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Novel Biodegradable Pseudopolyrotaxane as Drug Carrier for Sulfamethazine: Synthesis, Characterization and Antimicrobial Efficacy

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Abstract: Polymers are widely used in pharmaceutical applications to improve the physical properties of drugs. At present time polyrotaxanes which contain cyclodextrin have significant uses in different fields particularly in drug delivery. Antibiotics are type of drugs used in medical treatment of bacterial infections but often have some side effects. In this study we aim to improve the physicochemical and efficiency properties of sulfamethazine drug (D) by loading into the prepared pseudopolyrotaxane, Chitosan/β-CD. The chemical structure of the obtained polymers and the drug after loading into the pseudopolyrotaxane Chitosan/β-CD/D were confirmed by utilizing the spectral analysis FT-IR, 1H-NMR. The morphological characterizations of the products were examined by SEM, the crystallinity of the products was investigated by XRD. Minimal inhibitory concentration (MIC) values of sulfamethazine, Chs/β-CD and Chs/β-CD/D determined against some Gram-negative and Gram-positive bacteria (E. coli, S. aureus). The results of this study established that loading sulfamethazine on the newly designed polymer not only showed higher antimicrobial and antibiotic efficacy compared to the pure drug, but also modified the physical and the chemical properties of the sulfamethazine drug itself.
Keywords: Sulfamethazine, β-cyclodextrin, polyrotaxanes and Chitosan polymers.

1 Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides made from the enzymatic degradation of starch by cyclodextrin glucanotransferase (CGTase). The most common and commercially successful cyclodextrins are the α-, β- and γ-CDs which consist of six, seven and eight glucose molecules, respectively. They were discovered for the first time by Villier from the *Bacillus amylobacter* digest of potato starch [1,2].

The presence of lone pair electrons on the oxygen atom that bound to the glucoside in the cavity make the interior of the cyclodextrin cavity includes a comparatively high electron cloud density that shows hydrophobic properties [3]. Also, hydroxyl groups outer side of the torus-shaped molecule make the molecule exhibit hydrophilic properties. During this approach, such variety of characteristic create it possible to embed a hydrophobic guest molecule or functional group with a suitable molecular size in the cavity of cyclodextrin, which could be a perfect biological material that is able to effectively bind with the guest molecule [4]. Within the past twenty years, a lot amount of work has been revealed on chitosan polymer and its potential use in drug delivery systems. Chitosan is a biodegradable [5], biocompatible polymer thought to be safe for human dietary use and approved for wound dressing applications. Chitosan has been used as a carrier in polymeric nanoparticles for drug delivery through numerous routes of administration [6]. Chitosan has chemical functional groups that can be modified to achieve specific goals, making it a polymer with an incredible vary of potential applications [7]. Nanoparticles (NP) prepared with chitosan and chitosan derivatives typically possess a positive surface charge and mucoadhesive properties such that can adhere to mucus membranes and release the drug payload in a sustained release manner. Moreover, it shows very low toxicity both in vitro and in vivo models. CD

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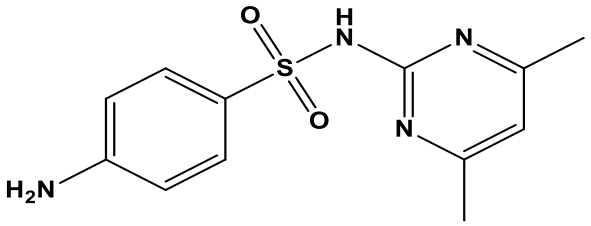
pseudopolyrotaxanes, prepared by threading a polymer chain or long molecule through many cyclodextrin rings [8], have fascinating supramolecular structures with uncommon properties. It has been shown that pseudopolyrotaxanes can be prepared with various synthetic polymers [9-14], biopolymers, conducting polymers, dyes, polypeptides, proteins, and enzymes, and that the huge variety of supramolecular structures are very useful in several numerous areas [15,16]. The nanostructure or microstructure and the functionality of the guest molecules are altered when encapsulated by cyclodextrin molecules; also, CD rings assist the stabilization and protection of the guest molecules and can control or sustain their delivery. CDs are natural, nontoxic, and slowly biodegradable, and consequently CD polyrotaxanes are very attractive candidates for good materials, controlled or sustained delivery systems, sensor devices, molecular switches, or alternative diagnostic systems [17-20]. Antibiotics are used to treat or stop microorganism infections, and generally protozoan infections. Once an infection is suspected of being accountable for an illness, but the responsible pathogen has not been identified, an empiric therapy is adopted [21]. This involves the administration of a broad-spectrum antibiotic based on the signs and symptoms presented and is initiated pending laboratory results that can take many days [22]. When the responsible pathogenic microorganism is already known, medical care will be started. This can typically involve the utilization of a narrow-spectrum antibiotic. The selection of antibiotic given also will be supported its price. Identification is critically vital because it will reduce the cost and toxicity of the antibiotic therapy and reduce the possibility of the emergence of antimicrobial resistance [23]. Sulfamethazine was the first type of synthetic chemical used to treat microorganism diseases in humans. Some medicine that are closely related to sulfamethazine, and they are sulfathiazole, sulfadiazine, and sulfamethoxazole. All these sulfa drugs are better than sulfamethazine since these sulfa drugs have half-lives time shorter than sulfamethazine, which in theory makes them a more robust chemical environmentally [24,25]. Sulfamethazine might cause nausea, vomiting, diarrhea and hypersensitivity reactions. Hematologic effects such as anemia, agranulocytosis, thrombocytopenia and hemolytic anemia in patients with glucose-6-phosphate dehydrogenase deficiency might also occur. [26]



Sulfamethazine drug (D)

From this point this study aim to synthesis of new biodegradable drug carrier for sulfamethazine drug containing chitosan and β-cyclodextrin to improve their physical, chemical and antibacterial efficiency.

2 Experimental

2.1 Chemicals and reagents

Sulfamethazine, β-cyclodextrin, chitosan with deacetylation degree above 85% and dimethylformamide (DMF) were purchased from Merck Co., Germany. Glacial acetic acid was purchased from Alfa Aesar GmbH &Co KG. All chemicals were used as received without additional purification.

2.2. Synthesis of the new carrier's polymer

2.2.1 Synthesis of pseudopolyrotaxane Chs/β-CD

β-Cyclodextrin solution was prepared by dissolving 1g of β-CD in 50 ml deionized water for 30 min. at 50°C, also chitosan solution was prepared by dissolving 1g of chitosan in 50 ml of (1% v/v) glacial acetic acid for 1hr at 50°C in a magnetic stirrer until clear solution was obtained after that two solution were mixed. The reaction mixture was stirred for 24hr at 50°C. The formed hydrogel was poured into a Petri dish and the solvent was evaporated at room temperature to give pseudopolyrotaxane Chs/β-CD as a pale brown powder (80% yield, m.p. over 300°C).

2.3 Loading Sulfamethazine (D) into Chs/β-CD to form Chs/β-CD/D

Sulfamethazine (D) was loaded into the pseudopolyrotaxane by dissolving 0.5 g of the Chs/β-CD in 30 ml DMF to form stable hydrogel by using the colloidal tectonic approach. Then, (0.25 g) of drug was dissolved in 10 ml DMF then add into the reaction mixture at room temperature. The reaction mixture was stirred for 24hr at 50°C until obtained homogenous solution. The formed emulsion was poured in a petri dish and left to dry at room temperature to obtain (pale yellow) solid, Chs/β-CD/D.

2.4 Characterization

The chemical structures of sulfamethazine drug **(D)** before and after loaded into pseudopolyrotaxanes **Chs/β-CD** were examined by FT-IR spectroscopy using infrared spectrometer (Jasco Model 4100 – Japan) at room temperature in the wave number range of 4000 – 400 cm^{-1}. And also examining by proton nuclear magnetic resonance (^{1}H-NMR).^{1}H-NMR spectra were recorded at 25°C using a Bruker AM-400 NMR spectrometer (Germany) at 400 MHz. For examining the phase structure and crystallite size of the samples, X-ray diffraction spectra were recorded at room temperature using a powder diffractometer (Brucker D8 Advance, Germany) with Cu K αradiation source, λ=

1.5406 ˚A and 2 θin the range (5–80 °). Scanning electron microscope SEM (JEOL SEM model JSM –5500 –Japan) was used to observe the morphological structures of the obtained materials with accelerated voltage 10 kV.

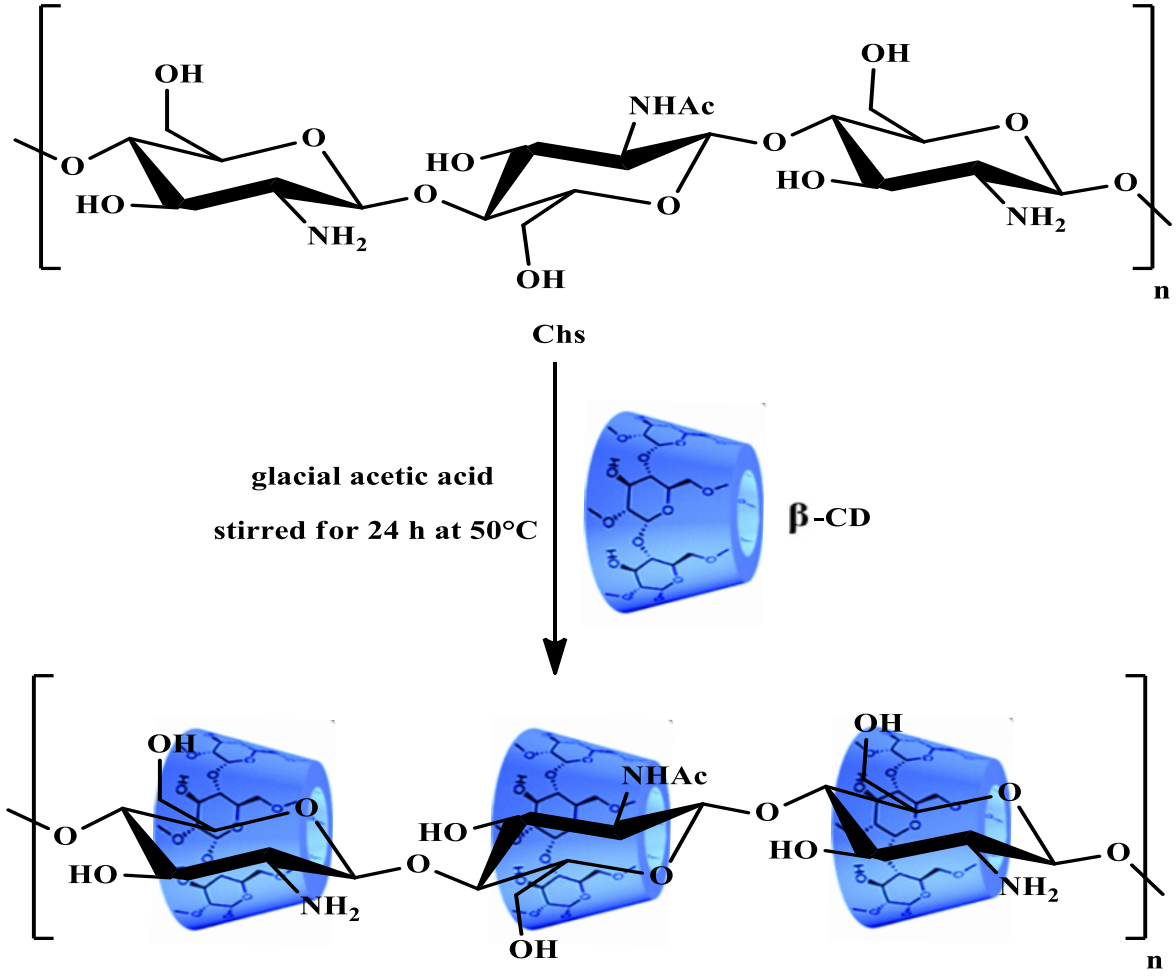
2.5 Antibacterial test

Antibacterial activity of sulfamethazine drug (D) and Chs/β-CD on *Staphylococcus aureus* (NCTC 8325- 4) and *Escherichia coli* (BAA-2471) was measured after overnight liquid cultures quantitatively by measuring the MIC (Minimal Inhibitory Concentration) which is the minimum concentration required from the antimicrobial agent to inhibit the growth of microorganism. Micro-titre plate (Nucleon, Germany) with two-fold dilutions of an antimicrobial agent is inoculated with a bacterial suspension with (105 CFU/mL) in 100 µL MHB (Mueller–Hinton broth) and incubated at 37°C. Inoculated and uninoculated wells of antibacterial free broth were included to check for the adequacy of the broth to support the growth of the organism and check of sterility. The results represent data from three independent experiments.

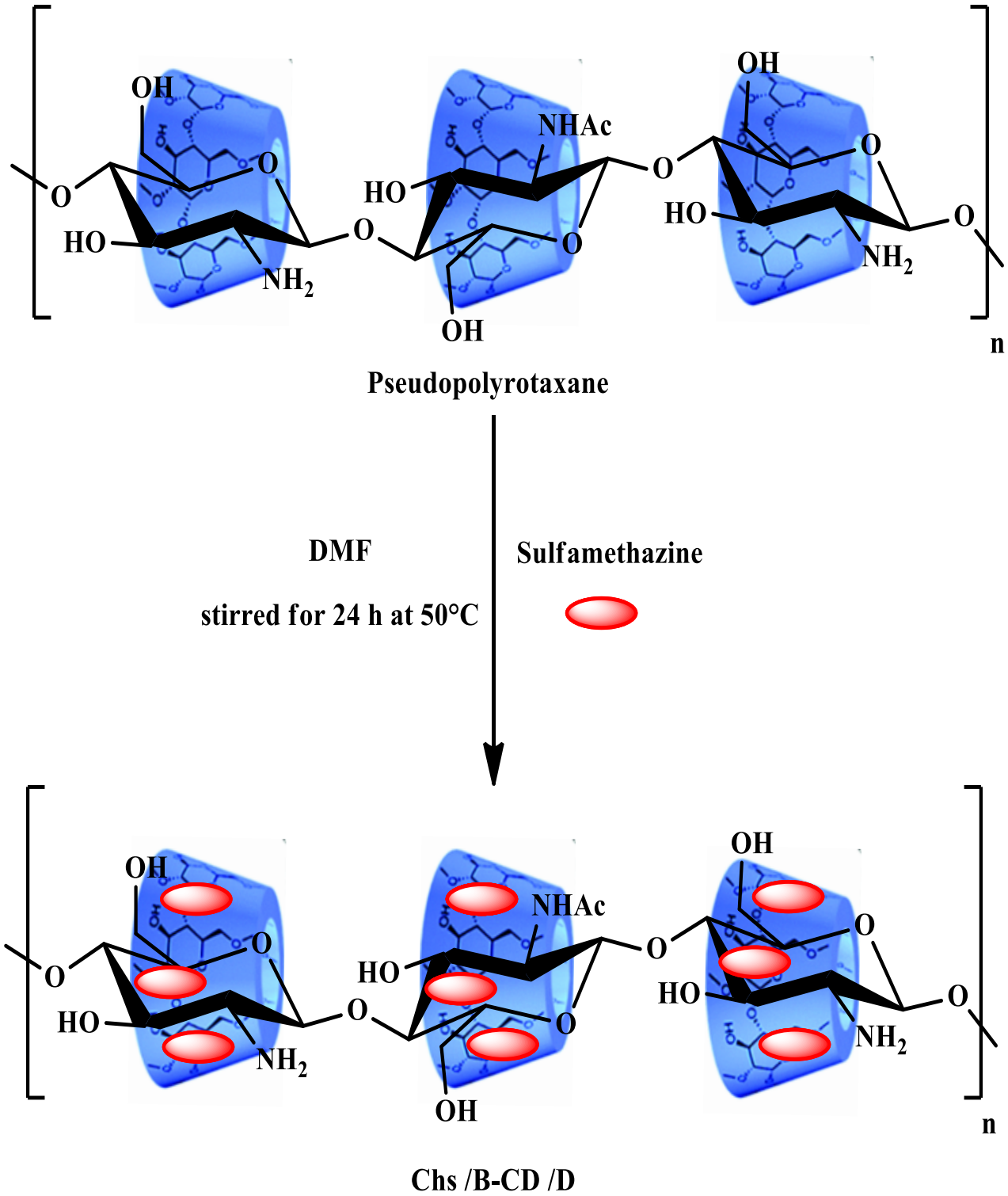
3 Results and Discussion

3.1 Chemistry

Scheme (1)illustrates the mechanism that has been proposed for the synthesis of the Chs/β-CD**.** The polymers chain of chitosan is inserted into the macrocyclic molecules of β-CD by threading method *Via* formation of hydrogen bonds and hydrophobic-hydrophilic interaction.
Scheme (2) showed the loading of the sulfamethazine drug into the Chs/β-CD to form Chs/β-CD/D by dissolving the inclusion complex into DMF then sulfamethazine drug is added to the mixture. The reaction mixture is heated with stirring for 24h at 50ºC until obtained homogenous solution. The formed emulsion was poured in a petri dish and left to dry at room temperature to obtain Chs/β-CD/D.



Scheme 1: Synthetic route for the synthesis of the pseudopolyrotaxane.



Scheme 2: Synthetic route for the loading of sulfamethazine into the pseudopolyrotaxane to form Chs/β-CD/D.

3.2 FT-IR-Studies

Pseudopolyrotaxane was synthesized as represented in Scheme 1 and its chemical structure was established by FTIR spectra. FT-IR spectrum shows several characteristic bands at 3382 cm^{-1} for (OH) groups, characteristic band due to CH aliphatic at 2826cm^{-1} also characteristic band at 1159 cm^{-1} due to C-O-C groups. The absorption band of ν[OH] symmetric stretching and ν[CH-aliphatic] was shifted to lower frequency compared to those in pure β-CD (Fig 1). Also, the absorption band for ν[O-H] stretching and ν[CH-aliphatic] was shifted to lesser frequency compared to those in pure β-CD. In addition, the absorption band for ν[C–O-C] and ν[C-O] stretching was shifted to higher frequencies. **Table 1** indicates the differences in the absorbance bands of pure β-CD and pseudopolyrotaxane. The shifting that the macrocyclic molecules of β-CD. The enhancement in frequencies is due to the insertion of the chitosan chain through the electron- cavity of the β-CD rings. In contrast, the decreasing in frequencies is due to the creation of Vander Waals forces, hydrogen bonds between the (OH), (C-O-C) groups of β-CD and the hydroxyl, amino groups of chitosan groups. The FT-IR spectrum of sulfamethazine drug showed band at 3372 cm^{-1} correlated to the amino groups (NH_2), and band at 2941 cm^{-1} correlated to (CH-aromatic). The absorption bands at 1349, 1333 cm^{-1} which characterized the vibration of (SO_2) group (Fig 1). The differences between absorbance bands of sulfamethazine drug and Chs/β-CD/D are summarized in **Table 1**. The difference in the intensity of sulfamethazine drug before and after loaded into pseudopolyrotaxane is due to the formation of Vander Waals forces and hydrogen bonds between NH_2 group, O and N atoms of pure drug and (OH,

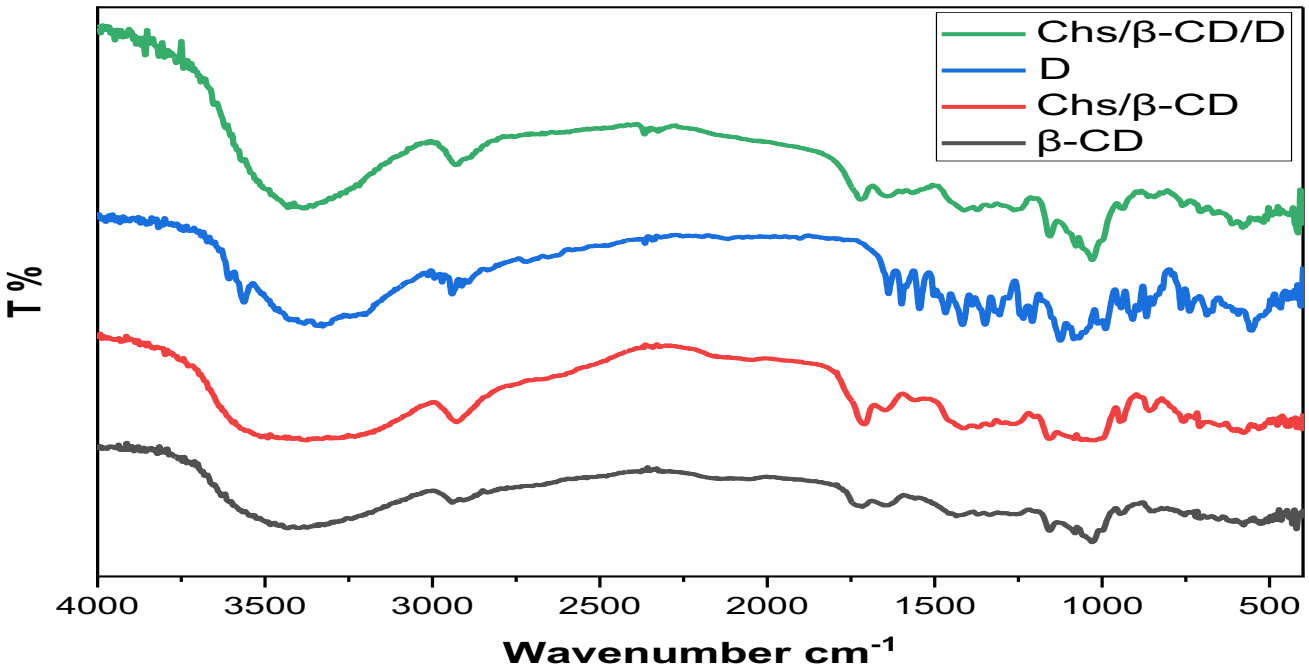


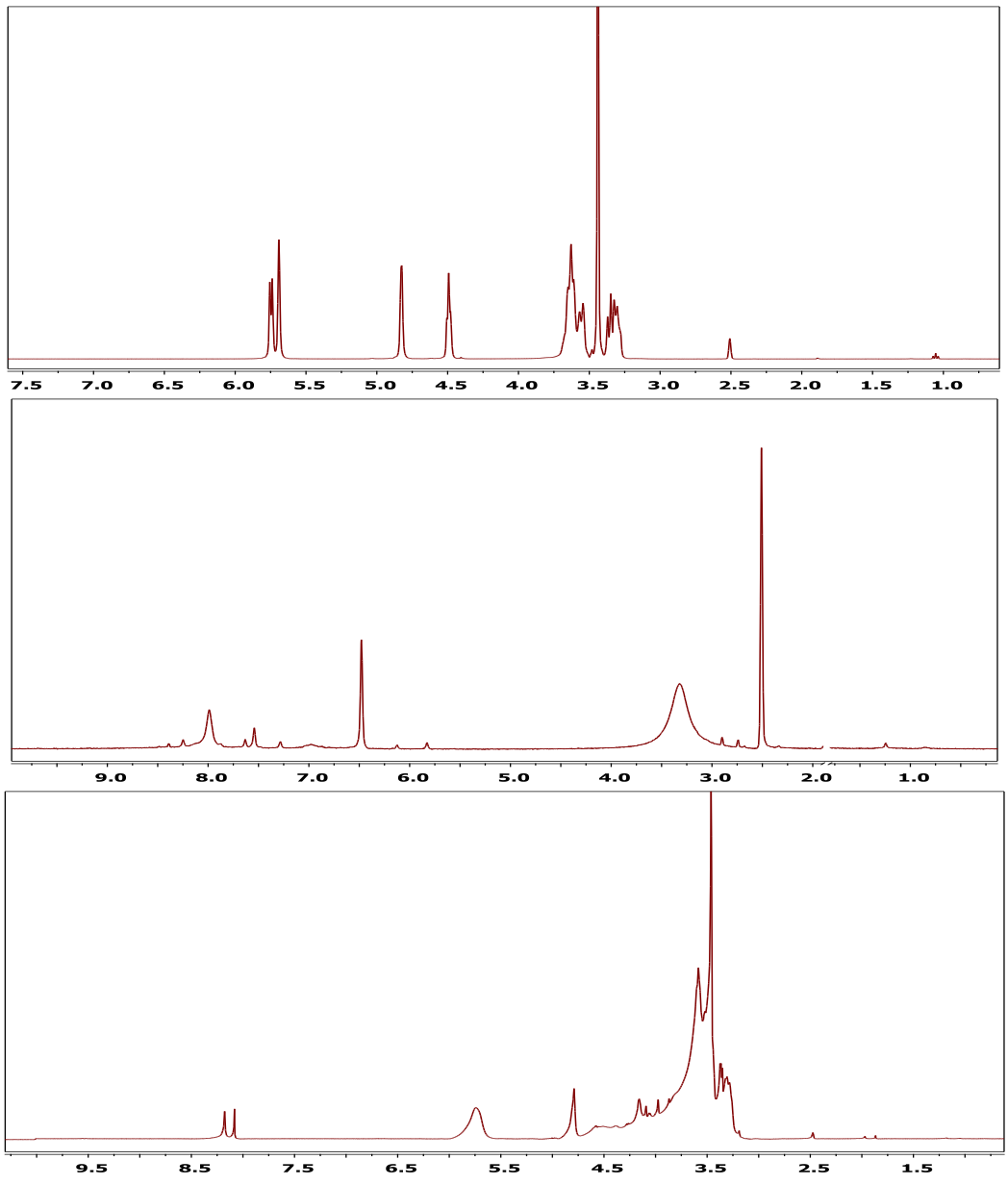
Fig 1: FT-IR spectra of β-CD, Chs/β-CD, D and Chs/β-CD/D.

Table 1: FT-IR difference between β-CD and Chs/β-CD and difference between pure Drug (D) and Chs/β-CD/D.

Functional group	Wavenumber, cm^{-1}		Δδ	Wavenumber, cm^{-1}		Δδ
	β-CD	Chs/β-CD		D	Chs/β-CD/D	
ν[O-H]- ν[NH_2] stretching	3400	3382	-18	3381	3372	-9
ν[CH-aliphatic]	2835	2826	-9	2837	2833	-4
ν[C-O-C] vibration	1156	1159	+3	-	-	-
ν[C-O] stretching	1023	1030	+7	-	-	-

3.3. [1]H- NMR analysis

The chemical structure of sulfamethazine drug (D), Chs/β-CD and Chs/β-CD/D was confirmed by ^{1}H-NMR spectroscopy as shown in (Fig 2). The ^{1}H-NMR spectra of β-CD showed appearance of signals due to the aliphatic CH-protons at the region 3.21 to 5.69 ppm. ^{1}H-NMR spectrum of sulfamethazine drug (D) showed appearance of signals due to two methyl group (CH_3) at δ 3.32 ppm, the NH protons appear at 5.8 ppm, the NH_2 protons appear at 6.5 ppm, also, the aromatic protons appear at the range 7.28-7.99 ppm.^{1}H-NMR spectrum of Chs/β-CD showed appearance of signals due to aliphatic protons of CD and chitosan at the range 3.18-4.78 ppm and the presence of OH secondary alcohols protons in 5.73 ppm, also appearance of protons due to (NH-CO-) groups in the region 8.08-8.17 ppm. ^{1}H-NMR spectra of Chs/β-CD/D indicates appearance of characteristic peak due to two methyl group (CH_3) of sulfamethazine drug (D) at δ 1.24 and 1.94 ppm. In addition to the presence of signals due to aliphatic protons of β-CD and chitosan in the region 3.0-4.3 ppm, and appearance of the aromatic protons in the region 7.5-9.5 ppm.



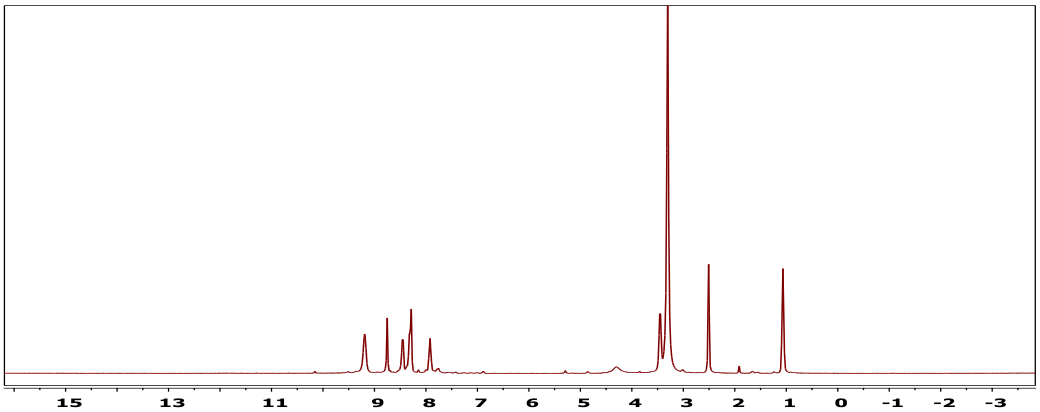


Fig 2. [1]H-NMR for B-CD, D, Chs/β-CD and Chs/β-CD/D in DMSO-d*6*

3.4 XRD analysis

Phase structure and its crystallite size of sulfamethazine drug (D), Chs/β-CD and Chs/β-CD/D were figured out by utilizing XRD analysis (Fig 3). XRD spectra were recorded at room temperature in the range 5°−80°. The characteristic diffraction peaks of sulfamethazine drug (D), Chs/β-CD and Chs/β-CD/D were observed at 2theta (Table 2). The loading of sulfamethazine drug (D) into pseudopolyrotaxane Chs/β-CD was confirmed by the change in the crystalline nature, were the crystallinity values of Chs/β-CD found 74.7 % and the crystallinity values of sulfamethazine drug (D) found 60.9 % and after loading into pseudopolyrotaxane Chs/β-CD found 62.6 %. The change in XRD spectra and crystallinity of sulfamethazine drug (D), Chs/β-CD and Chs/β-CD/D supported the synthesis of these materials.

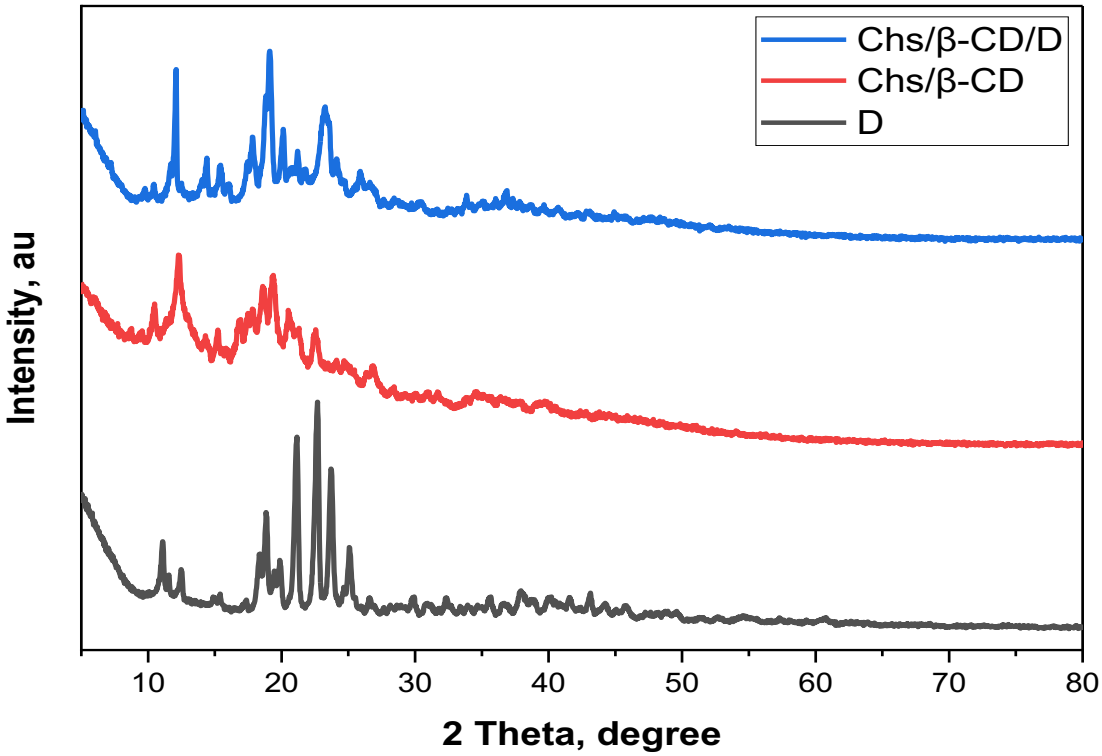


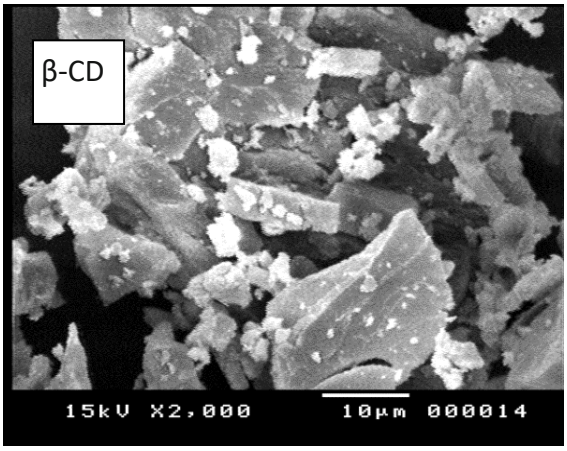
Fig. 3: XRD spectra of D, Chs/β-CD and Chs/β-CD/D.

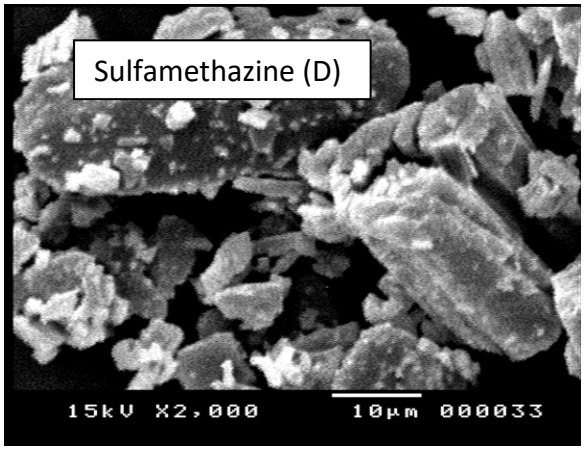
Table 2: XRD peaks and crystallinity of D, Chs/β-CD and Chs/β-CD/D.

	2 Theta, degree	Intensity, au	Crystallinity
D	18.84	49.01	60.9%
	21.14	78.30	
	22.68	91.82	
	23.71	65.88	
Chs/β-CD	10.43	59.95	74.7%
	12.27	80.54	
	18.58	68.33	
	19.37	72.62	
Chs/β-CD/D	12.09	73.09	62.6%
	17.81	46.91	
	19.12	80.25	
	23.27	58.62	

3.5 Surface Morphology

The morphological structure of β-CD, sulfamethazine drug (D), Chs/β-CD and Chs/β-CD/D were elucidated in (Fig 4). These images show a completely difference between them. The surface morphology structure of β-CD and sulfamethazine is different from Chs/β-CD and Chs/β-CD/D, this give good indication for the formation of inclusion complex**.** In addition to successfully loading of sulfamethazine drug into the pseudopolyrotaxane**.** The SEM image of β-CD was appeared as three-dimensional block structure with an irregular shape, while the SEM image of sulfamethazine drug was showed an irregular brick-like morphology with rough edges and caves. But the SEM image of Chs/β-CD was appeared as homogeneous amorphous morphology, while the SEM image of Chs/β-CD/D was showed an irregular block-like structure, and the original morphology of the individual compounds disappeared, which is solid evidence of the crystal. The change in SEM image of sulfamethazine drug (D), Chs/β-CD and Chs/β-CD/D supported the synthesis of these materials.





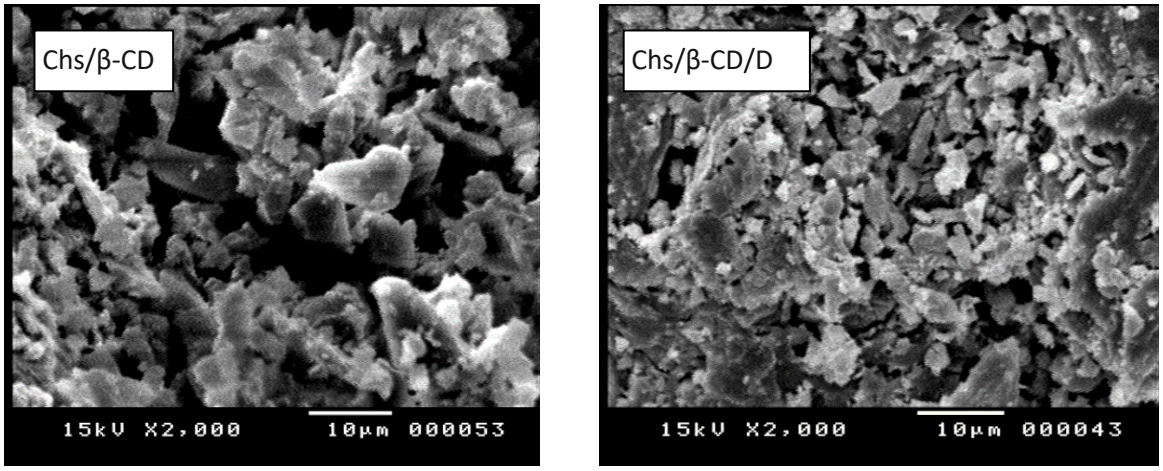


Fig. 4: SEM images of β-CD, Sulfamethazine drug (D), Chs/β-CD and Chs/β-CD/D at magnification of 2000X.

3.6 Antimicrobial performance evaluation

- Minimal inhibitory concentration (MIC)

The MIC values of the tested compounds Chs/β-CD and Chs/β-CD/D determined against S. aureus & E. coli ranged from (16-0.125 µg/ml) [27]. The obtained values were compared with MIC values of sulfamethazine drug (D) that used as standard drug. The most effective compounds against E. coli was Chs/β-CD/D which inhibit bacterial growth with MIC value 4 µg/ml, followed by Chs/β-CD with MIC value 8 µg/ml, compared to sulfamethazine drug (D) (16 µg/ml) (Fig 5). On the other hand, there is no change in activity against S. aureus between sulfamethazine drug (D) with MIC value 8 µg/ml and after loaded onto prepared inclusion complex (Chs/β-CD) to form Chs/β-CD/D (Fig 6).

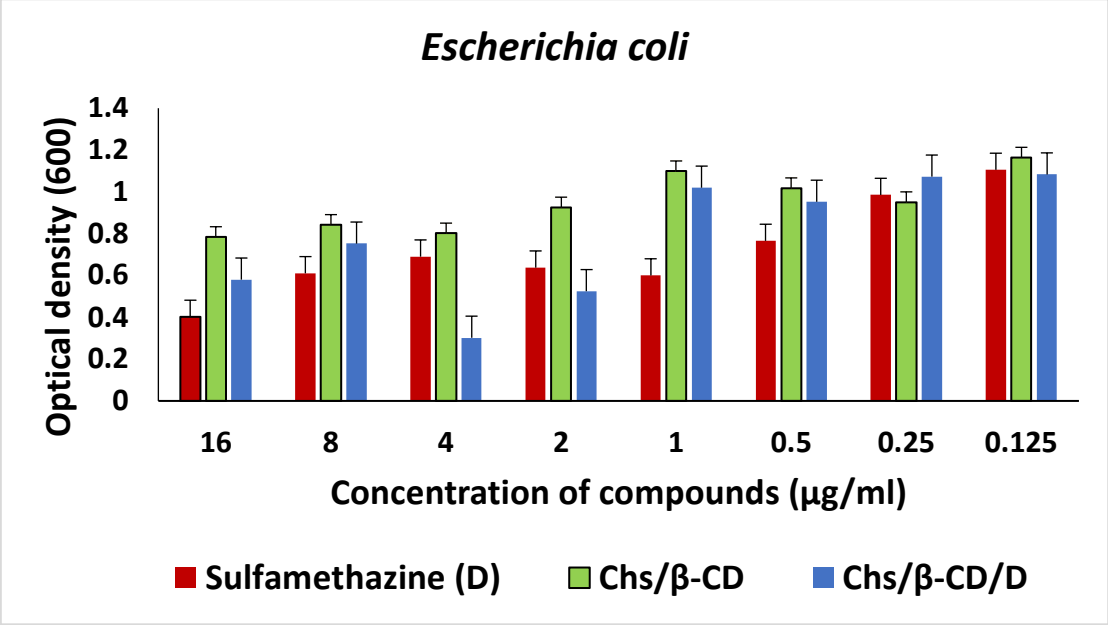


Fig. 5: Antibacterial activities of Sulfamethazine drug (D), Chs/β-CD and Chs/β-CD/D against *E. coli.* OD600 represents bacterial growth. Graph displays standard error of the mean of triplicate wells from one of three similar experiments.

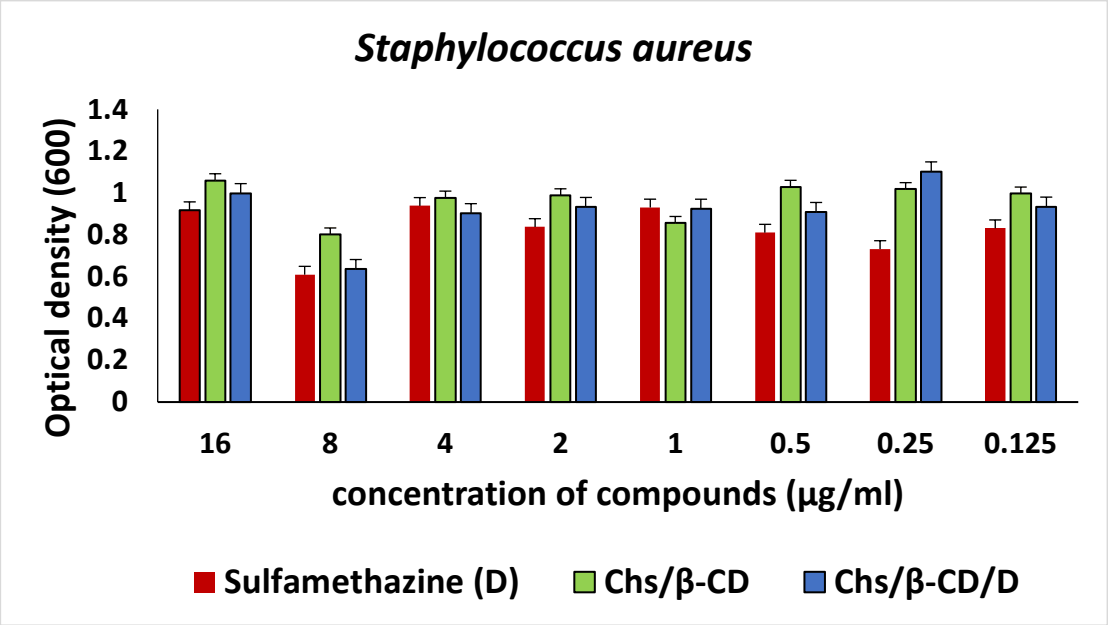


Fig. 6: Antibacterial activities of Sulfamethazine drug (D), Chs/β-CD and Chs/β-CD/D against *S. aureus*. OD600 represents bacterial growth. Graph displays standard error of the mean of triplicate wells from one of three similar experiments.

4 Conclusions

Sulfamethazine is an antibiotic used to treat a number of bacterial infections while the common side effects of sulfamethazine include nausea, vomiting, diarrhea and hypersensitivity reactions. For these reasons in this study we aim to improve their physical, chemical and antibacterial efficiency by loading the sulfamethazine into the prepared inclusion complex Chs/β-CD. The drug and the obtained materials were characterized by several methods, FTIR, ^{1}H-NMR, XRD and SEM. Moreover, the biological activity of the products was investigated against some Gram-negative and Gram-positive bacteria. The results indicate the prepared drug delivery system for the sulfamethazine drug could modify the antimicrobial, antibiotic efficacy, the physical and chemical properties of the sulfamethazine.

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