-carrier and the DOX loaded through electrostatic interaction mechanism between COO⁻ of Cs-Ga NPs and DOX as we have mentioned for the previous studies reported by Koide, (1998) Chandran et al. (2017) and (Soares, et al. (2016) [35,37].

On the other hand, DOX physically loaded in the Cs NPs through adsorption on the surface of nanocarriers causes high initial burst release in the first hour till 12 hr in both pH 7.4 and pH 5.5 media followed by weak sustained release for

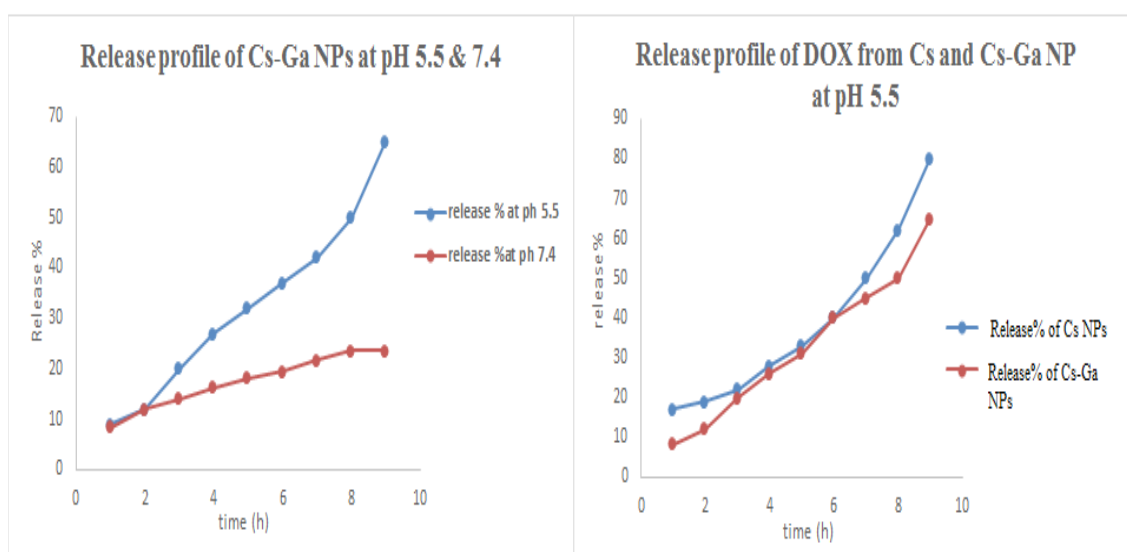


Fig. 4. Release profile of DOX from Cs and Cs-Ga NPs at different pH values.

DOX depending on degradation rate of chitosan nanoparticles in acidic media.

Conclusion

In this study, we successfully prepared for the first time (to the best of our knowledge) an eco-friendly, colloidal pH-responsive chitosan-glutamic (Cs-Ga) nanocarriers for doxorubicin hydrochloride (DOX) via ionic gelation pathway. This new adduct of L-glutamic acid and chitosan was investigated and its structure was confirmed via (IR, TEM, SEM and DLs) with particle size in the range of 23-37 nm. In addition, zeta potential measurements were recorded.

The best particle size was obtained with Cs-Ga: TPP ratio 2.5:1(w: w) after studying the effect of different ratios of Cs-Ga: TPP to obtain the best

particle size. Cs-Ga /DOX nanoparticles were prepared and the effect of drug concentrations on Cs-Ga NPs was studied and showed that the best DOX concentration was 20% with encapsulation efficiency 69%.

Also, the in-vitro release of DOX at different pH values (5.5, 7.4) indicated that Cs-Ga NPs are suitable as biocompatible and colloidal pH responsive nano-carrier and enhanced the controlled DOX release.

These results are considered just a promising start for wide use of biocompatible amino acids as conjugates with different polymers to improve their features for numerous applications.

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دراسة عن الإنطلاق المحكم لعقار الدوكسوروبسين هيدروكلوريد المحمل على جسيمات نانومترية محضرة من تفاعل الكيتوزان و الحامض الأميني

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تناولت هذه الدراسة تصميم نظام علاجي فريد يتكون من كوكبتيل يشتمل على حبيبات بوليمرية نانومترية ناتجة من تفاعل الكيتوزان و الحامض الأميني (الجلوتاميك) و المحمل بعقار الدوكسوروبسين هيدروكلوريد المضاد لمرض السرطان عبر مسار تكوين جل أيوني وكانت كفاءة التغليف ٦٩٪. و متوسط حجم الحبيبات ذات الشكل الكروي المتجانس ٢٠-٣٧ نانومتر ، وذات جهد موجب محدد من خلال جهاز جهد زيتا. وأكدت نتائج التحليل الطيفي للأشعة تحت الحمراء تكون رابطة أميدية عند شريط امتصاص ١٦٤٤ سم-١. تم فحص إنطلاق عقار الدوكسوروبسين في المختبر لكل من الرقم الهيدروجيني ٥,٥ و ٧,٤ ليكون ثنائي الطور مع الإفراج عن انفجار متبادل يليه إطلاق (افراز) مستمر لمدة ١٦٨ ساعة لتصل إلى ٥٨٪ عند الرقم الهيدروجيني ٥,٥ وتصل إلى ٢٥٪ في الرقم الهيدروجيني ٧,٤ وتقترح هذا الدراسة تقديم توليفة واعدة لحبيبات نانومترية من حمض جلوتاميك و كيتوزان كناقل للأدوية المضادة للسرطان متجاوب مع درجة الحموضة