Published by SET Publisher

Journal of Basic & Applied Sciences

ISSN (online): 1927-5129

Formulation and Characterization of Poly (Acrylic Acid)-Co-Chitosan Nanoparticles as pH-Thermo-Responsive System to Control Delivery

Abir Derbali[1,2,*], Djallel Bouzid[1,2] and Olivier Boyron[3]

[1]*National Polytechnic School of Constantine, BP 75, A, Novel ville RP Constantine, Algeria*

[2]*Laboratory of Process Engineering for Sustainable Development and Health Products, BP 75, A, Nouvelle Ville RP, Constantine, Algeria*

[3]*Catalysis, Polymerisation, Processes and Materials, 43Bd du 11 Nov.1918 (B.P.82007)69616 Villeurbanne, CEDEX, France*

Article Info:

Keywords:
Chitosan,
control release,
poly (acrylic acid),
pH responsive nanoparticles.

Timeline:
Received: October 10, 2022
Accepted: November 05, 2022
Published: November 15, 2022

Citation: Derbali A, Bouzid D, Boyron O. Chitosan nanoparticles as pH-thermo-responsive system to control delivery. J Basic Appl Sci 2022; 18: 72-86.

Abstract:

The present study aims to develop a pH thermosensitive nanocarriers as a drug delivery system to better controll drug release. Nanoparticles was developed by the combination of smart polymers, chitosan and poly(acrylic acid) were chosen as biodegradable vectors to encapsulate and transport the drug. The used method was based on the polymerization of acrylic acid using reticulated chitosan as a template. Analysis of particle size, Zeta potential, and size distribution revealed that most of the resulting nanoparticles had an average diameter less than 100nm, with a high Zeta potentiel about -29.7 mV and a narrow size distribution. In addition, the developed system showed an encapsulation efficiency around 97%. *In vitro* release test was achieved using different buffer solutions with pH equal to 1.2, 3.6, 4.2, 4.8, 6.8 and 7.4. The release profiles showed that nanoparticles provide drug protection at different pH values. They responded at pH = 3.6 and provided sustained controlled release of up to 62.62% over 8 hours. The results reveal that the prepared nanoparticles can be used as drug delivery carriers. They can improve therapeutic efficiency of the drugs used in the treatment of inflamed tissues where the pH is around 3.6 as in the Crohn disease.

DOI: https://doi.org/10.29169/1927-5129.2022.18.09

*Corresponding Author
E-mail: derbaliabir94@hotmail.com

1. INTRODUCTION

Several drugs with proven therapeutic efficacy have limited use because of their undesirable side effects caused by the conventional approaches [1]. Extensive research in the pharmaceutical field is devoted to develop approved strategies for the treatment of current diseases [2]. The anti-inflammatory drug has been widely used in the treatment of several diseases due to their effectiveness in relieving pain [3]. However, their sides effects limited their use, This is the reason why research focused, for long time, on the use of intelligent materials and biodegradable polymers [4,5], in order to developed an appropriate systems to carry drugs and controlled their release [6,7].

In recent years, the oral route is considered as the most suitable and convenient mode to administer drugs. Therefore, it is important to develop a new drug delivery system to avoid side effects and offers better efficacy for oral drug delivery [8]. Several strategies have been developed in nanomedicine field in order to enhance drug effectiveness and better target drug, nanoparticles carried drug in a suitable form are able to vehicle drug at the specific sites and avoiding its accumulation at healthy cells [9] which could improve therapeutic effect and reduce the cytotoxicity of drug.

Polymeric nanoparticles show a potential interest in targeting drug, gene transfection and imaging technology. Several studies have proven that drug nanoparticles carriers are a promising strategy to target drug delivery, because of their ability to improve the therapeutic efficiency with less sides effects, compared with the conventional forms [8,10]. Nanoparticles systems have proven their ability to encapsulate and protect proteins, drugs and small molecules against enzymes and hostile environments [8,10]. Moreover, biodegradables nanoparticles are capable to transport drug to its specific site with a sufficient concentration to eliminate the damage, because of their capacity to penetrate the physiological barriers due to their small size [11]. Several research studies have considered the use of nanotechnology in chemotherapy for cancer treatment [1,10], because nanoparticles have proven their ability to lead treatment to targeted cells while avoiding accumulation in healthy tissues, which can enhance the activity of anticancer drugs. Other studies have been focusing on developing a fluorescent micelle to treat cancer, they reported results that have proven the ability of micelle prove to diagnosis tumors *in vivo* and track drug distribution in tissues [12]. Some research work is investigating to develop magnetic DNA nanoparticles to target tumors, the developed nanoparticles have showed a potential efficiency in targeting gene therapy and in inhibiting tumor growth *in vivo* [7], magnetic DNA nanoparticles exhibited high cellular uptake and high stability compared with naked DNA [7].

Inflamed tissues, as in Crohn disease, present acidic environment compared with healthy tissues [13,14], so the development of drug delivery system which can release drug in acidic environment and hardly released it in neutral or basic environment is a necessary. Smart sensitive polymers have a major interest to target drug delivery because they can respond to changes in a specific internal stimulus such as pH, temperature and the presence of specific substances [15,16]. Chitosan is a hydrophilic, cationic polymer with great biocompatibility and high drug loading efficiency. Several studies improved its pH and thermo-sensitivity [17,18]. However, its limited application to controlled drug delivery and its insolubility [19] make its complexation with another polymer necessary to improve its physico-chemical properties and consequently obtain a desire nanoparticle. Poly (ethylene glycol) (PEG) is used to increase the solubility of both chitosan and sodium diclofenac (DS). Sodium tri-polyphosphate (TPP) proved its ability to crosslinked chitosan with anionic interactions and show a pH sensitivity [20,21].

Poly (acrylic acid) (PAA), is widely used due to its hydrophilicity, which play a major role in increasing the biocompatibility of the formed nanoparticles and enhancing the solubility of hydrophobic drug. Moreover, the pH- sensitivity of PAA which is an important factor make it used for controlling the drug release [20], and better localized the drug diffusion. The use of sodium tri-poly-phosphate, as crosslinking agent, and PEG affect the crosslinked density and the swelling behavior of the formed nanoparticles, allowing a better controlled drug release.

In the present study, we have developed a multi-stimulus nanocarriers which loaded and protect drug, a biodegradable and biocompatible polymer such as chitosan, PAA and PEG were used to developed nanoparticles. The combination between various polymeric network structure is needed to enhance the surface properties of the developed system [22]. Several studies have been carried out using chitosan and PAA due to their pH and thermo-sensitivity [6,23,24] The swelling behavior and the inter and intra molecular interactions between the polymeric chains

and the surrounding medium allowed to controlled drug release and minimize their side effects.

2. MATERIALS AND METHODS

2.1. Materials

Chitosan (CS; Mw 80 kDa; DD>80 %), Poly ethylene Glycol (PEG 4000), Sodium Tripolyphosphate (TPP), Acrylic acid (AA; d= 1,051ml/g; M=72.06 g/mol), Ammonium persulfate (APS), Sodium Diclofenac and all other chemicals were of analytical grade and purchased from Sigma Aldrich

2.2. Preparation of CS-TPP-PAA Nanoparticles

Chitosan was dissolved in distillated water containing (1%wt/v) of acetic acid and left stirred for 24 hours at room temperature, the solution was then filtered on micro filter (0,45 µm) to remove all insoluble impurities form chitosan solution (1% wt/v, pH= 4.2). The CS-TPP-PAA nanoparticles were prepared by polymerization of acrylic acid in presence of chitosan using TPP (0,1% wt/v; pH= 3) as crosslinking agents. Ammonium persulfate (APS, 1g) was dissolved in 30 ml of CS to initiate polymerization of acrylic acid, the reaction mixture was placed in water bath at 60°C under stirring [27]. After, 5 ml of drug (1% wt/v), dissolved in PEG 4000, was added in the monomer solution under stirring. Then, TPP was added drop by drop to ensure the crosslinking of chitosan. Next, variable amounts of AA (0.1, 0.4, 0.8, 1.6, 3.2) (g) were added drop by drop to solution to show the effect of AA on the CS-PAA NPs properties. The mixture was continuously stirred for 1 hour [30] and then it was cooled at ambient temperature. The pH of the reaction mixture was neutralized to pH 8 by addition of 1 N NaOH [24,25]. In order to stop the polymerization of AA, 200 ml of methanol was added to the reaction mixture under stirring for 24 hours. The nanoparticles were precipitated in methanol solution and filtered to remove residual monomer and non-encapsulated DS. Distillated water was added after that to remove methanol from NPs. The obtained nanoparticles were filtered with pore size 0.22µm to recuperate nanometric particles. Then, the final product was dried by saponification technique using 1N NaOH solution at 100°C for 1hour [27]. All experimental series carried out were repeated three times.

2.3. Fourier Transform Infrared Spectroscopy

Infrared spectrum of polymers and CS-TPP-PAA nanoparticles were analyzed with FTIR (Shimadzu IRAffinity-IS) spectrophotometer.

2.4. Particle Size, Zeta Potential and Size Distribution

The average particles size, Zeta potential and Size distribution were measured by Zeta-sizer granulometer (HORIBA Scientific ZS 100) on the basis of dynamic light scattering technique at 25°C with detection angle of 90° [26].

2.5. Encapsulation Efficiency% and Drug Loading Capacity %

The absorbance of free DS in supernatant methanol was measured by UV-vis spectrophotometer at 284±2 nm. The amount of free DS was determined from the calibration curve and calculated with the following formula:

$$\text{Encapsulation efficiency}\,\% \frac{\text{initial amount DS - Free amount DS}}{\text{initial amount DS}} \times 100 \quad (1)$$

The obtained nanoparticles were weighted with analytical balance, the drug loading capacity was calculated with the following formula:

$$\text{Drug loading capacity}\,\% \frac{\text{Masse of drug in nanoparticles}}{\text{Masse of nanoparticles}} \times 100 \quad (2)$$

2.6. Swelling Studies

To study the swelling behaviors of CS-TPP-PAA nanoparticles as a function of pH and temperature, 5mg of dry nanoparticles were immersed in 10ml of different buffered solutions pH (1.2, 3.6, 4.2, 4.8, 6.8, 7.4). The samples were stored in an autoclave at 25°C,37°C and 40°C for 48 hours [27]. The size of the swelling samples obtained were then measured by zeta sizer.

2.7. Colloidal Stability

A series of experiments were executed to determine the colloidal stability of CS-TPP-PAA-NPs. In these experiments, the NPs were stored in the reaction mixture at 4°C after stopping polymerization and another one was stored in its dry state. After1,6 and 12 months, the samples were characterized for average particle size and Encapsulation Efficiency.

2.8. *In Vitro* Drug Release from CS-TPP-PAA Nanoparticles

The Drug release studied was carried out in water bath, the temperature was kept at 37°C±1°C with 100 rpm of

magnetic stirring, various buffer solution was used for the release experiments (1.2, 3.6, 4.2, 4.8, 6.8, 7.4). 3 mg of CS-TPP-PAA nanoparticles were placed into a capsule and then immersed in 150 milliliters of buffer medium with various pH values. the released DS was determined by UV-visible spectrophotometer analysis at 274±2 nm.

$$\text{Release rate } \% \frac{\text{Release drug from hydrogel}}{\text{Total drug in the hydrogel}} \times 100 \qquad (3)$$

3. RESULTS AND DISCUSSION

3.1. Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Infrared spectrum of CS-TPP-PAA nanoparticles identified the formed hydrogel and confirmed the drug encapsulation into the polymeric matrix. Figure **1** shows that CS-TPP-PAA nanoparticles hydrogel have a similar spectrum as chitosan. However, some modifications have been observed, the characteristic band at 1651 cm^{-1} due to NH groups of chitosan and the characteristic band at 887cm^{-1} due to P-O groups of TPP disappeared, while the band at 3201.833 cm^{-1} became larger, which indicate that hydrogen bonding enhanced with the occurrence of P=O stretching at 1254cm^{-1}. These modifications confirmed the crosslinking of chitosan by TPP and the formation of nanoparticles [25]. Another pics at 1534cm^{-1} and 1731cm^{-1} characterizing the carboxylic groups of PAA have been observed. Furthermore a characteristic band at 1412 cm^{-1} is due to the stretching vibration in carboxylate anion COO^- of the carboxyl group of PAA, which react with the protonated amino groups of chitosan by electrostatic interaction during polymerization [26]. Both absorption of the carboxylate and alcoholic – OH stretching bands are appeared in the wide range of 3500–2550 cm^{-1} [27].The pics at 1900 cm^{-1} due to the presence of NH_3^+ have been disappeared on the CS-TPP-PAA nanoparticles spectrum, due to the reaction of the amino groups of chitosan with both anionic groups of TPP and AA [28]. Two pics at 1110cm^{-1} and 2900cm^{-1} appeared in the CS-TPP-PAA spectrum indicated that PEG was reacted with the chitosan chain [29].

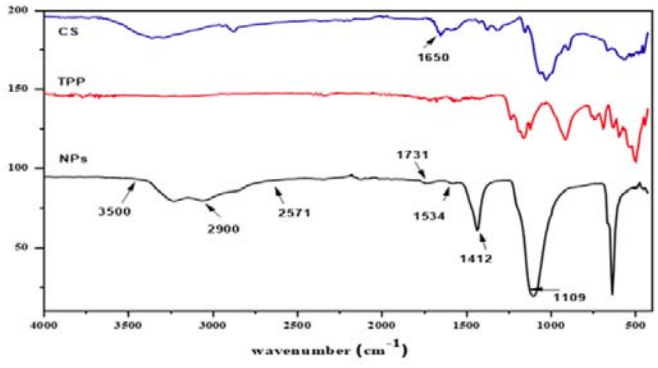


Figure 1: FTIR Spectrum of CS, TPP and CS-TPP-PAA nanoparticles.

3.2. Particle Size and Zeta Potential

Acrylic acid concentration and chitosan: TPP mass ratio are an important parameter used to define the physico-chemical characteristics of CS-TPP-PAA gel, the effect of acrylic acid content on the average size of the CS-TPP-PAA-nanoparticles is studied. Chitosan: TPP mass ratio was maintained (10:1) as an optimum based on previous published works [30]. The amount of AA was changed from 0.1g to 3.2g. Figure **2** shows the influence of AA on the average size of nanoparticles. We observed that their average diameter is ranging from 80.84±16.64nm to 218.2±15nm. Moreover, an increase in the AA from 0.1g to 1.6g in the reaction mixture leads to a significant decrease in particle size from 218.2±15nm to 80.84±16.64nm. The results ishowed that the average size of nanoparticles prepared with (0.8g, 1g, 1.6g, 3.2g) of acrylic acid is less than 100nm. Such nanoparticles can diffuse in cells and reach the target. Moreover, reactive carboxylate ions COO- increase the negative charge density of the matrix and then increase the electrostatic repulsions between the similar charge leading to the formation of a small and stable form of nanoparticles. In this range of AA concentration (0.8g, 1g, 1.6g, 3.2g), a slight decrease in the average size was observed. This change in the average size of NPs can be explained by the fact that the polymerization of acrylic acid was carried out using chitosan as a support on which AA polymerize. It seems that the average particles size depends on the concentration and the average molecular weight of polymeric chain surrounding the drug, and then the copolymer chain is restricted by the CS template [31]. A significant increase in the average size of CS-TPP-PAA nanoparticles to 218.2±15nm and 128.1±24.96nm was observed for 0.1g and 0.4g of AA, respectively. This increase was mainly due to the low Zeta potential of nanoparticles which lead to a decrease in the electrostatic repulsion between negative charges. This increase results from the tendency of nanoparticles to accumulate. Figure **2** indicates that an acrylic acid concentration over 1.6g, leads to a slight increase in particle size. This can be explained by the fact that the

increase of the electrostatics repulsions causes a polymerization of the rest of AA on its own chain [24]. This increases the length of the polymeric chain and the particle size.

The results of the Zeta potential of the CS-TPP-PAA nanoparticles shows that the obtained nanoparticles have generally a negative charge ranging from -8 (mV) to -29.7 (mV). This is due to the free anionic groups of PAA, which did not interact with the amino groups of chitosan. The high Zeta potential of nanoparticles ensures the stability and suitability of nanoparticles. In addition, high charged particles are typically used to increase their reactivity with the charged tissues which enhances their absorption and improves the drug efficiency.

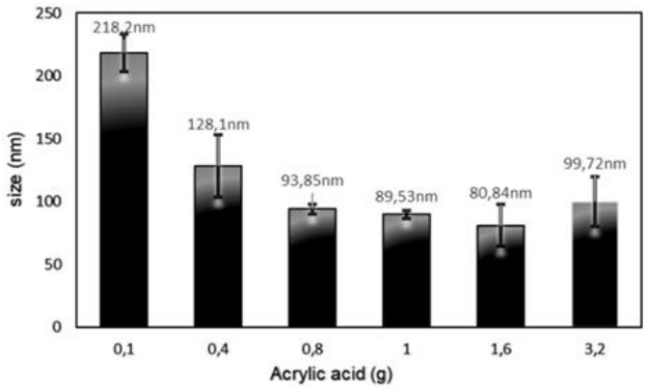


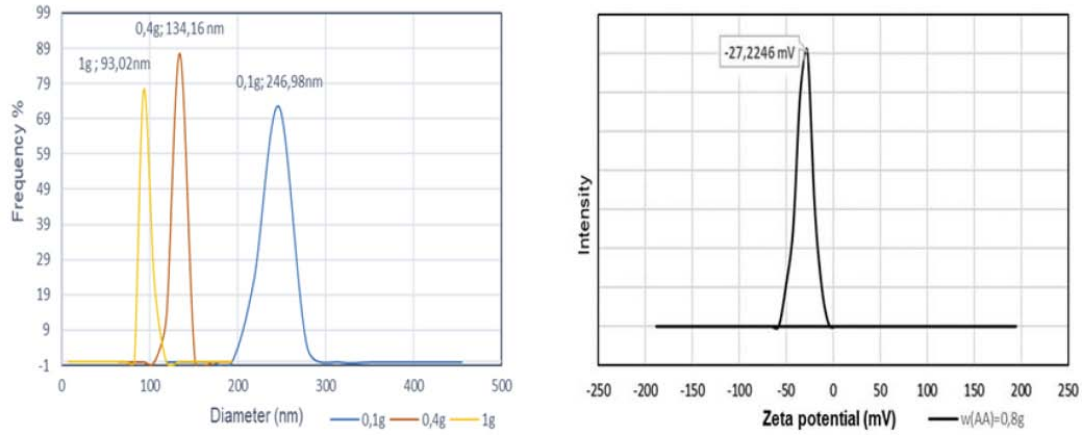
Figure 2: Effect of Acrylic acid on the particles size.

Furthermore, the electrostatics repulsions between the charged surfaces decrease the tendency of nanoparticles to aggregate. We found that when acrylic acid amount increased from 0.1g to 1g, the Zeta potential of CS-TPP-PAA nanoparticles increased significantly from -8 mV±4 to -29.7 mV±3.42. This can be due to the presence of carboxylate ions of PAA excesses than amino groups of CS. The excessive carboxylic charges are absorbed into the CS-TPP-PAA nanoparticles, which increases the zeta potential. Nevertheless, the surface charge of nanoparticles became almost stable at -29.7 mV, when the acrylic acid was increased from 0.8g to 1.6g. Onthe other hand, a change in the particle zeta potential has been observedwhen the AA amount increased to 3.2g, and the surface charge of nanoparticles become positive at +56,1 mV±4.

3.3. Size Distribution

The particles size distribution of CS-TPP-PAA nanoparticles were analyzed by Zeta Seizer ZS 100. The results represented in Figure **3** show the polymer nature and nanoparticles composition used to define the physico-chemical characteristics of the polymeric matrix, including the average diameter and size distribution.

The results illustrated in Figure **3a** represent the relationship between the acrylic acid concentration and the size distribution. It indicates that an increase an acrylic acid from 0.1g to 1g decreases the average diameter of nanoparticles with a narrower distribution. The sample with acrylic acid concentration 0.1g presents a large particle size distribution with a great average diameter. In this concentration range, we obtain a heterogenous population of CS-TPP-PAA nanoparticle with a high tendency to agglomerate compared with the other formulations. Furthermore, an increase in the acrylic acid concentration leads to a decrease in the average diameter. This indicates that



a **b**

Figure 3: (**a**) Size distribution of CS-PAA-TPP nanoparticles with different amount of acrylic acid 0.1g, 0.4g and 1g. (**b**) Zeta potential distribution of CS-PAA-TPP-NPs with 0.8g of acrylic acid.

acrylic acid concentration is an essential parameter which directly affect the degree of non-uniformity of nanoparticles and the stability of nanocarriers population. Similarly, the Zeta potential histogram in Figure **3b** present a narrow Zeta potential distribution with charge surface from -8mV to -30± mV to ±56.1m

3.4. Encapsulation Efficiency %and Loading Capacity%

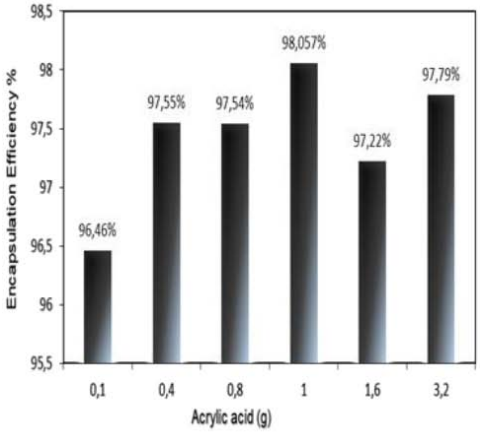
The made nanoparticles yielded an excellent encapsulation loading efficiency with over 90% of Drug, as shown in Figure **4a**, and a greatly controlled release ability, which can contribute to the high hydrophilic property of the drug. As shown in Figure **4b**, an increase in the AA amount from 0.1g to 1g leads to an increase in the loading rate capacity% from 17.19% to 55.70%, which is due to the increase of attraction between acrylic acid and DS [32]. Also, an increase in crosslinking density with increasing acrylic acid amount leads to create more free volumes which proved the penetration of drugs molecules through the polymers network and enhanced the drug loading. Previous studies confirmed the great loading capacity of crosslinked chitosan nanoparticles [26].

3.5. pH and Thermos-Responsiveness of CS-TPP-PAA Nanoparticles

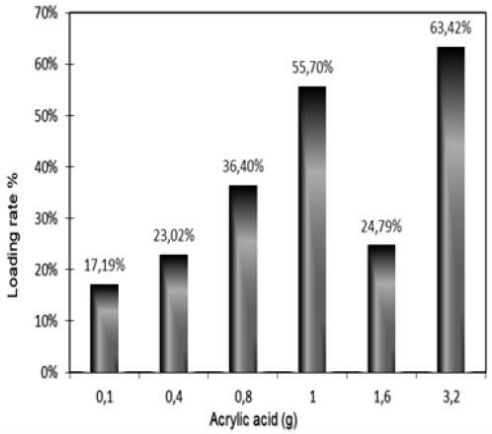
3.5.1. Effect of pH of Buffer Solutions on the Swelling behavior of Nanoparticles

In order to identify the response of the nanoparticles and their sensitivity to the pH of the reaction mixture, the swelling behavior of CS-TPP-PAA nanoparticles for different buffer solutions at different pH value, at 37°C, were studied. The swelling of nanoparticles is proposed to be caused by the difference between the osmotic pressure due to the mobile ions in the polymeric matrix and ions in the surrounding solution [33]. The pH sensitivity of nanoparticles is affected by their composition. The pH responsive polymers such as PAA is usually used to develop systems which maintain the drug in an inactive form until the polymeric matrix has been cleaved [34]. A vacuum will be created through which the drug diffuses. Also, the inter and intra-molecular interactions and the repulsion of charges along the polymer chains [31] affect the character of the polymeric matrix. In order to increase the pH sensitivity of nanoparticles, sodium tripolyphosphate, as crosslinking agent, with a pH sensitive crosslinker is used. The experimental results showed in Figure **5a**, **b**, **c**, **d**, **e** indicate that in gastric pH condition pH=1.2 the CS-TPP-PAA nanoparticles contract, and the average diameter decrease compared with the other buffer solutions. At lower pH (pH< 2), chitosan is completely insoluble and the free carboxylic groups of PAA were completely non dissociated in the neutral form of COOH due to the pKa value of acrylic acid equal to 4.7 which added more compactness to the network [35].

However, no significant swelling effect was observed at low pH. This may be since TPP is more reactive in this range of pH. The PO4 groups of the crosslinking agent were in ionized form which increase the electrostatic repulsion between PO4-and OH- and the electrostatic attraction between PO4- and amino groups of chitosan NH3+. Therefore, the nanoparticles retract and present a small diameter at low pH conditions. Thus, the drug remains confined into the nanoparticles. It was observed that swelling conformation of CS-TPP-PAA was significantly increased at pH 3.6. The average diameter of nanoparticles was also increased. Acrylic



a



b

Figure 4: (**a**) Encapsulation Efficiency % of DS loaded CS-PAA-TPP nanoparticles (**b**) Loading capacity rate% of nanoparticles.

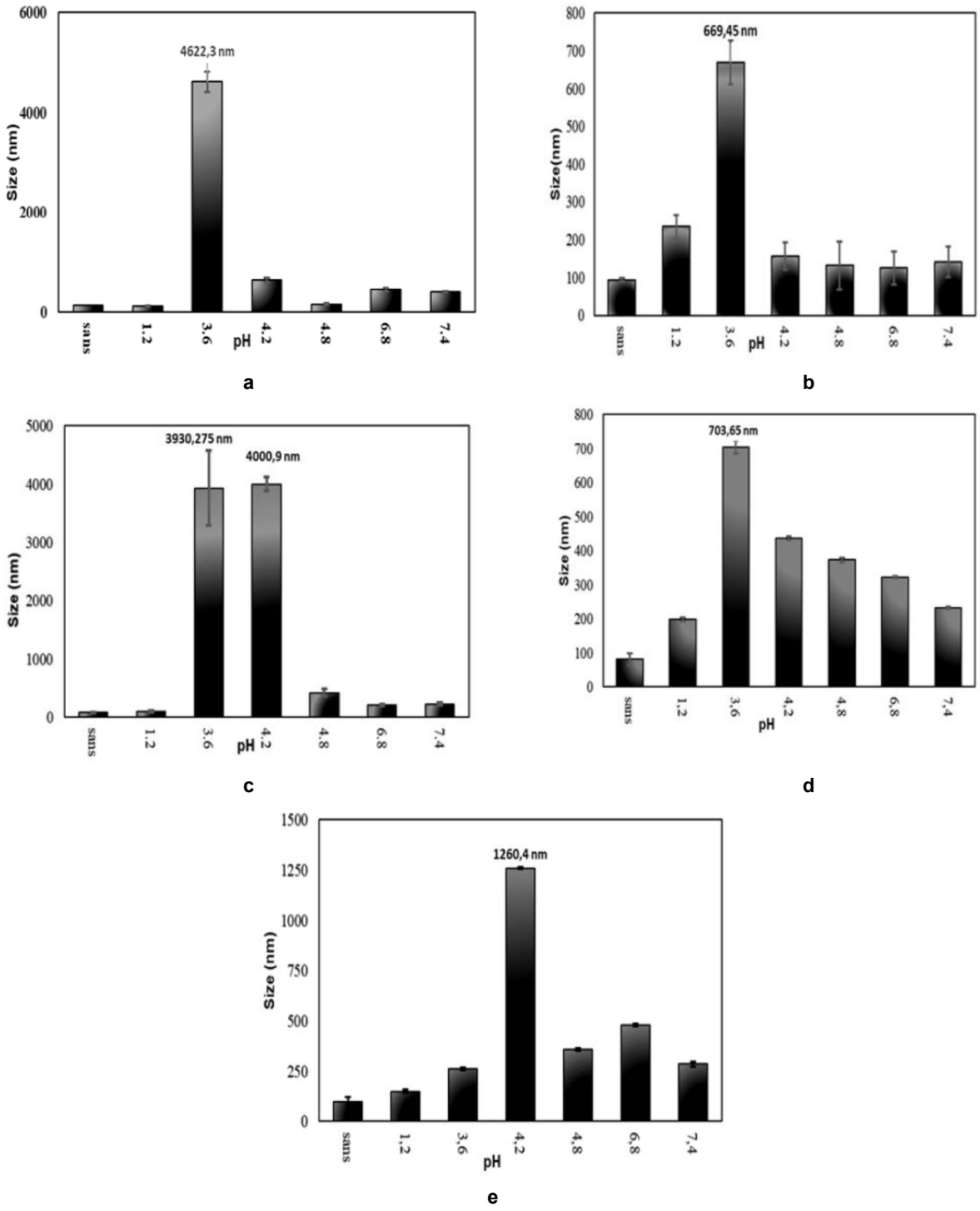


Figure 5: Swelling behaviors of CS-PAA-NPs with different acrylic acid amount: (**a**) wt(AA)=0.4g, (**b**) wt(AA)= 0.8g, (**c**) wt(AA)=1g, (**d**) wt(AA)=1.6g, (**e**) wt(AA)=3.2g in different pH Buffer solutions.

acid monomer is in excess which increases the hydrophilicity of the gel and consequently increases the formation of hydrogen bonds between PAA and the OH- in water surrounding the nanoparticles. Then the polymeric chains expanded and cleaved of their chemical bonds [26]. Moreover, the dissociation of carboxylate units of PAA to form COO- could cause a strong electrostatic repulsion with PO4- groups because in this range of pH the most reactive groups of TPP were ionized, which can increase the repulsions forces between them and the COO-. The repulsion between the polymers created a free volume in the gel network and leads to an increase in the swelling conformation of CS-TPP-PAA nanoparticles. This

facilitates the diffusion of solvent into the network and increases the uptake water capacity. Thus pH 3.6 amino groups of chitosan were protonated, but its proportion is so low and its attraction with COO- became weakened. The relaxation in the polymeric chains at this range of pH allowed the incorporated drug dissolution [36]. This result show that the developed polymeric system is a pH responsive which can be used for the targeting and the controlled drug release.

In contrast, at higher pH at the range of 4.8, the swelling behavior of CS-TPP-PAA decreases and the matrix formed a compact structure. However, the electrostatics repulsion between the ionized amino groups and carboxylate anions can produce a little vacuum in the polymer network and consequently more water can be absorbed.

Figure **5** indicates that the swelling behavior of CS-TPP-PAA gel decreases at pH values of 6.8 and 7.4. This can be explained by the fact that at pH values between 6 and 8, most of the amino groups of CS were in the neutral form (NH2) which restricts the equilibrium degree of swelling [30] and decreases the mobility of polymer chains in chitosan copolymer [37].

The swelling behavior depends on the ionization degree of the polymers chains in the gel, and their interactions with the ions present in the buffer medium. Another factor which contributes to the swelling degree of CS-TPP-PAA nanoparticles is the pKa of the formatted hydrogel which depend on the pKa of the cationic character of chitosan and the anionic character of acrylic acid in the other side. The crosslinking agent affect the crosslinked density of the matrix and therefore its swelling degree. Figure **5** shows that the amount of AA affects the swelling behavior of nanoparticles and consequently their particle size which is in good agreement with the results of previous studies [32]. An increase in the amount of acrylic acid in the composition of nanoparticles leads to an increase in the hydrophilicity of the systems due to the monomer nature. This increases the hydrogen bonds between PAA and the reaction mixture [37] allowing an increase in the swelling degree of nanoparticles and thus their particles size.

FTIR spectra of CS-TPP-PAA nanoparticles swelling at different pH buffer solution are showed in Figure **6**. The difference between nanoparticles FTIR spectra is located in the carbonyl band region. A significant intense pic appeared in FTIR spectra of nanoparticles swelling at pH=1.2 and 1126.11cm-1 indicating the crosslinked density of amide groups of chitosan by PO4- of sodium tripolyphosphate. The same pic was found in FTIR spectra of nanoparticles swelling at higher pH, but with less intensity due to the protonation of PO4- . In contrast with Figure **6**, an increase in the pH of the swelling medium to 3.6 and 4.2 leads to an increase in the intensity of the pic at 1583.29 cm-1 and a decrease in the carbonyl band of acrylic acid at 1683 cm-1. This is due to the ionization of COOH in acrylic acid chains to COO- which causes the swelling of the gel at this range of pH. A slight decrease in the carbonyl band at 1683cm-1 was observed when the pH increased to 4.8, 6.8 and 7.4; whereas the carbonyl amide band at 1583.29 cm-1 increased resulting in the increase in the swelling degree of nanoparticles.

3.5.2. Effect of Temperature on the Swelling behaviors of Nanoparticles

Generally, damaged cells present an elevated temperature towards 40-42°C [35]. In this part, thermo-sensitivity of CS-TPP-PAA nanoparticles was studied. CS-TPP-PAA nanoparticles were incubated in pH

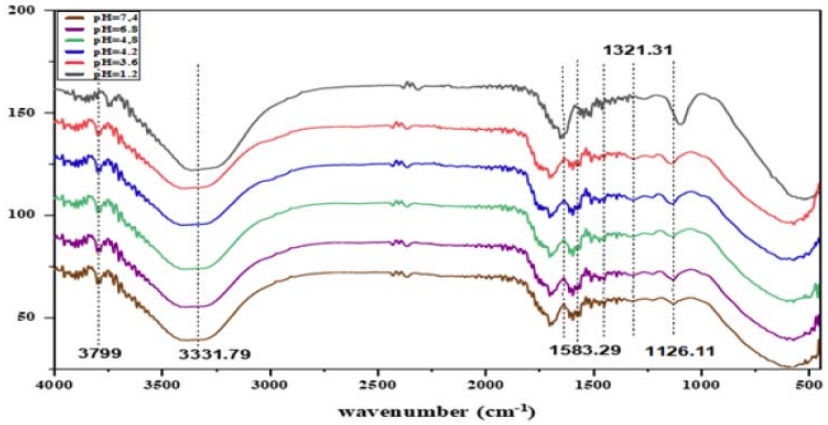


Figure 6: FTIR specrum of swelling gel at different buffer sollutions.

buffer solutions at different temperature (25°C, 37°C, 40°C) for 48 hours. Figure **7** shows the influence of temperature on the average particles size. Such an intelligent responsive system can be used as a vector to carry drugs to a specific site and then control their release. Figure **7** shows that all formulations have a small diameter at 25°C, which is due to the lower critical solution temperature (LCST) of both chitosan and acrylic acid (23°C and 25°C respectively). Formulations achieved with 0.8g and 1.6g of AA Figure **6b**, **6d** showed a slight swelling conformation and a slight increase in the particle size when temperature increases from 25°C to 37°C. This can be explained by the displacement of the LCST of the polymer network at 37°C due to an imbalance in the hydrophilic/hydrophobic equilibrium. For pH=3.6, particles are larger (from 669.45nm± 58.82 nm to 4442.82nm ±112.3nm and from 703.65nm ±19.2nm to 6944.3nm± 54nm, when temperature increased from 37°C to 40°C. In these cases, hydrogen bonds are favored between the polymers chains and the solvent. it is very important that nanoparticles remain stable at physiological body temperature 37°C which reduces drug sides effects and limits drug uptake in healthy tissues which allows minimizing the toxicity of the nanoparticles [2].

On the other hand, the nanoparticles formulated with 1g, and 3.2 g of acrylic acid showed a different behavior than the others one. They showed a high swelling degree when the temperature was elevated from 25°C to 37°C. It can be seen from Figure **7c** that nanoparticle formulated with 1g of AA initiate a thermal response with a maximum swelling degree at 37°C, with particle size larger than 4000 nm, 45 times larger than its original size. Furthermore, the same nanoparticles present a lower thermal-sensitivity when the temperature was elevated to 40°C, they swell less and their particles size slightly increase to 2312±142.03 nm. However, the nanoparticles with 3.2g of AA in their composition present an increase in their swelling degree when the temperature increases as illustrated in Figure **7a** and **7e**. The swelling and the increase in the particle size were explained by the change in the solvation state of the polymeric system. The external structure of nanoparticles was altered due to the thermal sensitivity of some chemical bonds in the polymeric chains leading to their cleavage. Thus, a relaxation in the polymeric chain, which allows the solvent to diffuse in the voids. Then, the incubated nanoparticles show an increase in the swelling conformation and an increase in their mean diameter. The swelling behavior of nanoparticles charge, the hydrophilic/hydrophobic character of polymeric matrix and also the crosslinked density which is clearly illustrated in Figure **7**.

3.6. Colloidal Stability

The colloidal stability of the formulated nanoparticles was carried out on samples stored at 4°C over 12 months. Results displayed in Table **1** show the change in the average particle size and the encapsulation efficiency after 1,6 and 12 months of storage period. The results indicated that the nanoparticles formed with 1g and 3.2g of acrylic acid were stable compared with the other formulations. A slight increase in the particle size from 89.53 ±3nm (day 0) to 94.21±25.37nm (after 30 days) to 96.12± 30.5nm after (180 days) to 99.15±25nm with a slight decrease in the encapsulation efficiency to 92.347% after 12 months in formulation with 1g of acrylic acid. Similarly, a slight increase in average particle size from 97.72±19.6nm (day 0) to 103±29nm with an encapsulation efficiency of 93.5667% (after 360 days) in nanoparticles with 3.2g of acrylic acid in their composition. This slight change is due to the colloidal stability of particle size with high Zeta potential -29.7mV and +56.4 mV, respectively. On the other hand, it can be seen regarding the formulation with 1.6g of acrylic acid, a surprising increase in the mean particle size compared to the rest of formulations from 80.84±16.6nm (day-0) to 144.25±34.6 nm (after 180 days) to 230.4±47.26 nm (after 360 days) with a Zeta potential of -29.7 mV. This can be attributed to a rearrangement on the surface charge achieved by the polymeric chain which decreases the charge density and leads to an increase in the aggregation tendency, and thus formulates large agglomerates. Accordingly, an increase in the average size from 93.85±4.05nm (day 0) to 126.36±61.6 nm (after 180 days), and a small increase to 133.43±35 nm (after 360days) was observed. This remarkable increase in the average size describes the non-uniformity and the time instability of the nanocarriers population with 0.8g and 1.6g of acrylic acid. It can be seen that for formulations with concentrations of 0.1g and 0.4g of acrylic acid, the change in the average particle size becomes less important than the change in other formulation which can be explain by the high crosslinked density between the polymer's chains in the network. However, a great decrease in the encapsulation efficiency was observed in these two formulations. Hence, from the abovementioned results, it can be concluded that the anionic charge from the

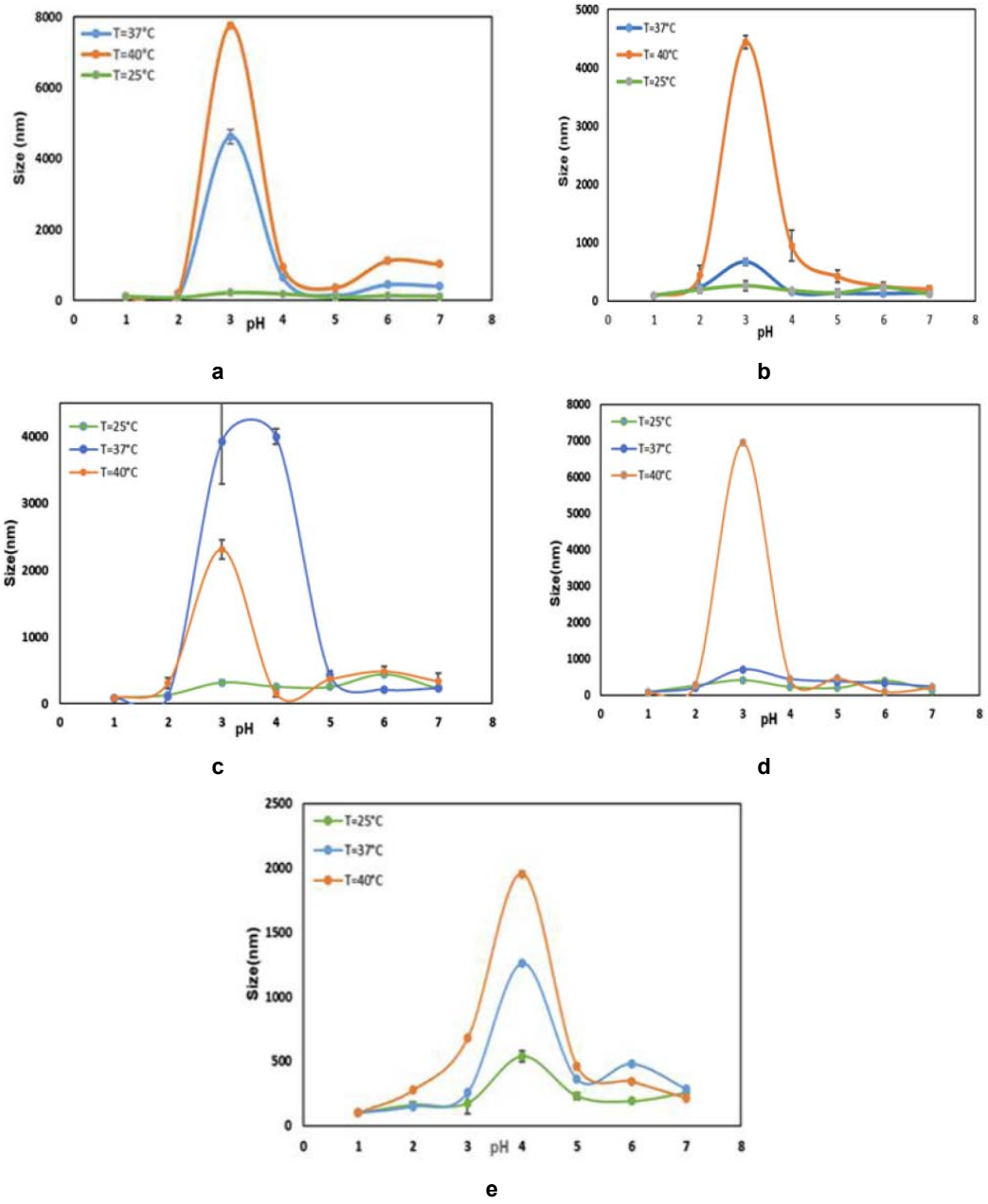


Figure 7: Effect of temperature on the swelling behavior of CS-TPP-PAA nanoparticles: (**a**) nanoparticles with wt(AA) = 0.4g, (**b**) nanoparticles with wt(AA) = 0.8g, (**c**) nanoparticles with wt(AA) = 1g, (**d**) nanoparticles with wt(AA) = 1.6g. (**e**) nanoparticles with wt(AA)=3.2g.

carboxylic groups, of acrylic acid in the copolymer network, and the crosslinked density affect the colloidal stability of the CS-TPP-PAA nanoparticles.

3.7. *In Vitro* Drug Release

Nanoparticles based on crosslinked Chitosan, PAA and PEG offer many advantages for oral drug administration [31,38]. In this work, stimulus responsive nanocarriers which improved its high pH and ionic strength sensitivity were developed. Moreover, the pH responsive nanocarriers enhance the stability of drug through the TGI and provide maximum bioavailability at target cell level and minimal sides effects [37]. The pH-responsive behavior of CS-TPP-PAA nanoparticles was evaluated in various buffer solutions at pH values 1.2, 3.6, 4.2, 4.8, 6.8 and 7.4 at physiological temperature 37°C. However, CS-TPP-PAA showed a prolonged control release of drug *in vitro*. The developed system responds to a specific change in pH, which produces an alteration on their structure and then released the encapsulated drug. Beyond this range of pH, nanocarriers assure a great protection of the drug from hostile environment when pH=1.2, and

Table 1: Study of the Colloidal Stability of Average Size of CS-PAA-TPP Nanoparticles

CS%(g /ml)	AA(g)	Average size (nm) ± SD				Encapsulation Efficiency %			
		t=0	After month	After 6 months	After12 months	t =0	After month	After 6months	After12 months
1	0.1	218.2±5	219.57±32	225.0±15	238.22±10.87	96.46	95.78	50.10	47.46
1	0.4	128.1±24.9	130.5±23	134.7±19.2	143.5±15	97.55	57.29	57	22.98
1	0.8	93.85±4.05	97.45±27	126.36±61.6	133.43±35	97.53	97.41	92.87	90.36
1	1	89.53±3	94.21±25.4	96.12±30.5	99.15±25	97.2	96.31	95.51	92.35
1	1.6	80.84±16.6	85.8±20.8	144.25±34.6	230.4±47.26	97.8	95.85	75.29	58.634
1	3.2	99.72±19.6	99.9±10.12	99.95±14	103±29	97.8	96.89	95.96	93.5667

prevent its release when pH equal to 4.2, 4.8, 6.8 and 7.4.

The release kinetic% for 8 hours was studied. The results are shown in Figures **8**, **9**, **10** and **11**. Release profiles indicate that the drug release depend on the pH condition and the proportion of monomers in the matrix composition.

In Figure **8b**, where the amount of acrylic acid is 0.4g in the composition of the polymeric matrix, maximum and fast rate release of drug at pH =3.6 about 62,62 % is observed. Thus, no significant release rate was observed at pH= 1.2, 4.2, 4.8, 6.8 and 7.4 in Figure **8a**.

Moreover, the entrapped drug needs up to 8 hours to be totally released from nanocarriers at pH =3.6. As illustrated in all release profiles, Figure **8a**, **c**, Figure **9f**, **h** and Figure **10j**, a maximum of 3% of drug was released in acidic condition pH= 1.2 over 8 hours. This weak and slow released rate can be explained by the behavior of acrylic acid at pH < 2. The carboxylic groups of AA were protonated to COOH, the hydrogen bonds between the PEG and non-ionized acrylic acid increased the crosslinked density to the nanocarriers network [39,40]. At higher pH value of 4.8, 6.8 and 7.4, amino groups of chitosan are in the neutral form of NH2. Reactive groups of sodium tripolyphosphate are

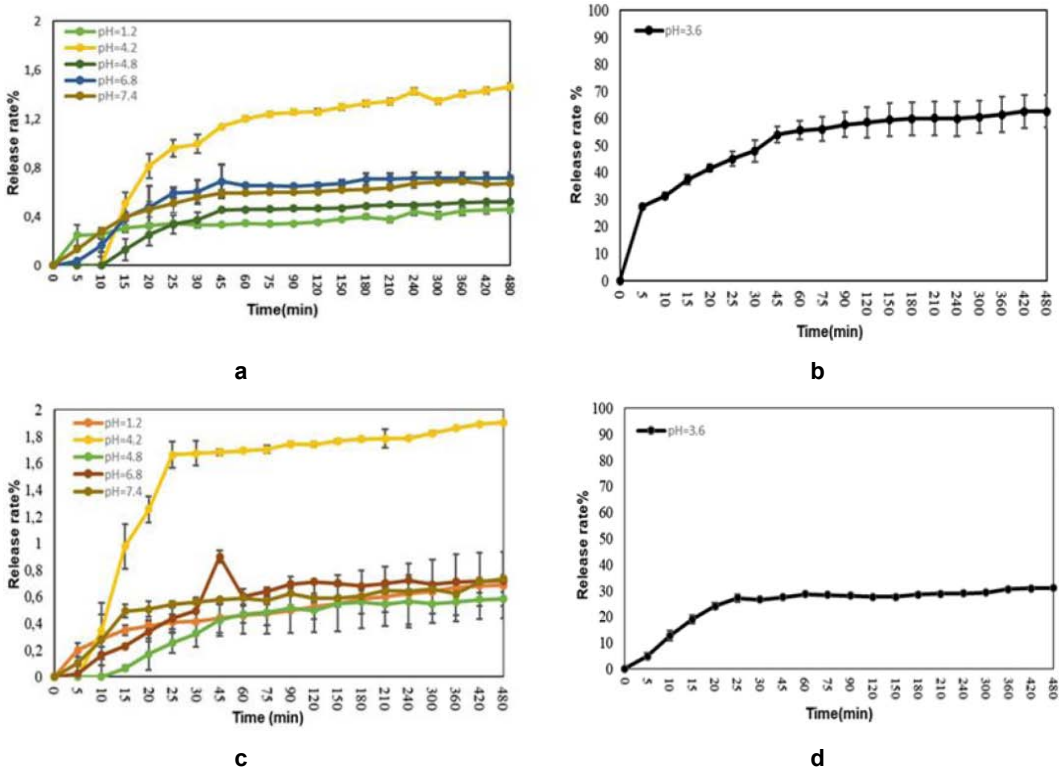


Figure 8: Release amount (%) of drug from CS-TPP-PAA nanoparticles, (a) Nanoparticles with 0.4g of acrylic acid at pH= 1.2, 3.6, 4.2, 4.8, 6.8 and 7.4), (b) Nanoparticles with 0.4g at pH 3.6, (c) Nanoparticles with 0.8g of acrylic acid at pH =1.2, 3.6, 4.2, 4.8, 6.8 and 7.4), (d) Nanoparticles with 0.8g at pH 3.6.

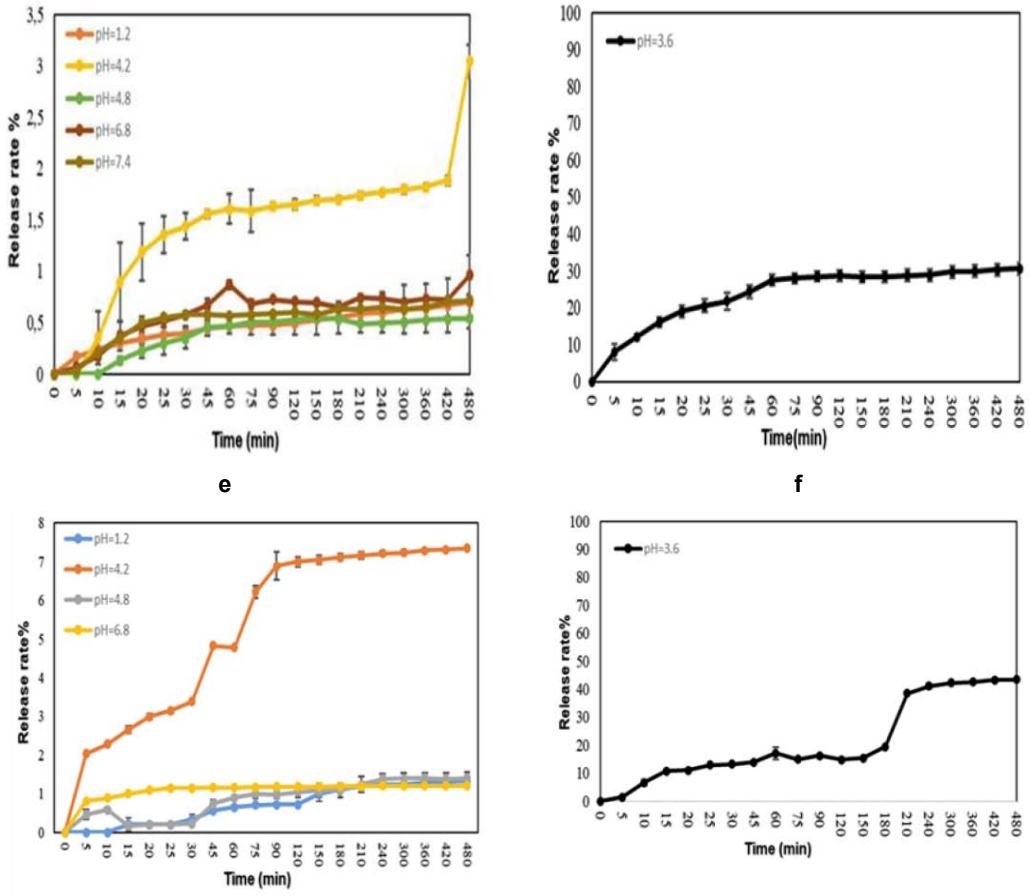


Figure 9: Release amount (%) of drug from CS-TPP-PAA nanoparticles, (**e**) nanoparticles with 1g of acrylic acid at pH =1.2, 3.6, 4.2, 4.8, 6.8 and 7.4), (**f**) nanoparticles with 1g of acrylic acid at pH 3.6, (**g**) nanoparticles with 1.6g of acrylic acid at pH =1.2, 3.6, 4.2, 4.8, 6.8 and 7.4), (**h**) nanoparticles with 1.6g of acrylic acid at pH 3.6.

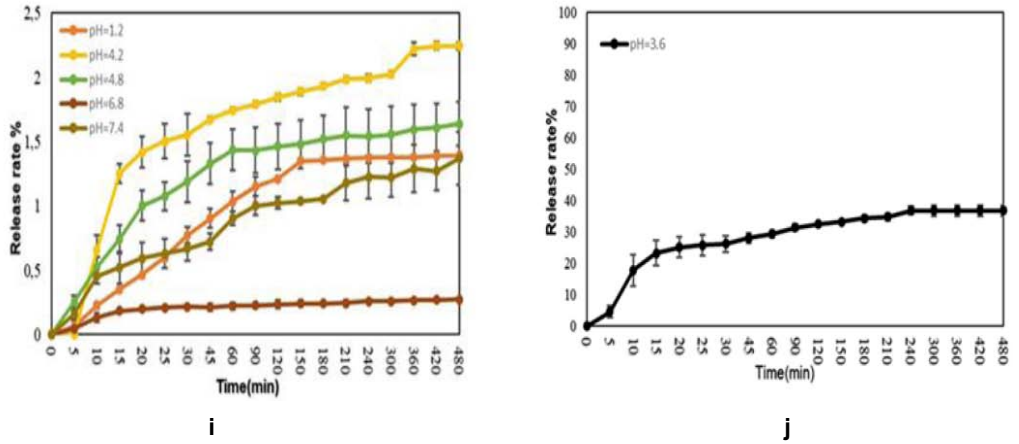
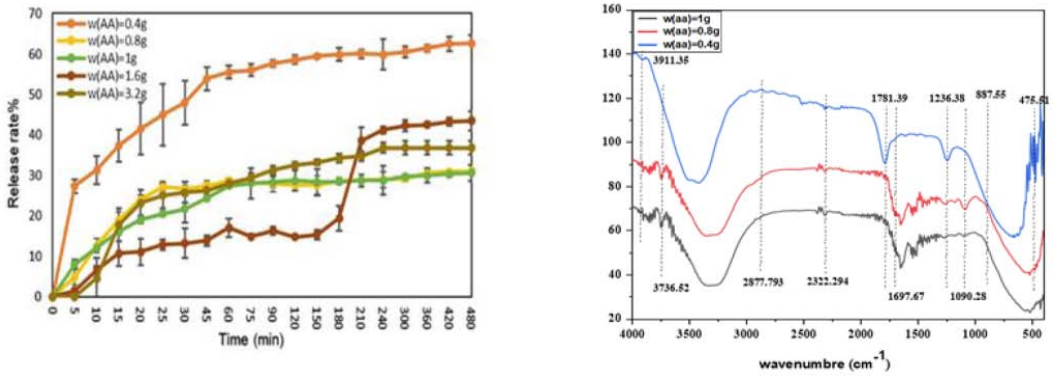


Figure 10: Release amount (%) of drug from CS-TPP-PAA nanoparticles with 3.2g of acrylic acid (**i**) at pH=1.2, 3.6, 4.2, 4.8, 6.8 and 7.4), (**j**) at pH= 3.6.

totally not ionized. Previous studies suggest that the drug became imprisoned in the collapsed polymer chains [16], which reduces the swelling behavior of the CS-TPP-PAA nanoparticles in this range of pH. Furthermore, the decrease in the repulsion forces between the negative charge decreases the swelling of the nanoparticles which prevents the release of drug.

The results indicate a small drug release from nanoparticles at pH=4.2. The largest release amount



a b

Figure 11: Effect of acrylic acid amount (**a**) on the on the release amount of drug (**b**) FTIR spectrum of CS-TPP-PAA nanoparticles at pH=3.6.

reaches 7.35% of the loaded drug from the CS-TPP-PAA nanoparticles with 1.6g of AA in their composition. Drug release is primarily controlled by the swelling behavior of the polymeric matrix and Fickian diffusion [39]. Figure **9h** illustrate a sudden change in the release amount (180min, 240min). At first, the releases were accomplished by Fickian diffusion of the drug through the shell while the effect of the swelling behavior was negligible. Between 180min and 240min, both swelling of the nanoparticles and the Fickian diffusion predominate, leading to an increase in the release rate of the drug. The results in Figure **11a** reveal that the acrylic acid amount incorporated in the polymeric chains has a significant effect on the drug release amount. Nanoparticles with less acrylic acid in their composition 0.4g, show a fast and maximum release 62.62% over 8 hours compared with the other nanoparticles. Nanoparticles formulated with 3.2g of AA release a higher percent of encapsulated drug 36.79 % over 8 hours compared to nanoparticles with 0.8g and 1g of AA which release 31.01% and 30.59% of loaded drug, respectively. This result can be due in part to the tiny size of nanoparticles formulated with 3.2g of AA, which increases the surface contact between nanoparticles and dissolution medium, and so improve the drug diffusion to the solvent, in other part, the increase in the hydrophilicity of nanoparticles network by increasing the acrylic acid amount, contribute to create more reactive sites in polymeric chains, which increase interactions with the different ions present in the formulation.

Figure **11b** shows the FTIR spectra of nanoparticles with different amount of AA at pH=3.6. The intensity and boarding of the band at 3911.35cm-1-2877.79cm-1 in spectra of nanoparticles with 0.4 g of AA is due to O-H stretching and the free carboxylic groups of acrylic acid. This result confirms the high swelling degree of nanoparticles at this pH. This broad band is replaced by two new characteristic bands as it shown by the spectra of nanoparticles having 0.8g and 1g of AA in their composition. The change achieved in this region was explain by an inter-associated banding between PEG and PAA [41] indicated at 3736cm-1. Further, the pic at 1090.28cm-1 appeared at spectra of nanoparticles having 0.8g and 1g of AA due to hydrogen bonds forming between PEG and chitosan [42], increasing the crosslinked density of nanoparticles network. This peak disappeared from the spectra of nanoparticles with 0.4 g of AA which indicates the cleavage or the absence of hydrogen bonds in this structure. Also, stretching P=O appear at 1236.38 cm-1 is intense in FTIR spectra of nanoparticles with 0.4g of acrylic acid.

An important difference between the nanoparticle's behavior appeared on the C=O region at the range of 1781.39cm-1 to 1522cm-1. The picsat 1781.39cm-1 and 1697.67 cm-1 are due to free C=O stretching in AA. The pics at 1641cm-1 and at 1522cm-1 correspond to the hydrogen bonded intermolecular in AA [42]. It can be seen on Figure **11b** that the spectra of nanoparticles with 0.4g of AA have only the pic at 1781.39 cm-1. However, FTIR spectra of nanoparticles having 0.8g and 1g of AA in their composition show that an increase in the acrylic acid amount leads to a decrease in the intensity of the pic at 1697.67cm-1 and an increase in the intensity of the pic at1522cm-1 which is due to the exchange in hydrogen banding from AA to free C=O in acrylic acid [42].

4. CONCLUSION

CS-TPP-PAA nanoparticles show a great potential for

pH-targeted drug delivery. The results demonstrated that the obtained nanoparticles showed high encapsulation efficiency, up to 90%, and high Zeta potential which ensure their colloidal stability. The properties of formulated nanoparticles such as particle size, Zeta potential and size distribution were demonstrated. Moreover, the swelling behavior of the CS-TPP-PAA nanoparticles was evaluated at many pH values and temperatures. The results shows that the amount of acrylic acid in the polymeric network affects the characteristics of nanoparticles such as the size, zeta potential, its pH and thermal responsiveness. The pH-thermo-responsive character of the CS-TPP-PAA nanoparticles has been demonstrated with a FTIR study. The *in vitro* drug release demonstrates that the CS-TPP-PAA system protect drug in gastric acid environment and in intestinal condition, 62.62% of loading drug has been released from nanoparticles with 0.4g of acrylic acid in their composition at pH=3.6 over 8 hours due to the swelling behavior of the polymeric system at this range of pH.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

The authors thank the " The General Direction of Scientific Research and Technological Development (DGRSDT)" for its support for the achievement of this work.

ABBREVIATIONS

AA = Acrylic acid

CS = Chitosan

DNA = Deoxyribonucleic acid

PAA = Poly (acrylic acid)

TPP = Sodium tri-poly(phosphate)

PEG = poly (ethylene glycol)

NPs = nanoparticles

DS = Sodium diclofenac

APS = Ammonium persulfate

TGI = Gastro-intestinal-target

FTIR = Fourier Transform Infrared spectroscopy

wt = weight

v = volume

REFERENCES

[1] Cabeza L, Perazzoli G, Peña M, Cepero A, Luque C. Cancer therapy based on extracellular vesicles as drug delivery vehicles. J Control Release 2020; 327: 296-315. https://doi.org/10.1016/j.jconrel.2020.08.018

[2] Moradi F, Soltani M, Souri M. Controlled anti-cancer drug release through advanced nano-drug delivery systems: Static and dynamic targeting strategies. J Control Release 2020; 327: 316-349. https://doi.org/10.1016/j.jconrel.2020.08.012

[3] Jelvehgari M, Barar J, Valizadeh H, Loveymi BD, Ziapour M. Preparation of Diclofenac Sodium Composite Microparticles with Improved Initial Release Property 2010; 17(2).

[4] N. I. I. N. Polymer-polymer. Reactions--4. complex formation between polyacrylic acid and monosubstituted poly (ethylene glycol) 1992; 28(5) 475-479. https://doi.org/10.1016/0014-3057(92)90120-Q

[5] Jiang WH, Han SJ. Study of Interaction between Polyethylene Glycol and Chitosan by Viscosity Method 1998; 1996: 1275-1281. https://doi.org/10.1002/(SICI)1099-0488(199806)36:8<1275::AID-POLB2>3.0.CO;2-R

[6] Pandey R, Bhairam M, Shukla SS, Gidwani B. Colloidal and vesicular delivery system for herbal bioactive constituents 2021. https://doi.org/10.1007/s40199-021-00403-x

[7] Chen HHY, Domb AJ. Engineering of magnetic DNA nanoparticles for tumor-targeted therapy 2013.

[8] Nanomaterials in Advanced Medicine. John Wiley & Sons, ISBN: 9783527345496 2019.

[9] Mohammadi Z, Samadi FY, Rahmani S, Mohammadi Z. Chitosan-Raloxifene nanoparticle containing doxorubicin as a new double-effect targeting vehicle for breast cancer therapy 2020. https://doi.org/10.1007/s40199-020-00338-9

[10] Domb AJ. Polymeric nanoparticles for therapy and imaging 2014.

[11] Defense N, Defense N, Defense N. Improving drug loading efficiency and delivery performance of micro- and nanoparticle preparations through optimising formulation variables Chiao-Hsi Chiang * Hossein Hosseinkhani Wen-Sheng Cheng Cheng-Wei Chen Chun-Hsiang Wang Yi-Lei Lo 2013; vol. 10: pp. 996-1006. https://doi.org/10.1504/IJNT.2013.058125

[12] Majumder N, Das NG, Das SK. Polymeric micelles for anticancer drug delivery 2020; 11: 613-635. https://doi.org/10.4155/tde-2020-0008

[13] Press AG, *et al.* Gastrointestinal pH pro ® les in patients with inflammatory bowel disease 1998; pp. 673-678. https://doi.org/10.1046/j.1365-2036.1998.00358.x

[14] Angela E, Driscoll CMO, Mcallister M, Fotaki N. Gastrointestinal diseases and their impact on drug solubility: Crohn' s disease. European Journal of Pharmaceutical Sciences 2020; 152. https://doi.org/10.1016/j.ejps.2020.105459

[15] Agrawal M, *et al*. Stimuli-responsive In situ gelling system for nose-to-brain drug delivery. J Control Release 2020; 327: 235-265. https://doi.org/10.1016/j.jconrel.2020.07.044

[16] Deloney M, Smart K, Christiansen BA, Panitch A. Thermo-responsive, hollow, degradable core-shell nanoparticles for intra- articular delivery of anti-inflammatory peptide. J Control Release 2020; 323: 47-58. https://doi.org/10.1016/j.jconrel.2020.04.007

[17] Ajdary M, Keyhanfar F, Aflatoonian R, Amani A, Amjadi F. Design and evaluation of a novel nanodrug delivery system for reducing the side effects of clomiphene citrate on endometrium 2019. https://doi.org/10.1007/s40199-019-00310-2

[18] Tang CHS. Characterization and anti-tumor effects of chondroitin sulfate - chitosan nanoparticles delivery system 2014.

[19] Kumar T, Thakur A, Alexander A, Badwaik H, Tripathi DK. Modified chitosan hydrogels as drug delivery and tissue engineering systems: present status and applications. Acta Pharm Sin B 2012; 2(5): 439-449. https://doi.org/10.1016/j.apsb.2012.07.004

[20] Wu B, *et al.* The e ff ect of hydrogen bonding on diffusion and permeability in UV-cured Polyacrylate-based networks for controlled release. J Control Release 2020; 327: 150-160. https://doi.org/10.1016/j.jconrel.2020.07.039

[21] Abedini F, Abraham J. Overview on natural hydrophilic polysaccharide polymers in drug delivery 2018; pp. 1-10. https://doi.org/10.1002/pat.4375

[22] Performance S, *et al.* Poly (vinyl alcohol) Double Network Hydrogels with Tunable Mechanics and High 2019.

[23] Liu J, Wang W, Wang A. Synthesis, characterization, and swelling behaviors of chitosan- g -poly (acrylic acid)/ poly (vinyl alcohol) semi-IPN superabsorbent hydrogels 2011. https://doi.org/10.1002/pat.1558

[24] Elliott JE, MacDonald M, Nie J, Bowman CN. Structure and swelling of poly (acrylic acid) hydrogels: Effect of pH, ionic strength, and dilution on the crosslinked polymer structure. Polymer (Guildf) 2004; 45(5): 1503-1510. https://doi.org/10.1016/j.polymer.2003.12.040

[25] Lehr CM, Bouwstra JA, Tukker JJ, Junginger HE. Intestinal transit of bioadhesive microspheres in an in-situ loop in the rat-A comparative study with copolymers and blends based on poly (acrylic acid). J Control Release 1990; 13(1): 51-62. https://doi.org/10.1016/0168-3659(90)90074-4

[26] Hu Y, Jiang X, Ding Y, Ge H, Yuan Y, Yang C. Synthesis and characterization of chitosan - poly (acrylic acid) nanoparticles 2002; 23: 3193-3201. https://doi.org/10.1016/S0142-9612(02)00071-6

[27] Mahdavinia GR, Pourjavadi A, Hosseinzadeh H, Zohuriaan MJ. Modified chitosan 4. Superabsorbent hydrogels from poly (acrylic acid-co-acrylamide) grafted chitosan with salt- and pH-responsiveness properties. Eur Polym J 2004; 40(7): 1399-1407. https://doi.org/10.1016/j.eurpolymj.2004.01.039

[28] Nam SY, Lee YM. Pervaporation and properties of chitosan-poly (acrylic acid) complex membranes 1997; 135: 161-171. https://doi.org/10.1016/S0376-7388(97)00144-0

[29] Chang SJ, Niu CC, Huang CF, Kuo SM. Evaluation of chitosan-g-PEG copolymer for cell anti-adhesion application. J Med Biol Eng 2007; 27(1): 41-46.

[30] Hussain Z, Sahudin S. Preparation, characterisation and colloidal stability of chitosan- tripolyphosphate nanoparticles: optimisation of formulation and process parameters 2016; 8(3).

[31] Nge THI, Yamaguchi M, Hori N, Takemura A, Ono H. Synthesis and Characterization of Chitosan / Poly (acrylic Acid) Polyelectrolyte Complex 2002; pp. 1025-1035. https://doi.org/10.1002/app.10010

[32] Bashir S, Teo YY, Naeem S, Ramesh S, Ramesh K. pH responsive N-succinyl chitosan / Poly (acrylamide-co-acrylic acid) hydrogels and in vitro release of 5-fluorouracil 2017; pp. 1-24. https://doi.org/10.1371/journal.pone.0179250

[33] Rieka J, Tanaka T. Swelling of Ionic Gels: Quantitative Performance of the Donnan Theory 1984; pp. 2916-2921. https://doi.org/10.1021/ma00142a081

[34] Toit LC, Choonara YE, Kumar P, Pillay V. Polymeric networks for controlled release of drugs: a patent review 2016; 3776.

[35] Povea MB, Monal WA, Valerio J, Rodríguez C. Interpenetrated Chitosan-Poly (Acrylic Acid-Co-Acrylamide) Hydrogels. Synthesis, Characterization and Sustained Protein Release Studies 2011. https://doi.org/10.4236/msa.2011.26069

[36] Das SS, *et al*. Stimuli-Responsive Polymeric Nanocarriers for Drug 2020.

[37] Materials A. Preparation and Characterization of Chitosan / Polyacrylic Acid / Ag-Nanoparticles Composite Membranes 2015; 60014618(9): 1149-1154.

[38] Liu L, Yao W, Rao Y, Lu X, Gao J. pH-Responsive carriers for oral drug delivery: challenges and opportunities of current platforms 2017; 7544. https://doi.org/10.1080/10717544.2017.1279238

[39] Serra L, Doménech J, Peppas NA. Drug transport mechanisms and release kinetics from molecularly designed poly (acrylic acid-g-ethylene glycol) hydrogels. Biomaterials 2006; 27(31): 5440-5451. https://doi.org/10.1016/j.biomaterials.2006.06.011

[40] To D, Id HAL. La libération des principes actifs, developpement de deux approches 2015.

[41] Alkan C, Günther E, Hiebler S, Himpel M. Complexing blends of polyacrylic acid-polyethylene glycol and poly (ethylene-co-acrylic acid) -polyethylene glycol as shape stabilized phase change materials. Energy Convers Manag 2012; 64: 364-370. https://doi.org/10.1016/j.enconman.2012.06.003

[42] Lu X. Phase Behavior of Blends of Poly (ethy1ene glycol) and Partially Neutralized Poly (acrylic acid) 1996; 1: 3022-3029. https://doi.org/10.1021/ma00113a002