

CHITOSAN NANOPARTICLES AS A DRUG DELIVERY SYSTEM FOR 1,2,4-TRIAZOLE DERIVATIVES: RELEASE AND ANTIMICROBIAL ACTIVITY.

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Abstract: -

Chitosan (CS) 1,2,4-triazole derivatives including in the formulation tripolyphosphate (TPP) as crosslinking agent was prepared by a simple one step method using ultrasonic technique. The resultant nanoparticles were characterized by x-ray diffraction (XRD), Fourier transform infrared analysis (FT-IR), thermal gravimetric analysis (TGA), derivative thermal gravimetric analysis (D-TGA), transmission electron microscopy (TEM) and scanning electron microscopy (SEM). The results indicate that spherical nanoparticles with an average size of 21, 14.6, 23.95, 29.5 nm and their standard deviation (SD) were ± 5 , ± 5.3 , ± 5.3 , ± 7.9 nm for CSTPP, CSTPPIII, CSTPPIV and CSTPPV, respectively were successfully prepared. The in vitro release and mechanism of 1,2,4-triazoles at different pH values was investigated. From values of R^2 the mechanism of drug release is following zero order mechanism at pH 6.8; however in pH 2, CSTPPIII, CSTPPIV and CSTPPV are following Hixson-Crowell, zero order and Korsmeyer-Peppas equation, respectively. The antibacterial activity against the gram negative bacteria and gram positive bacteria was examined.

Keywords: - Chitosan nanoparticles, 1,2,4-Triazole derivatives, Sodium tripolyphosphate, Ionic gelation, In vitro release, antimicrobial activity.



INTRODUCTION

Nanoparticle systems offer many advantages in drug delivery by improving the stability of the drug against chemical and enzymatic degradation, extending the therapeutic effect of the drug at the target site and improving bioavailability.^[1] CS is a naturally occurring nontoxic, cationic polysaccharide composed of randomly distributed (1→4) linked D-glucosamine and N-acetyl-D-glucosamine units and presents well-documented favorable properties for drug delivery such as biodegradable, bioadhesive, promotes wound-healing and has bacteriostatic effects.^[2]

CS contains abundant amino and hydroxyl groups which facilitate the formulation of nanoparticles via physical and chemical crosslinking. Several techniques have been developed to prepare CS nanoparticles as emulsion crosslinking, ionic gelation and spray drying technique. Crosslinking is usually achieved by reaction with dialdehydes such as glutaraldehyde and epichlorohydrin. However, they are toxic and can cause irritation to mucosal membranes.^[3]

Ionic crosslinking of CS is a typical non-covalent interaction, which can be realized by association with negatively charged multivalent ions such as TPP. For pharmaceutical application, physical crosslinking is more favorable since the crosslinking is reversible and may largely avoid potential toxicity of the reagents used in chemical crosslinking.^[4] Optimization of the fabrication conditions and comprehensive properties of the resultant CS nanoparticles is still an ongoing important topic in this field. The size of nanoparticles affects not only the release performance but also governs the interaction of the particles with different biological tissues where they are introduced.

The chitosan–tripolyphosphate (CSTPP) nanoparticles, composed of food-safe ingredients, has shown its capacity for the encapsulation and delivery of Bovine serum albumin (BSA)^[5], hydrophilic and hydrophobic drugs^[6], rutin^[7], essential oil^[8], catechins,^[9] Amoxicillin trihydrate and Metronidazole.^[10]

1,2,4-Triazoles have an effective antibacterial activity against both Gram-positive and Gram-negative bacteria, anti-fungal, antitubercular, anti-inflammatory, anti-convulsant, antiviral, antitumor and antispastic agents.^[11]

To reduce the effect of 1,2,4-triazole toxicity upon using for long term application, the objective of this work was directed to produce CS/1,2,4-triazole nanoparticles including in the formulation TPP as crosslinking agent by a simple method using ultrasonic technique. Reduced size would improve the loading capacity and effect on the release rate. The morphology and composition of the nanoparticles was investigated using XRD, FT-IR, TEM, SEM, TGA and D-TGA. Moreover, the release kinetics was evaluated. In addition the efficiency against Gram positive and Gram negative bacteria was studied.

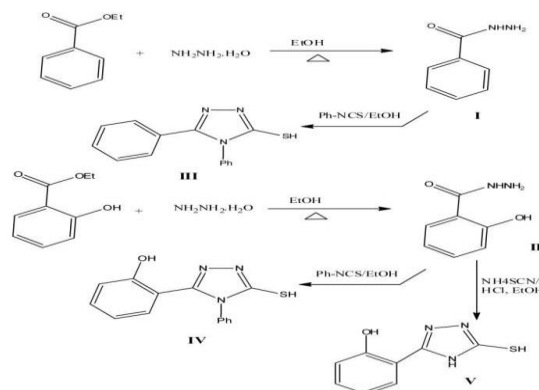
Materials and Methods

Materials

CS (Across organics, USA, Mw. 100,000-300,000; deacetylation degree (DD) 90%), TPP, phenyl isothiocyanate (Across organics, USA), dimethyl sulfoxide (DMSO) (Sigma-Aldrich), Hydrazine mono hydrate (Laboratory rasayan), Nutrient agar (Alpha chemiecke), ethyl benzoate (fabricaclone Aleman), ammonium thiocyanate (Win Lab), methyl-2-hydroxy benzoate, sodium hydroxide, concentrated hydrochloric acid (HCl) and acetic acid, mono, di sodium phosphate, acetic acid (Adwic, Egypt) were purchased and used as received. Thin layer chromatography (TLC) was carried out with silica-gel precoated plastic sheets.

Instruments

FT-IR spectra were recorded on Bruker, Tensor 27 FT-IR spectro-photometer with frequency range 4000 cm^{-1} to 400 cm^{-1} using KBr pressed disc technique. XRD were carried out using GRN, APD 2000 PRO X-Ray diffraction, equipped with a Ni-filtered Cu-K α radiation ($\lambda=1.54 \text{ \AA}$) at a scanning rate of 0.02° per second and diverged slit 0.3° (Central Lab. Tanta University, Egypt). TGA data were obtained using Shimadzu thermal analyzer system at a heating rate of 10 °C/min, sample weight of 5-6 mg, under nitrogen (20 ml/min) flow. The range investigated from 25-800 °C. TGA-50 furnace with a M3 microbalance, and TA72 Graph ware software were employed for thermal analyses (Microanalysis center, Cairo University, Giza, Egypt). TEM micrographs were taken with a JEOL JEM-1230 electron microscope at an accelerating voltage of 100 KV (Faculty of Medicine, Tanta University, Egypt). SEM micrographs were taken with JEOL JSM 6360 LA Analytical SEM at an accelerated voltage 10 KV. SEM samples were prepared by coating with gold (Science Research and Technological applications city, New Burg El Arab city, Alexandria, Egypt). UV-Vis absorption spectra were obtained using a Shimadzu UV-visible 2101 PC spectrophotometer. All melting points (m.p.) measured in open capillary tubes using Stuart Scientific melting point apparatus SMP3 and are uncorrected.



Scheme 1: Preparation of 1,2,4-triazole derivatives (III-IV).

Methods

4,5-Diphenyl-4H-1,2,4-triazole-3-thiol (III), 2-(5-mercapto-4-phenyl-4H-1,2,4-triazol-3-yl) phenol (IV) and 2(5-mercapto-4H-1,2,4-triazol-3-yl) phenol (V) were prepared (Scheme1) according to recommended methods as follows

Preparation of III and IV

A mixture of 2-substituted benzohydrazide (I, II) (0.01 mol), phenyl isothiocyanate (0.01 mol) in 20 ml absolute ethanol was refluxed (TLC). The solvent was distilled under reduced pressure; the residual solid was refluxed with sodium hydroxide (0.12 mol) for 3 h. The resulting solution was acidified with HCl. The product was separated, dried and recrystallized from dilute ethanol to give (III, IV). **Compound (III)** yield 89%, m.p. 290- 292 °C, **(IV)** yield 88%, m.p. 300- 301 °C.^[12,13]

Preparation of V

A mixture of 2-hydroxy benzohydrazide (II) (0.01 mol), ammonium thiocyanate (0.03 mol), and 4 ml hydrochloric acid in 200 ml ethanol was refluxed (TLC). The solvent was distilled under reduced pressure; the residual solid was refluxed in 4% NaOH for 3 h. The resulting solution was acidified with HCl. The product was filtered off, dried and recrystallized from dilute ethanol to give (V) yield 85%, m. p. 287- 288 °C.^[13]

Preparation of CSTPP nanoparticles

CSTPP were prepared by ionotropic gelation of CS in the presence of TPP anions using (CS:TPP; 2.3:1) molar ratio. TPP (1 g dissolved in distilled water) was added to CS solution (CS 1 g dissolved in 100 ml of 1% acetic acid) during sonication for 15 minutes at 30 °C. The mixture was stirred for 45 minutes, CSTPP nanoparticles were centrifuged the supernatant was discarded and the deposit was re-dispersed in distilled water for three times and dried at 40 °C.

Preparation of CSTPPIII, CSTPPIV and CSTPPV nanoparticles.

(0.0011 mol) of 1,2,4-triazole derivatives III was added to CS solution (1 g was dissolved in 80 ml of 1% acetic acid) and stirred for 30 minutes. TPP (1 g dissolved in distilled water) was added during sonication for 15 minutes followed by stirring for 2 h. The products were separated using centrifuge and dried at 40 °C to afford CSTPPIII. The same method was used to prepare CSTPPIV and CSTPPV.

Adsorption efficiency

The amount of 1,2,4-triazole derivatives in the initial solution and supernatant was determined by UV-Vis spectrophotometer to determine the adsorption efficiency (Table1).

Drug Release

Phosphate buffer solutions (PB) were prepared by dissolving di and mono sodium phosphate in deionized water, the pH was adjusted at 6.8. For pH 2 drops of concentrated HCl were added to deionized water until pH adjusted to 2.

Release Measurements

10 milligrams of samples in 1 ml buffer were dialyzed in dialysis membrane bag which is put in 100 ml of the PB (pH 6.8) or acidic solution (pH 2). The concentration of 1,2,4-triazole derivatives were determined by using UV- Vis spectrophotometer at λ_{\max} =261, 263 and 255 nm for **III, IV and V**, respectively. At each time, 3 ml of the buffer was collected for analysis.

In vitro release kinetic and mechanism

Data obtained from *in vitro* release studies were analyzed by fitting to the following equations

Zero-order release kinetics: $W = W_0 + k_0 t$

First-order release kinetics: $\log (100 - W) = \log 100 - k_1 t$

Higuchi model: $W = k_H t^{1/2}$

Hixson-Crowell Kinetics: $(W - 100)^{1/3} = 100^{1/3} - k_{Hix} t$

Korsmeyer-Peppas equation: $F = \frac{M_t}{M_\infty} = k_a t^n$

Where k_0 , k_1 , k_H , k_{Hix} , k_a are drug release rate constants, W is the percent released of drug at time t , W_0 is the cumulative drug release at $t=0$, $F=M_t/M_\infty$ is a fraction of 1,2,4-triazole derivatives released at time t into dissolution media, M_t is the concentration of triazoles with time t , M_∞ is the maximum amount absorbed, n is the release exponent that shows the release of 1,2,4-triazoles transport mechanism.

In Vitro Antimicrobial Activity

The microbial strains were obtained from Bacteriological laboratory of Botany department, Microbiology section, Faculty of science, Tanta University. The Gram-negative bacteria (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027) and Gram-positive bacteria (*Staphylococcus aureus* ATCC 6633, *Staphylococcus epidermidis* ATCC 12228) were used to examine the antimicrobial activity of the nanoparticles. The bacterial strains were maintained on nutrient agar (3 g peptone; 5 g NaCl; 5 g beef extract; 28 g nutrient agar per liter, pH=7.4). 1,2,4-Triazole derivatives (III-V), CS, CSTPP, and CS/ 1,2,4-triazole/TPP (CSTPPIII, CSTPPIV, CSTPPV) were screened for their antimicrobial activity against the

above bacterial strains. The bacteria strains were maintained on nutrient agar and nutrient broth. All media were sterilized in autoclave before experiments. The inoculum's concentrations were 6.5×10^5 CFU. After incubation for 24 h, the agar plates were checked for the diameter of inhibition zone. The antimicrobial activity of the tested samples was determined by well diffusion method. Powder samples (20 mg/ml) were dispersed in DMSO. DMSO was used as negative control. The plates were incubated at 37 °C for 24 h, after which they were examined for inhibition zones development. A caliper was used to measure the inhibition zones. Three replicates were carried out. The antimicrobial effect of triazole derivatives, CS and triazole loaded nanoparticles on the microorganism was determined by the size of the inhibitory zone.

Results and discussion

The present work develops a simple one step process for synthesis CS/1,2,4-triazole/TPP nanoparticles using ultrasonic technique. First CS was dissolved in acetic acid and mixed with 1,2,4-triazole derivatives to obtain 1,2,4-triazole/CS solution followed by addition of TPP to prepare cross-linked CS nanoparticles.

IR spectra analysis

Fig 1 represent FT-IR spectra of CS, TPP, CSTPP, triazole derivatives (III-V) and triazoles loaded nanoparticles (CSTPPIII, CSTPPIV and CSTPPV). In the spectrum of CS, the band at 3437 cm^{-1} is assigned to OH and NH_2 , 2921 cm^{-1} (C-H stretching), 1649 and 1425 cm^{-1} (C=O stretching amide I and NH stretching amide II), 1383 cm^{-1} (CH_3 in amide group), 1153 cm^{-1} (anti-symmetric stretching of C-O-C bridge) 1095 cm^{-1} , 1010 cm^{-1} (skeletal vibration involving the C-O stretching).^[14] In the spectrum of TPP, the band at 1217 cm^{-1} is assigned to (P-O stretching), 1149 and 898 cm^{-1} (symmetric and anti-symmetric stretching vibrations in PO_2 group respectively), 1069 cm^{-1} (symmetric and anti-symmetric stretching vibrations in PO_3 group and antisymmetric stretching of the P-O-P Bridge).^[15] In the spectrum of triazoles (III-V), the peaks at 660 - 770 cm^{-1} are assigned to mono substituted and di substituted aromatic ring, 2930 cm^{-1} related to C-H aliphatic, 3038 cm^{-1} and 3326 cm^{-1} assigned to C-H aromatic and O-H group, respectively.

FT-IR spectrum of CSTPP nanoparticles shows absorption band at 1542 cm^{-1} due to NH_3^+ vibration. Moreover, the band at 3437 and 1649 cm^{-1} related to OH and C=O stretching (amide I) were shifted to 3447 and 1624 cm^{-1} in CSTPP, implying electrostatic interaction between NH_3^+ groups of CS and phosphoric groups of TPP within the nanoparticles.^[16] The spectrum of CSTPPIII, CSTPPIV and CSTPPV loaded triazoles nanoparticles show that the peaks at 3437 cm^{-1} related to O-H group was shifted to 3419 , 3328 , 3391 cm^{-1} , respectively implying the inter action between CS and TPP within the nanoparticles. Moreover, peaks were observed at 1549 , 1543 and 1565 cm^{-1} implying the inter action between NH_3^+ groups of CS and phosphoric groups of TPP within the nanoparticles in CSTPPIII, CSTPPIV and CSTPPV, respectively. It worth noting that a mixture of TPP, CS and III powders (Fig 1 CS/TPP/III) showed the same characteristic peaks for these compounds without any shift that confirm the interaction in nanoparticles.

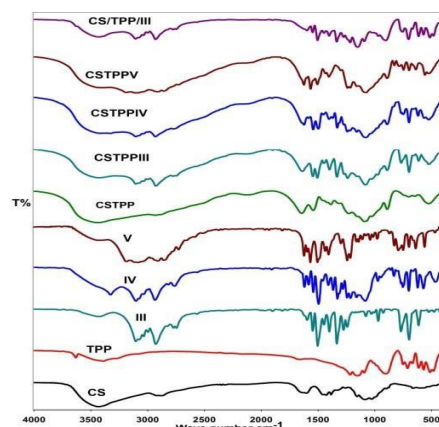


Figure 1: FT-IR of CS, TPP, 1,2,4-triazoles (III-V), CSTPP, CSTPPIII, CSTPPIV, CSTPPV and CS/TPP/III.

Crystallographic assay

Fig 2 shows the XRD patterns of CSTPP loaded by triazole derivatives as well as those of the parent CS, TPP, and 1,2,4-triazole derivatives (III-IV). CS shows two characteristic peaks at $2\theta = (10^\circ$ and $20.3^\circ)$. The presence of plenty of $-\text{OH}$ and $-\text{NH}_2$ groups in CS structure forms strong inter- and intra-molecular hydrogen bonds, thereby showing certain regularity in the CS structure.^[14] XRD of TPP showed many sharp peaks at $2\theta = (18.5, 18.9, 19.2, 19.8, 21.8, 29.1^\circ)$ indicates that TPP is in crystalline form.

In case of CSTPP nanoparticles a diffraction peak was observed at $2\theta = 17.20^\circ$ with broad peak at $2\theta = 24.37^\circ$. This could be attributed to modification in the arrangement of molecules in the crystal lattice, due to formation of electrostatic interaction between NH_3^+ groups of CS and phosphoric groups of TPP.

As compared with CSTPP nanoparticles, CSTPPIII, CSTPPIV and CSTPPV nanoparticles, sharp peaks characteristic to III, IV, V at $2\theta = 9.1, 12.1, 19.4, 20.1, 25.6, 26.8^\circ$ derived from triazole derivatives were observed confirming the presence of III, IV and V within CSTPP nanoparticles. The broadness of the peak at $2\theta = 17.2^\circ$ indicates that the incorporation of III, IV and V resulted in a change in the CSTPP packing structure. The ionic cross-linking of CS with TPP in CSTPP, leads to the destruction of the native CS packing structure. The broad peak of CS nanoparticles may be caused by the cross-linking reaction between CS and TPP, which may destroy the crystalline structure of CS.^[16] CS nanoparticles are

comprised of a dense network structure of interpenetrating polymer chains cross linked to each other by TPP counter ions.^[17] The XRD implicated greater disarray in chain alignment in the nanoparticles after crosslinks.^[18] It is well-known that the width of XRD diffraction peak is related to the size of crystallite, the broadened peak usually results from imperfect crystal.^[16] Crystalline sizes of samples were estimated using Scherer's equation (1)

$$D = \frac{k\lambda}{\beta \cos \theta_{(1)}}$$

Where k = constant ($0.89 < k < 1$), λ wavelength of the X-ray, B =FWHM (full width at half maximum width of the diffraction peak, θ =diffraction angle. The average diameters of the nanoparticle were calculated. The highest three peaks were chosen to calculate the average diameters that were found to be 41.4, 25.4, 30.7 nm for CSTPPIII, CSTPPIV and CSTPPV, respectively.

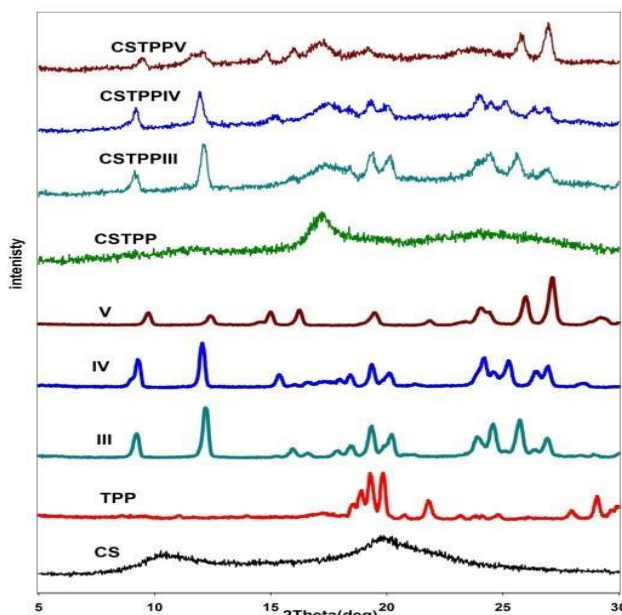


Figure 2: XRD of CS, TPP, 1,2,4-triazoles (III-V), CSTPP, CSTPPIII, CSTPPIV and CSTPPV.

Morphology

The morphology of the particles was characterized by TEM observation (Fig 3). TEM images showed that the CSTPP has spherical shape with size average 21 nm and SD of ± 5 (Fig 3a). The size distribution obtained by TEM indicated the average size of CSTPPIII, CSTPPIV and CSTPPV nanoparticles were 14.6, 23.95, 29.5 nm and their SD are ± 5.3 , ± 5.3 and ± 7.9 , respectively (Fig 3b,c,d).

It was reported that ionic gelation of CS using TPP produce particles with spherical shape and diameter ranging from 309.8- 402.2 nm in oil loaded CSTPP nanoparticles^[8], and ranging from 322 -814 nm as entrapped with rutin depending on CS ratios.^[7] In another publication, particle size of CSTPP nanoparticles loaded with Bovine serum albumin (BSA) was in range between 615 to 116 nm depending on concentration of calcium alginate.^[5] Ionically Cross-linked CSTPP microparticles were prepared for the controlled delivery of Pyrimethamine was produced in microparticles range.^[6] Moreover, loading of Amoxicillin trihydrate and Metronidazole for the treatment of gastric ulcer using CS gave microparticles in range of 10 μm to 25 μm .^[10]

The morphology of the CSTPP and CSTPPIII nanoparticles was further characterized by SEM. Fig 4a,b depicts porous surface morphology. A spherical shape is observed for CSTPP with average size of 84 nm SD ± 7 nm. These pores may allow water molecules to diffuse into the nanoparticles to form the large pores causes swelling of the nanoparticles.

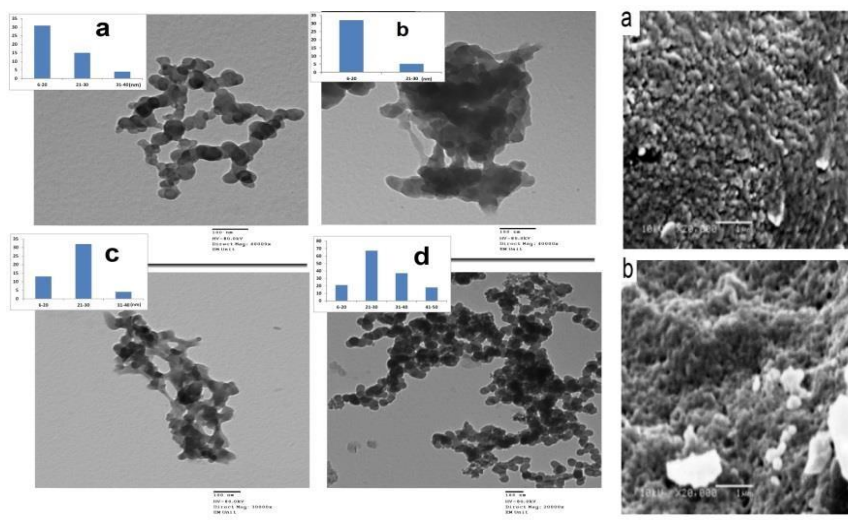


Figure 3: TEM of a: CSTPP, b: CSTPPIII, c: CSTPPIV and d: CSTPPV. Figure 4: SEM of a: CSTPP and b: CSTPPIII.

Thermal properties

To investigate the weight loss of nanoparticles loaded 1,2,4-triazole derivatives, TGA was performed under nitrogen atmosphere (Fig 5a, b). Three steps weight loss were observed in CSTPP (Fig 5b). The first step at 50-110 °C can be attributed to the loss of moisture and bound water from the nanoparticles. The second weight loss from 200 to 350 °C can be assigned to degradation of the saccharide rings. The third step at 575 to 798 °C assigned to decomposition of the acetylated and deacetylated units of the polymer.^[19] The temperatures corresponding to the maximum slopes of each weight change step are clearly observed when the first derivative of the TGA curve with respect to temperature, the so-called D-TGA, is plotted (Fig 5b). The temperatures which give the highest rate of weight loss at each step (i.e., peaks in the D-TGA thermogram) are usually considered as degradation temperatures (Td) of components in the material. D-TGA for CSTPP showed degradation at 57.87, 229.39 and 747 °C.^[8]

TGA of CSTPPIII, CSTPPIV and CSTPPV showed four steps of weight loss. The first step of weight loss of CSTPPIII, CSTPPIV and CSTPPV in range from 27.46 to 171.49 °C (Fig 5a) and D-TGA showed Td at 61.08, 62.95 and 64.06 °C, respectively (Fig 5b), this was attributed to the loss of absorbed and bound water. The second step of weight loss in range from 171 to 262 °C (Fig 5a) and D-TGA showed Td at 232.31, 232.03 and 224.96 °C, respectively (Fig 5b), this can be assigned to degradation of the saccharide rings, depolymerization and decomposition of the acetylated and deacetylated units of the polymer. The third step in range from 262 to 375 °C (Fig 5a) and D-TGA showed Td at 287.01, 297.28 and 286.03 °C, respectively (Fig 5b), this step confirms the incorporation of the 1,2,4-triazole derivatives into nanoparticles. The last step at 375 to 798 °C assigned to decomposition of the acetylated and deacetylated units of the polymer. The percentage weight loss at this temperature range was thus used to compute the amount of loaded 1,2,4-triazole derivatives (Table 1). In addition, the loading percentage of III-V on nanoparticles was calculated using UV-Vis Spectrophotometer (Table 1).

Table 1: Adsorption efficiency and percentage of loaded 1,2,4-triazole derivatives into nanoparticles calculated using TGA and UV-visible spectrophotometer.

Code	Adsorption efficiency	Percentage of loaded 1,2,4-triazole derivatives into nanoparticles	
		By using TGA	By using UV-visible Spectrophotometer
CSTPPIII	70.4 %	22.5 %	22 %
CSTPPIV	71.6 %	23.05 %	22.09%
CSTPPV	57.2 %	23.07 %	19.086 %

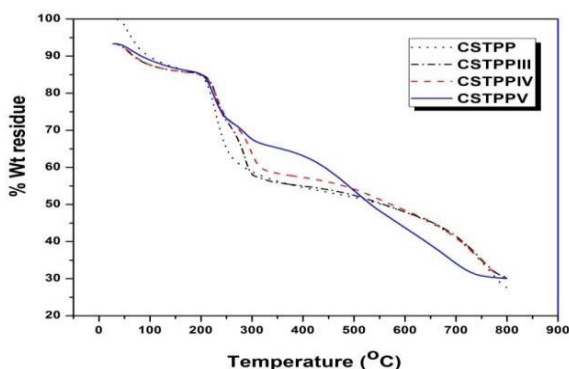


Figure 5a: TGA of CSTPP, CSTPPIII, CSTPPIV and CSTPPV.

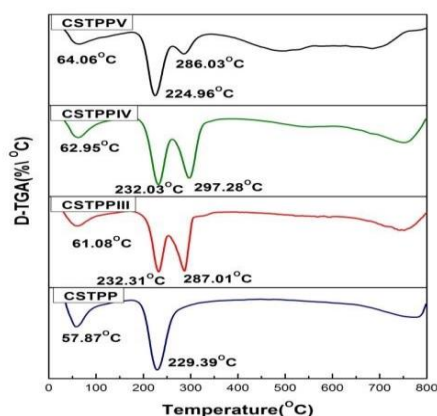


Figure 5b: D-TGA of CSTPP, CSTPPIII, CSTPPIV and CSTPPV.

Antimicrobial Activity

The capability of the 1,2,4-triazole derivatives, CS, CSTPP and CS-triazole-TPP nanoparticles to inhibit the growth of the selected micro-organisms on solid media is shown in Fig 6. All compounds can't inhibit the growth of *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. However, all compounds show antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. CS and CSTPP have similar antimicrobial effect against *Escherichia coli* and *Staphylococcus aureus*. The antibacterial activity of CS under acidic environment may result from its polycationic structure due to the protonation of $-NH_2$ on the C-2 position of the D-glucosamine. Positively charged CS can bind to bacterial cell surface which is negatively charged and disrupt the normal functions of the membrane, by promoting the leakage of intracellular components or by inhibiting the transport of nutrients into cells.^[20] Phosphate ion reacts with Gram-positive bacteria by the same mechanism of CS without need to acid medium since phosphate present as ion in CSTPP nanoparticles. Compound III and CSTPPIII nanoparticles have the similar antimicrobial effect against *Escherichia coli* and *Staphylococcus aureus* (11.5 and 19 mm, respectively). However IV and V show little higher antimicrobial activity in comparison with CSTPPIV, CSTPPV nanoparticles, respectively. It was worth mentioning that the concentration of III, IV, and V in CSTPPIII, CSTPPIV, and CSTPPV is 22.5- 23% (Table1). This indicates the nanoparticle has higher activity due to the small particle size.

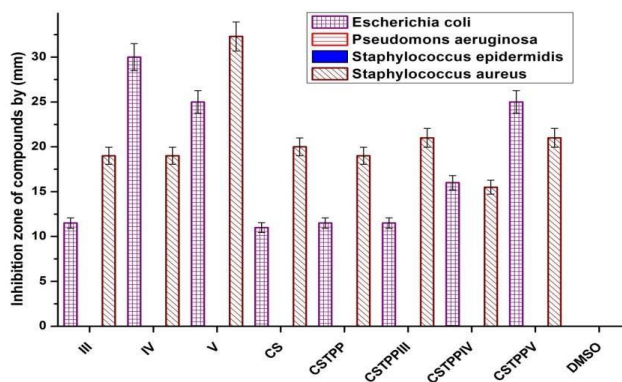


Figure 6: Inhibition zones of DMSO, 1,2,4-triazoles (III-V), CS, CSTPP, CSTPPIII, CSTPPIV and CSTPPV.

Release Profiles

The *in vitro* release of triazoles (III-V) from CSTPP nanoparticles was studied in different pH values (2 and 6.8) for 50 h at 40 °C. The concentration was measured during time intervals by UV-Vis spectrophotometer. A gradual release was observed at pH 2 and 6.8 (Fig 7). The maximum release of triazoles from nanoparticles in both pH values was observed to be in the range 20- 45% after 70 h based on the release profile at pH 2 and 6.8. This is probably due to the existence of interaction between NH₂, OH groups in CS and OH in triazoles that delay the release. It was reported that the maximum release of rutin from CSTPP was 20%. This indicates that 80% of rutin was still trapped within the particles.^[7] Also, it was reported that 45-55% of catechin was released from CSTPP depending on the mass ratio of TPP after 12 h emersion in distilled water at 37 °C.^[9]

The release of triazoles from nanoparticles was pH dependent; they showed significantly faster release in strong acidic media (pH 2) than slightly acidic media (pH 6.8). There are a plenty number of amino and hydroxyl groups on CS molecules, that provides favorable functional groups to biological molecules. In strong acidic media, the release is relatively rapid in comparison with weak acidic media. This is attributed to the electrostatic repulsion between NH₃⁺ groups that increase intra particles places in the nanoparticle. In contrast to weak acidic media NH₂ groups that mainly attached to the surface of CS slow down the release rate of the biological molecules.

It is worth nothing that CSTPPIV, CSTPPV showed a fast release rate in comparison with CSTPPIII, this may be attributed to the presence of OH group that favor the release. The hydrophobicity in CSTPPIII combined with the crosslinking density imparted delay for the release. Moreover, CSTPPV showed a larger cumulative amount release than CSTPPIV. The fast release is due to an increase in the hydrophilicity due to presence of phenyl group in CSTPPIV.

Similar results were obtained in CSTPP entrapped with rutin that showed no release after 2 hours in simulated gastric fluid (SGF) (pH 1.4) indicating that rutin-CS interactors remained relatively strong.^[7] In contrast, it was reported that catechin release from CSTPP in SGF to range between 5 to 15% depending on TPP% and the interaction between catechin and CS.^[9] Another study recorded that 20- 40% of bovine serum albumin was released from CS TPP nanoparticles after 20 min in simulated intestinal fluid (SIF) with enzymes.^[5]

Release kinetics for nanoparticles was analyzed by assessing the fitting of release data to the above kinetic equations (Fig 8, Fig 9). By applying kinetic equations, the release constant (k_v , k_1 , k_H , k_{Hix} and k_a) are obtained from plot of kinetic model in addition to correlation R² and for Korsmeyer-Peppas equation the release exponent value n was obtained (Table 2). From value of R² the mechanism of drug release in CSTPPIII, CSTPPIV and CSTPPV at pH 2 is following Zero-order kinetics, however, in pH 6.8 is following HixsonCrowell, Zero-order kinetics and Korsmeyer-Peppas equation, respectively. From Korsmeyer-Peppas equation the value n was found to be more than 0.5 then the release through anomalous non fickian diffusion.

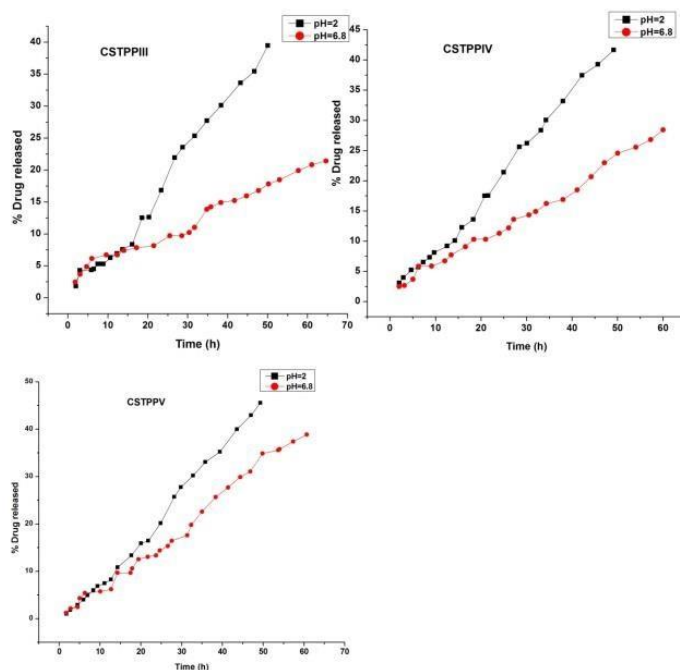


Figure 7: Release of 1,2,4-triazoles (III-V) from CSTPP nanoparticles during time at pH 6.8 and 2.

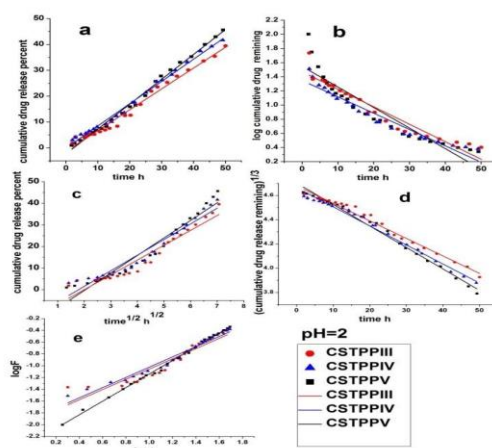


Figure 8: Kinetics of 1,2,4-triazoles (III-V) released from CSTPP nanoparticles (a): Zero-order, (b): Firstorder, (c): Higuchi model, (d): Hixson-Crowell, (e): Korsmeyer-Peppas equation at pH 2.

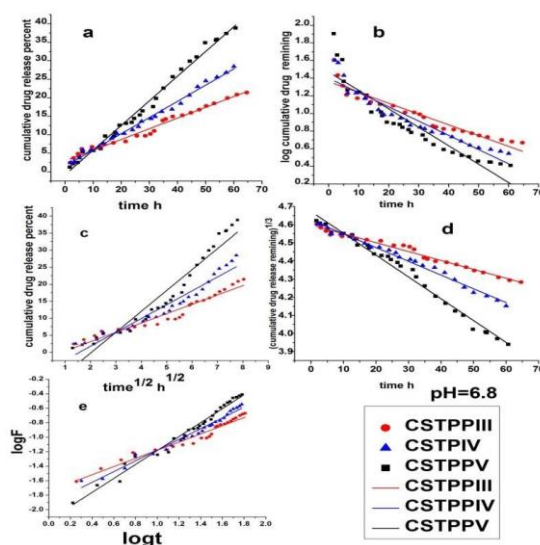


Figure 9: Kinetics of 1,2,4-triazoles (III-V) released from CSTPP nanoparticles (a): Zero-order, (b): Firstorder, (c): Higuchi model, (d): Hixson-Crowell, (e): Korsmeyer-Peppas equation at pH 6.8.

Table 2 Controlled release kinetics of 1,2,4-triazoles (III-V) released from CSTPP nanoparticles.

Code	pH	Zero order		First-order		Higuchi		Hixson-Crowell		Korsmeyer-Peppas		
		k_0	R^2	k_1	R^2	kH	R^2	$kHix$	R^2	$\log k$	n	R^2
CSTPPII	6.	0.289	0.9795	-	0.8982	2.7458	0.9365	-	0.9790	0.018	0.5636	0.9526
	8		8	0.0122	7	3	4	0.00489	4	1	5	3
	2	0.8046	0.9725	0.0247	0.9181	6.8751	0.9258	0.01144	0.9799	0.008	0.9555	0.9403
		5	6	3	9		9	4	6	2	6	6
CSTPPIV	6.	0.4376	0.9920	0.0161	0.8967	4.0722	0.9438	-	0.9885	0.012	0.7402	0.9784
	8		1	6	2	9	7	0.00757	9	1		4
	2	0.8594	0.9923	-	0.9308	7.2396	0.9402	-0.0157	0.9882	0.012	0.8676	0.9686
		2	2	0.0231	7		8		4	8	1	6
CSTPPV	6.	0.6644	0.9891	0.0209	0.859	6.1464	0.9293	-0.0120	0.9838	0.007	5226.0	0.9784
	8		2	1		7	3		3	2		4
	2	0.9603	0.9939	-	0.8544	8.1241	0.9428	-0.0167	0.9883	0.005	1.1519	0.9969
		7	8	0.0290	9	2	7		4	1	3	8

Conclusion

A one step route to synthesize CS/triazole derivatives/TPP nanoparticles was developed. This method is imperative in order to produce small particle size with an average size of 21, 14.6, 23.95, 29.5 nm and their SD were ± 5 , ± 5.3 , ± 5.3 , ± 7.9 nm for CSTPP, CSTPPIII, CSTPPVI and CSTPPVII, respectively. The release rate depend on the pH of the medium and the substituents on 1,2,4-triazoles. The drug release in CSTPPIII, CSTPPIV and CSTPPV at pH 2 is following Zero-

order kinetics, however, in pH 6.8 is following Hixson-Crowell, Zero-order kinetics and Korsmeyer-Peppas equation, respectively. By applying Korsmeyer-Peppas equation the release of triazole derivatives from nanoparticles follows non-fickian diffusion. Nanoparticles exhibited antibacterial activity against Gram positive and Gram negative bacteria.

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