Vol.64: e21190750, 2021
https://doi.org/10.1590/1678-4324-2021190750
ISSN 1678-4324 Online Edition

Article - Engineering, Technology and Techniques

Antibacterial and Cytotoxic Effects of Cyclodextrin-Triazole-Titanium Based Nanocomposite

Zari Fallah[1,2]
https://orcid.org/0000-0002-3824-0929

Mojtaba Mohseni[3]
https://orcid.org/0000-0002-5709-6600

Hossein Nasr Isfahani[2]
https://orcid.org/0000-0002-6006-9937

Ebrahim Zabihi[4,5]
https://orcid.org/0000-0003-4210-3836

Mahmood Tajbakhsh[1]*
https://orcid.org/0000-0002-4856-1937

Zeinab Abedian[4]
https://orcid.org/0000-0002-9324-2943

[1]University of Mazandaran, Faculty of Chemistry, Babolsar, Iran; [2]Shahrood University of Technology, School of Chemistry, Shahrood, Iran; [3]University of Mazandaran, Faculty of Science, Department of Microbiology, Babolsar, Iran; [4]Babol University of Medical Sciences, Health Research Institute, Cellular and Molecular Biology Research Center, Babol, Iran; [5]Babol University of Medical Sciences, School of Medicine, Department of Pharmacology, Babol, Iran.

Editors-in-Chief: Paulo Vitor Farago
Associate Editor: Andressa Novatski

Received: 2019.12.11; Accepted: 2020.10.07.

*Correspondence: tajbaksh@umz.ac.ir; Tel.: +98-11-35302301 (M.T.).

HIGHLIGHTS

- Observation of the antibacterial and cytotoxic effects of CD.COM due to the synergy between cyclodextrin, triazole and TiO_2 NPs.
- The antibacterial activity of CD.COM against both gram positive and gram negative bacteria.
- The CD.COM cytotoxicity against cancer cells and compatibility with fibroblast cells.

Abstract: In this paper, the antibacterial activity of triazole functionalized cyclodextrin (CD.Click) and cyclodextrin-triazole-titanium based nanocomposite (CD.COM) was evaluated. The results indicated that CD.Click and CD.COM perform a wide range of antibacterial activity against both gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria. The cytotoxic effect of CD.COM was investigated in vitro on cancerous cell lines (*cervical cancer*, *breast carcinoma* and *sarcoma osteogenic*) and fibroblast cells by MTT assay. The cell viability evaluation confirmed that the growth of cancerous cells is inhibited in a dose and time dependent way without any significant effect on the normal fibroblast cells.

Keywords: TiO_2; Triazole; *β*-Cyclodextrin; antibacterial; cytotoxic.

GRAPHICAL ABSTRACT

INTRODUCTION

Development of organic-inorganic nano-hybrids is an important research area in the field of nanotechnology. Biopolymers have been widely used in composite structures because of environmental and economic advantages containing renewable sources, compatibility, biodegradability and low cost. Polysaccharides, such as cellulose [1,2], cyclodextrins (CDs) [3,4] and starch [5] proved to be good candidates to provide suitable substrates for metal oxide nano-fillers.

CDs are nontoxic, biodegradable, water-soluble cyclic oligosaccharides consisting of 6 (*α*-CD), 7 (*β*-CD) or 8 (*γ*-CD) *D*-glucose units linked together via *α*-1,4-glycosidic bonds to form torus-like structure [6]. *β*-cyclodextrin (*β*-CD), produced from enzymatic conversion of starch with moderate truncated cone-shaped hole, is low priced commercially available and has been extensively used in industry [7]. The ability of solubilization, extraction of phospholipids [8] and cholesterol [9] from cell membranes and lysis of red blood cells and blood coagulation [10] made the *β*-CD to be applied in medicine.

Among various kinds of metal oxides, titanium dioxide nanoparticles (TiO_2 NPs) have attracted significant attentions due to their excellent physical and chemical properties along with safe and broad-spectrum antibiosis [11,12]. TiO_2 NPs are unique antibacterial agent because of their ability to disrupt bacterial cell walls as a result of their small size and large surface area [13,14]. TiO_2 NPs tend to agglomerate, so for solving this problem, they have been embedded on the support surface by chemical bonding. Modification of TiO_2 NPs with silane coupling agents is one way for creating the chemical binding with a support surface [15]. In addition, it provides the possibility of further chemical modifications to achieve more efficiencies [16].

The copper catalyzed alkyne-azide cycloaddition, known as click reaction, is a beneficial reaction for linkage of modified TiO_2 NPs with a support surface via triazole ring formation [17]. The triazoles are highly robust to metabolic degradation and capable to form hydrogen bonding enhancing their solubility and interacting with biomolecular targets [18,19]. So, triazole ring has significant biological properties such as antibacterial [20], antifungal [21], anti-inflammatory [22] antiviral [23] and anticancer [24-26] activities, and have been utilized in medicinal chemistry to produce medicinally interesting drug candidates with a wide range of applications.

Given that the cancer is the second leading cause of death in humans, a great deal of interest has been recently devoted to the synthesis of new compounds with the potential to adapt with normal cells and reduce cancer cells [27-29]. At the same time, due to the resistance of bacteria to antibiotics, it is necessary to produce substances with effective antibacterial properties.

The exploration of new agents with dual antibacterial and anticancer activities is necessary because of promising therapeutic potential due to their capability to decrease the threat of bacterial infections in the frequently immunocompromised cancer patient [30-32]. This class of compounds with dual antibacterial and anticancer effects not only controlling growth of cancer related bacterial infections but also protecting patients from the infection due to downplay of immune system [33].

Nanocomposites can perform significant improvement in various fields due to their better efficiency than any of the organic and inorganic raw materials. In this regards, novel cyclodextrin-triazole-titanium based nanocomposite (CD.COM) was synthesized hoping to observe the synergy of cyclodextrin, triazole and TiO_2 moieties. The CD.COM was prepared from nucleophilic reaction of the amino modified TiO_2 NPs (TiO_2/AS) with the triazole modified cyclodextrin (CD.Click) [4]. The antibacterial activity of CD.Click and CD.COM was investigated against gram negative (E. coli; *Escherichia coli* and P. aeruginosa; *Pseudomonas aeruginosa*) and gram positive (S. aureus; *Staphylococcus aureus* and B. subtilis; *Bacillus subtilis*) bacteria. In addition the cytotoxic effect of CD.COM was evaluated on cancerous cell lines (MCF-7; *breast carcinoma*, Saos; *sarcoma osteogenic* and Hela; *cervical cancer*) and fibroblast cells.

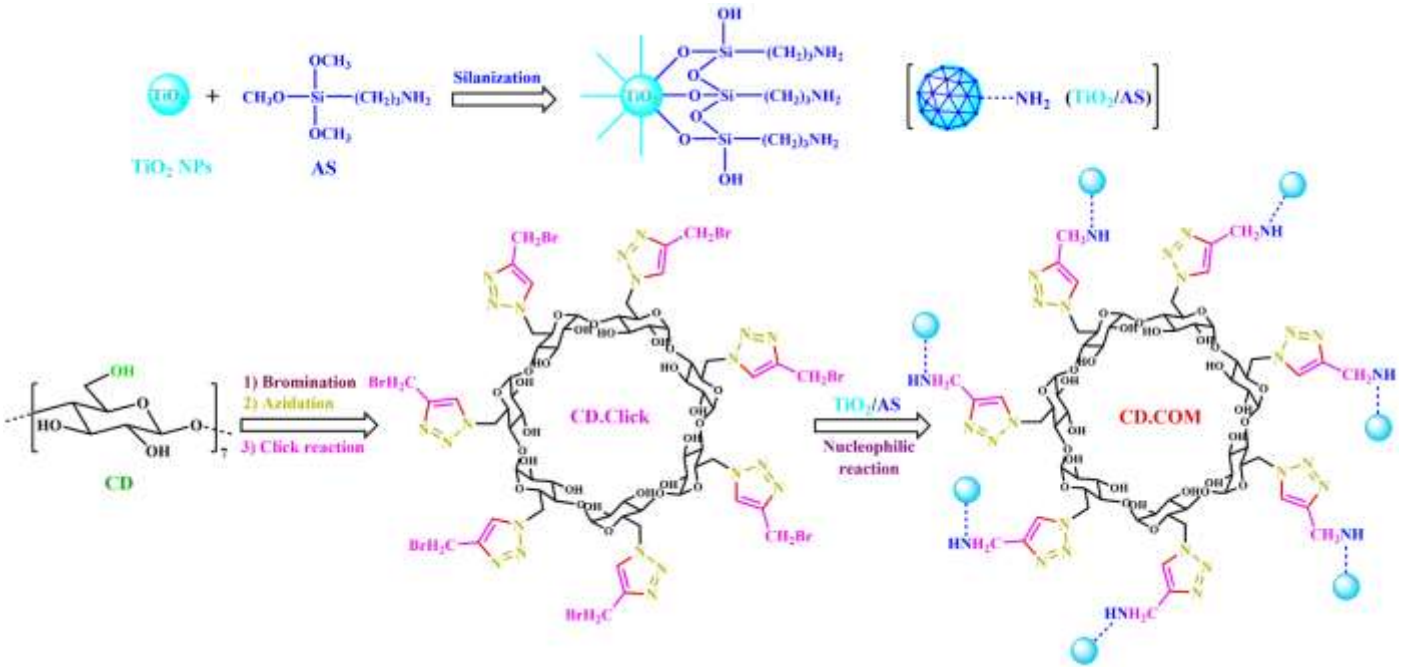
EXPERIMENTAL

Material and methods

The tested bacteria included E. coli PTCC 1330, P. aeruginosa PTCC 1074, S. aureus ATCC 35923, and B. subtilis PTCC 1023 were purchased from Persian Type Culture Collection (Iran). The reference antibiotics of chloramphenicol and gentamicin were obtained from Padtan Teb (Iran). Cancer cell lines of HeLa, MCF-7 and Saos were purchased from Pasteur Institute of Iran (Tehran) and the fibroblast cell line was obtained by non-enzymatic method from newborn human foreskin at Amirkola children's hospital (Babol, Iran). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was bought from Alfa Aesar (Lancashire, UK). Nutrient Muller Hinton Agar was obtained from Merck (Goettingen, Germany). Dulbecco's Modified Eagle's Medium (DMEM), penicillin and streptomycin were provided from Biowest (France). Fetal bovine serum (FBS) was obtained from Gibco (Germany). Dimethyl sulfoxide (DMSO) was obtained from Merck company (Germany). Cell viability was automatically measured by microplate reader (Rayto analyzer, CHINA) at wavelength of 570 nm.

Synthesis

The pathway of CD.COM synthesis is displayed in Scheme 1. The experimental details for synthesis of the CD.COM were reported in our previous paper [4]. The titania/γ-aminopropyltrimethoxysilane (TiO_2/AS) was synthesized via the reaction of 3-aminopropyltrimethoxysilane (AS) with anatase TiO_2 nanoparticles (TiO_2 NPs) [3]. The heptakis (6-bromo-6-deoxy)-*β*-cyclodextrin [CD-$(Br)_7$] was synthesized in the presence of triphenylphosphine (Ph_3P) by treating N-bromosuccinimide (NBS) with *β*-cyclodextrin (*β*-CD) [4]. Through the reaction of CD-$(Br)_7$ with sodium azide (NaN_3), the heptakis (6-azido-6-deoxy)-*β*-cyclodextrin [CD-$(N_3)_7$] was obtained [34]. The heptakis [6-(4-bromomethyl-1H-[1,2,3]triazole-1-yl)-6-deoxy]-*β*-cyclodextrin (CD.Click) was prepared through a click reaction of CD-$(N_3)_7$ with propargyl bromide (P.Br) [4].



Scheme 1. Synthetic pathway for the CD.COM preparation.

The CD.COM characterization details were reported in our previous papers [3,4]. A summary of the characterization methods (FTIR; Fourier transform infrared spectroscopy, XRD; X-ray diffraction, TGA; thermogravimetric analysis, EDX; energy-dispersive X-ray spectroscopy and FESEM; field emission scanning electron microscopy) for identifying and determining the purity of CD.COM are provided in this section.

FTIR

The FTIR spectra of *β*-CD, TiO_2 and CD.COM are presented in Figure 1. For the *β*-CD [4], the stretching and bending vibrations of O−H groups are appeared at 3393 and 1647 cm^{-1}, respectively. The absorption peaks at 2926 and 1369 cm^{-1} are related to the stretching and bending vibrations of $–CH_2$ groups. The absorption peaks for C−O stretching vibrations of the C−2/C−3, the C−O−C of glucopyranose unit and the C−O of C−6 are appeared at 1157, 1081 and 1029 cm^{-1}, respectively. Bands at 1419 and 942 cm^{-1} are attributed to the bending vibrations of $−CH_2$ and C−H of six-membered ring, respectively.

In the spectrum of TiO_2 [1], the absorbance peaks at 3200-3500, 1628, and 400-700 cm^{-1} are associated to the stretching and bending vibrations of hydroxyl groups on the surface of TiO_2 NPs, and the stretching vibrations of Ti−O−Ti, respectively.

The FTIR spectrum of CD.COM [3,4] displays all the expected absorption peaks of TiO_2, triazole ring and cyclodextrin. The appearance of intense broad band of C=N stretching at 1634 cm^{-1} could be ascribed to the triazole ring. A weak shoulder at ~3100 cm^{-1} is assigned to the =C–H stretching vibration of triazole ring.

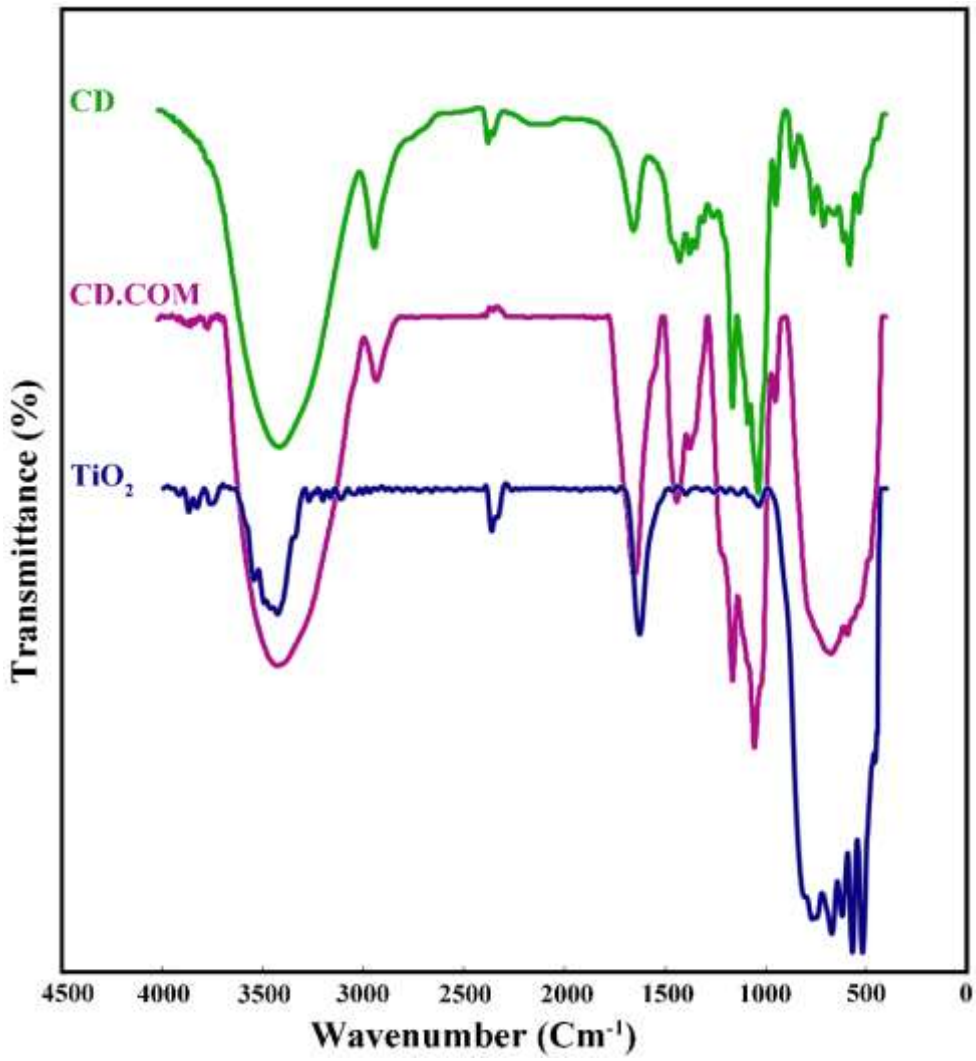


Figure 1. The FTIR spectra of samples.

XRD

XRD patterns of *β*-CD, anatase TiO_2 NPs and CD.COM are displayed in Figure 2. The XRD pattern of *β*-CD exhibits diffraction peaks at 2θ = 9.5º, 10.9º, 12.9º, 13.5º, 16.8º, 18.3º, 19.0º, 24.2º, 24.7º and 35.6º in agreement with crystalline form [3,4]. The XRD pattern of TiO_2 NPs shows 2θ angle and miller indices (hkl) values at 25.3° [101], 37.9° [004], 48.1° [200], 53.92° [105], 55.1° [211], 62.8° [204], 68.8° [116], 70.89° [220] and 75.38° [215], respectively [1]. The CD.COM XRD pattern indicates the presence of peaks for both constituents of the structure (TiO_2 and CD) [3,4]. The severity of cyclodextrin peaks in the composite pattern is very weak, which is probably related to the TiO_2 coating of the cyclodextrin surface.

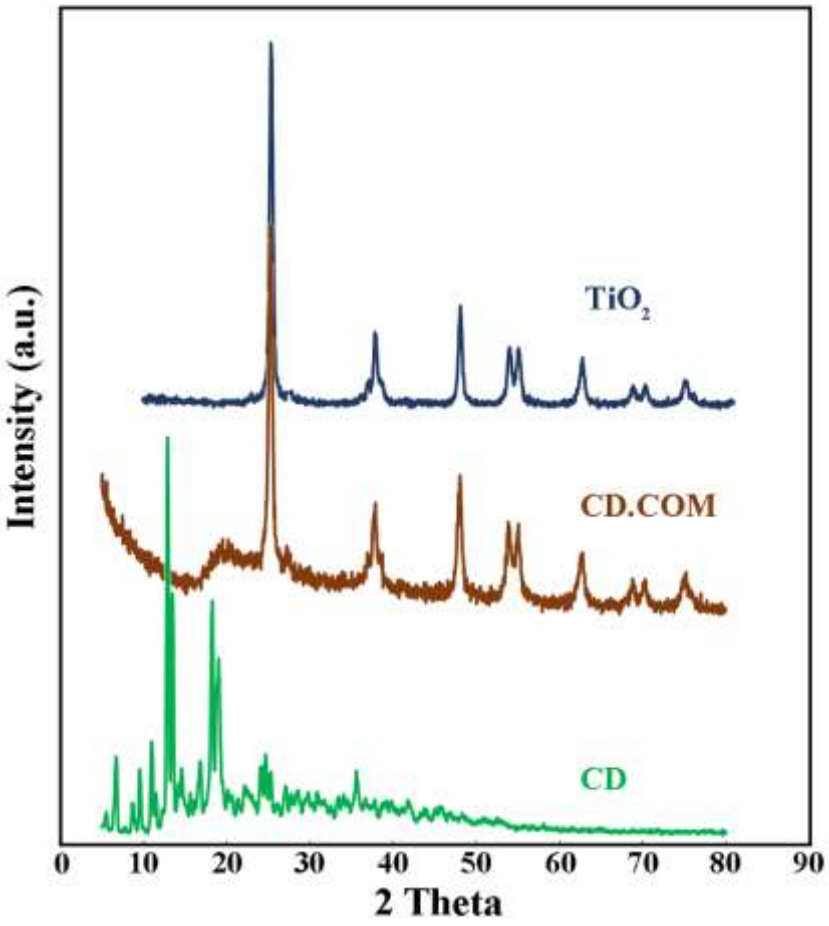


Figure 2. The XRD patterns of samples.

TGA

The thermal stability of samples was analyzed by TGA (Figure 3). TGA curve of TiO_2 NPs shows the 7 wt% weight loss over the full temperature range relating to loss of physisorbed water as well as dehydration of the surface OH groups. Thermal degradation of *β*-CD is started at θ > 250 ºC and pyrolysis takes place at θ > 300 ºC. It is obvious that the presence of modified TiO_2 NPs in the functionalized cyclodextrin structure enhances the thermal stability of CD.COM (char yield = 59.5%) [3,4].

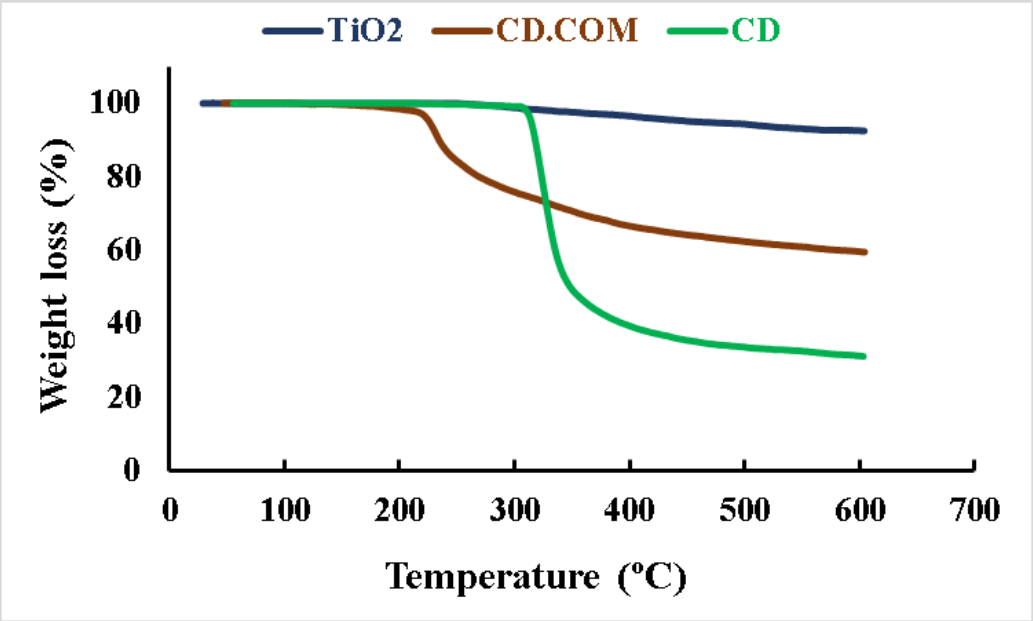


Figure 3. The TGA thermograms of samples.

EDX

The EDX spectra of TiO_2, *β*-CD and CD.COM are exhibited in Figure 4. The EDX spectra display the presence of appropriate elements for each sample. The existence of C, N, O, Si and Ti elements approves the presence of modified TiO_2 NPs with functionalized *β*-CD in the CD.COM structure [4].

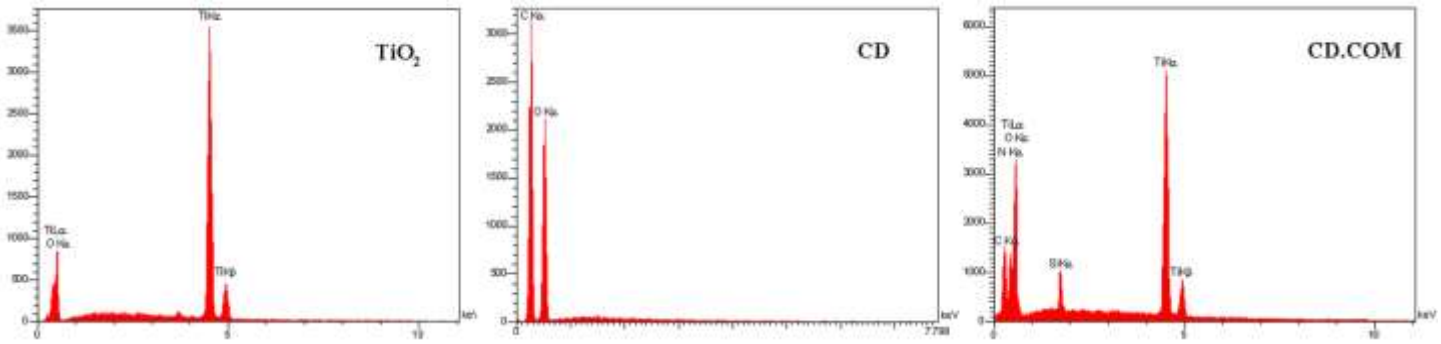


Figure 4. The EDX spectra of samples.

FESEM

Figure 5 represents the FESEM images of *β*-CD, TiO_2 and CD.COM. The FESEM image of CD.COM exhibits not only the creation of rough surface and surface order reduction compared with *β*-CD, but also the capture of modified TiO_2 NPs into the network of functionalized cyclodextrin [3,4].

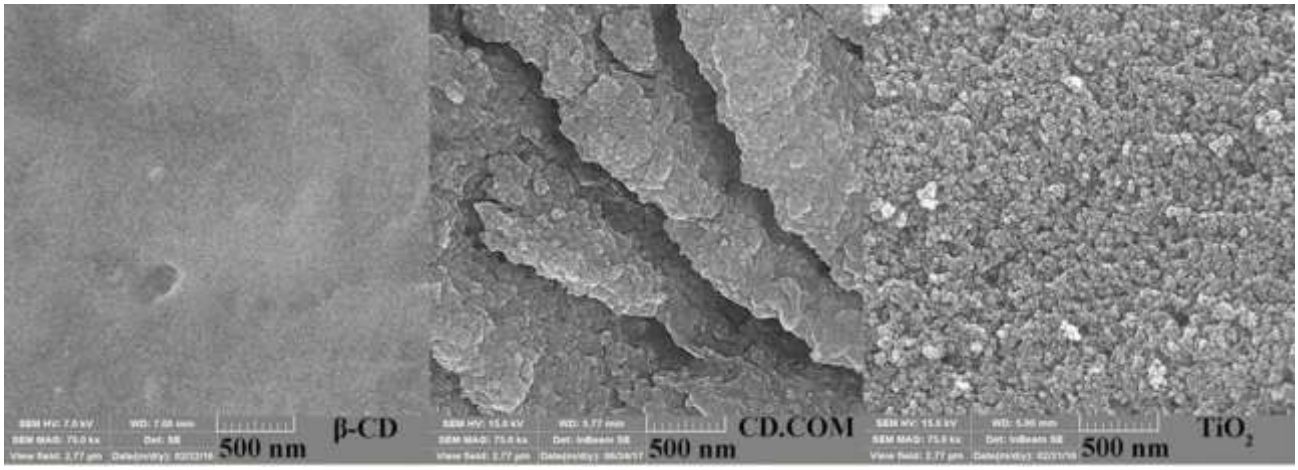


Figure 5. The FESEM images of samples.

Biological studies

Antibacterial investigation

Antibacterial effects of CD.Click and CD.COM were assessed using a disk diffusion method [35]. The CD.Click and CD.COM tablets (0.1 g, diameter: 13 mm) were placed on Muller-Hinton agar dishes infected with bacteria. The antibacterial activity of CD.Click and CD.COM was investigated against E. coli and P. aeruginosa as gram negative bacteria and S. aureus and B. subtilis as gram positive bacteria. The incubated bacteria in nutrient media at 37 °C for 24 h, led to bacterial concentration in the seeding culture of about 10^8 colony forming units (CFU)/mL. Afterwards, the prepared tablets of CD.Click and CD.COM were put on the seeded agar plate in a 37 °C incubator for 24 h. Discs of chloramphenicol (30 mg per disc) and gentamicin (10 mg per disc) antibiotics were applied as references for comparison. The diameter of clear zone inhibition (mm) around the samples after 24 h incubation was measured to evaluate the antibacterial efficiency. All samples/standards were run in triplicate.

Cytotoxicity investigation

Cytotoxicity assay is widely used to screen the cytotoxic effects of synthesized compounds to cells via the cell viability assessment. This is the simplest in vitro technique that was introduced for biocompatibility study of the new synthesized materials. This can be of vital importance during finding new pharmaceutical compounds to warrant the safety of users. The cytotoxicity assay assessing the cell viability by using the MTT. The MTT is a trustworthy and sensitive indicator for the metabolic activity of cells that measures the reducing potential of cell using a colorimetric reaction. The assay relies on the yellow water-soluble tetrazolium dye reduction of MTT to purple colored formazan crystals by the mitochondrial dehydrogenases. The produced formazan after dissolution in DMSO is examined spectrophotometrically, and the amount of cytotoxicity is estimated from the spectra of nanoparticle-treated and untreated cells. The CD.COM cytotoxicity was investigated in vitro against Hela, MCF-7, Saos and fibroblast cells by MTT. The cells (8×10^3 cells/well onto 96-well) were cultured in complete growth medium [DMEM; 10% FBS, 100 IU/mL penicillin and 100 µg mL^{-1} streptomycin] and then incubated at 37 °C in 5% CO_2 atmosphere for 24 h to permit cell attachment. Then cells were treated with the CD.COM at different concentrations (7.8-500 mg L^{-1}) within 24 to 48 h, along with replacing of the cell culture medium by a renewed medium and further incubated for 24 h. The cells without any treatment were served as a control and grown on each plate under the same conditions as treated cells. After incubation, the medium was removed, washed with PBS and then MTT reagent (50 µL; 5 mg L^{-1} in PBS) was added to each well and further incubated at 37 °C for 4 h. The cell viability is calculated from the following equation [36]:

$$\%\text{Cell Viability} = \frac{\text{Experimental OD}}{\text{Control OD}} \times 100 \quad (1)$$

Where experimental OD (OD: Optical density) is obtained by measuring the absorption of formazan solution in DMSO using microplate reader at 570 nm.

RESULTS AND DISCUSSION

Antibacterial evaluation

Nanoscience and nanotechnology by employing biomaterials as novel biocidal agents have shown promising potential for preventing bacterial colonization. Antibacterial activity of compounds has important applications in biomedicine and pharmacology area. Therefore, the CD.Click and CD.COM in vitro antibacterial operations were assessed against gram positive: B. subtilis and S. aureus, and gram negative: P. aeruginosa and E. coli bacteria. The images are shown in Figure 6, and Table 1 tabulates the inhibition of bacteria compared to conventional chloramphenicol and gentamicin antibiotics as controls. The synthesized compounds showed a wide-range of activity against gram positive as well as gram negative bacteria. E. coli's growing (the most prevalent intestinal contaminant) is more inhibited than S. aureus. The crucial process for disinfection is the destruction of exterior wall and bacteria membrane [37]. Microorganisms have different outer membrane [38], for example, the cell wall of E. coli is thinner than S. aureus which can be easily distorted [39]. It is worth mentioning that the antibacterial efficacy measured as region of bacterial growth inhibition halos revealed comparable activity between CD.Click and CD.COM. The antibacterial activity of CD.Click is likely related to the presence of cyclodextrin [40,41] and triazole rings [42], while in the CD.COM, the antibacterial effect depends on TiO_2 NPs [43] along with cyclodextrin and triazole rings participation. Although the composition of CD.Click in the CD.COM structure is ~75% (CD.Click: TiO_2/AS = 3:1), antibacterial activity has relatively increased which could be attributed to the antimicrobial properties of TiO_2 NPs.

Zhang and coauthors established that CDs can disrupt cell membranes and lyse the bacterial cells. One of the known physiological properties of CDs is their high binding affinity to form an inclusion complex with cholesterol molecules. Although the cell membrane of prokaryotes and associated strains do not contain cholesterol or cholesterol-like molecules, but genome sequencing of strains has many genes for flotillin-like proteins. It is supposed that the flotillin-like proteins form a complex with unknown lipid molecules in the cell membranes, and this complex plays a significant role in the building and stabilization of their structures. This suggests that strains possessing the flotillin-like proteins are affected by CDs via trapping the unknown lipid molecules [40].

The toxicity of TiO_2 NPs towards bacteria has been not only confirmed photochemically but also by a different mechanism in the dark [44]. NPs with positive or neutral zeta potentials carry the positive charges while the microorganisms carry negative charges; this causes electrostatic attraction between microorganisms and NPs leading to physical attachment and contact with cell [45]. NPs could create holes in the bacterial cell wall that associated with increased permeability, releasing the cell component and eventually cell death [46]. Therefore, TiO_2 NPs with high level of interaction with the bacterial cells surface due to their small size and high surface to volume ratio, perform good antibacterial activity [47].

The triazole rings with metabolic degradation stability and capability to hydrogen bond formation could favor binding to biomolecular targets and improve solubility [18]. The antibacterial activity of triazole rings with these favorable properties is related to inhibit the synthesis of cell membrane, cell wall and nucleic acids of bacteria [48].

According to literature review [49,50], it is assumed that in the CD.COM structure, active sites of –OH and –NH capture bacteria, TiO_2 NPs create cavities in the bacteria cell and cause permeability and disruption of bacterial cell walls, as well as CD disrupt and lyse the bacterial cell walls. The triazole ring moieties also degrade the cell membrane, cell wall and DNA, and finally cause the cell death. Thus the ability of CD.COM to bacterial inactivation was attributed to synergy between the cyclodextrin, TiO_2 NPs and biocidal triazole rings.

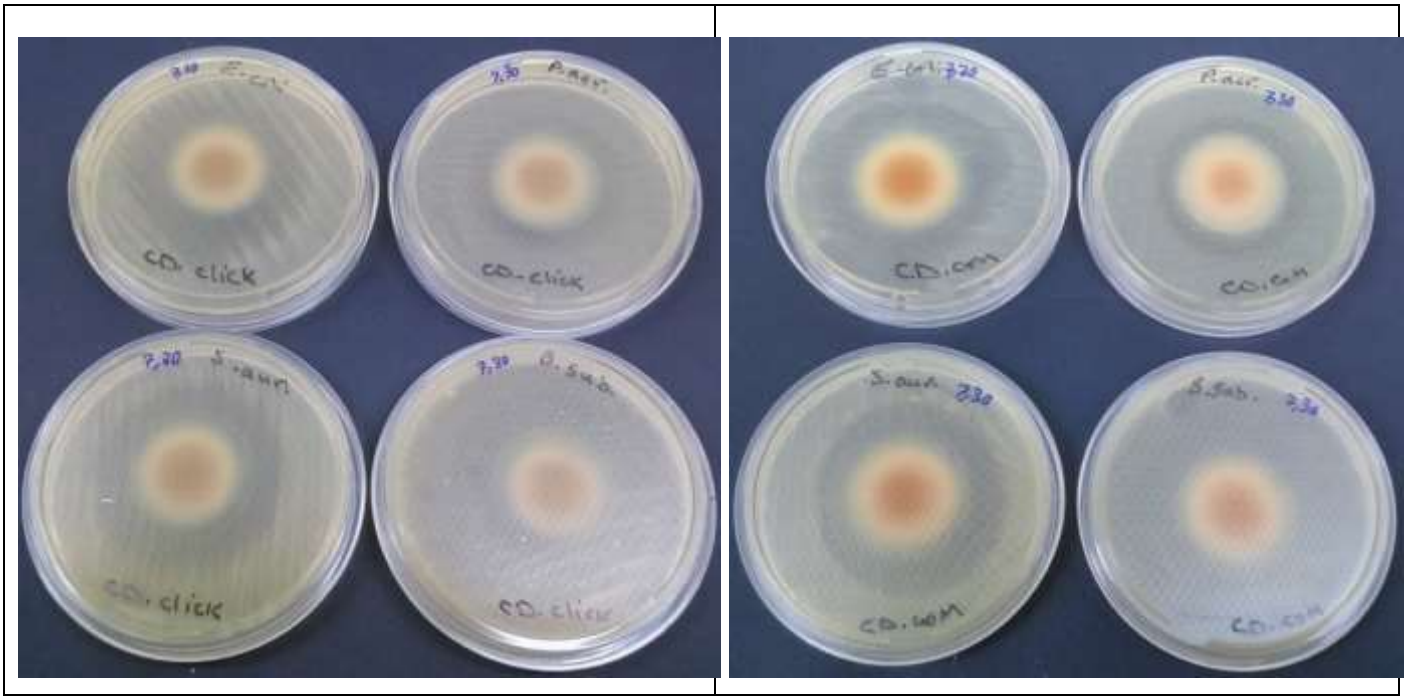


Figure 6. The images of antibacterial test.

Table 1. Antibacterial activity of the compounds using Kirby-Bauer test

Test Strain	Zone of growth inhibition (mm)			
	CD.COM[1]	CD.Click[1]	Gentamicin (10 µg/disc)	Chloramphenicol (30 µg/disc)
E. coli	48.5±1.3	45.0±1.4	19.6±1.1	20.7±1.5
P. aeruginosa	39.8±1.4	37.0±1.4	15.6±0.5	NE*
S. aureus	45.5±0.7	44.5±0.6	20.3±1.5	21.7±0.6
B. subtilis	50.5±0.7	49.5±0.9	26.0±1.7	22.3±1.2

[1] Film diameter: 13 mm & Film weight: 100 mg
* No Effect

Li and coauthors reported the inhibition zone of cellulose-TiO_2 hybrid (disc shape with 1.4 cm diameter) against S. aureus and E. coli bacteria that were 5.5 mm and 2.5 mm, respectively [51]. Tan and coauthors investigated the antibacterial effect of 1,2,3-triazole containing starch derivatives against E. coli and S. aureus by disc diffusion method [52]. All the triazole containing starch compounds exhibited a moderate antibacterial activity, and 6-carboxyltriazole-6-deoxy starch (CBTST) displayed the highest inhibition zone for E. coli = 10.27 ± 0.28 mm and S. aureus = 10.76 ± 0.44 mm. Antibacterial activity of cyclodextrins against *Bacillus* strains was reported by Zhang and coauthors using a diffusion method with filter paper disks (diameter 6 mm). The obtained results established the antibacterial effect of methyl-*β*-CD [40].

Studies conducted by our research group have demonstrated that the CD.COM showed higher antibacterial activity in comparison with cellulose-triazole-titanium based nanocomposite (Cell.Com; E. coli = 27.0 ± 1.4 mm, P. aeruginosa = 20.0 ± 1.4 mm, S. aureus = 26.0 ± 0.7 mm and B. subtilis = 21.5 ± 0.7 mm) [17]. This result is related to this fact that pure microcrystalline cellulose has not antibacterial activity [51], in spite of cyclodextrin. Comparing the results of our research work with those reported in the literature indicates that the synergistic effects of TiO_2 NPs, triazole ring and cyclodextrin have improved the CD.COM antibacterial activity.

Cytotoxicity evaluation

Considerable attention has been given on the synthesis of novel compounds with capability of biocompatibility with normal cells and killing cancerous cells. So, the cytotoxic effect of CD.COM was screened in vitro on cancerous cell lines (Hela, MCF-7 and Saos) and fibroblast cells by MTT assay. As can be seen in Figure 7, the CD.COM has no cytotoxic effect on fibroblast cells at concentration range of 7.8-500 mg L^{-1} over period of 24 h, while its toxicity is significant on the MCF-7 and Saos cancerous cell lines in concentrations above 15.6 mg L^{-1}. This toxicity is observed at a concentration dependent way in all three cancerous cell lines over a period of 48 h, while no significant toxicity is observed on fibroblast cells. These results show the promising properties of CD.COM against cancer cells and low toxicity to normal cells. The cytotoxic effect of CD.COM is mostly related to the triazole rings, because the 1,2,3-triazole moiety was reported to possess anticancer activity [53].

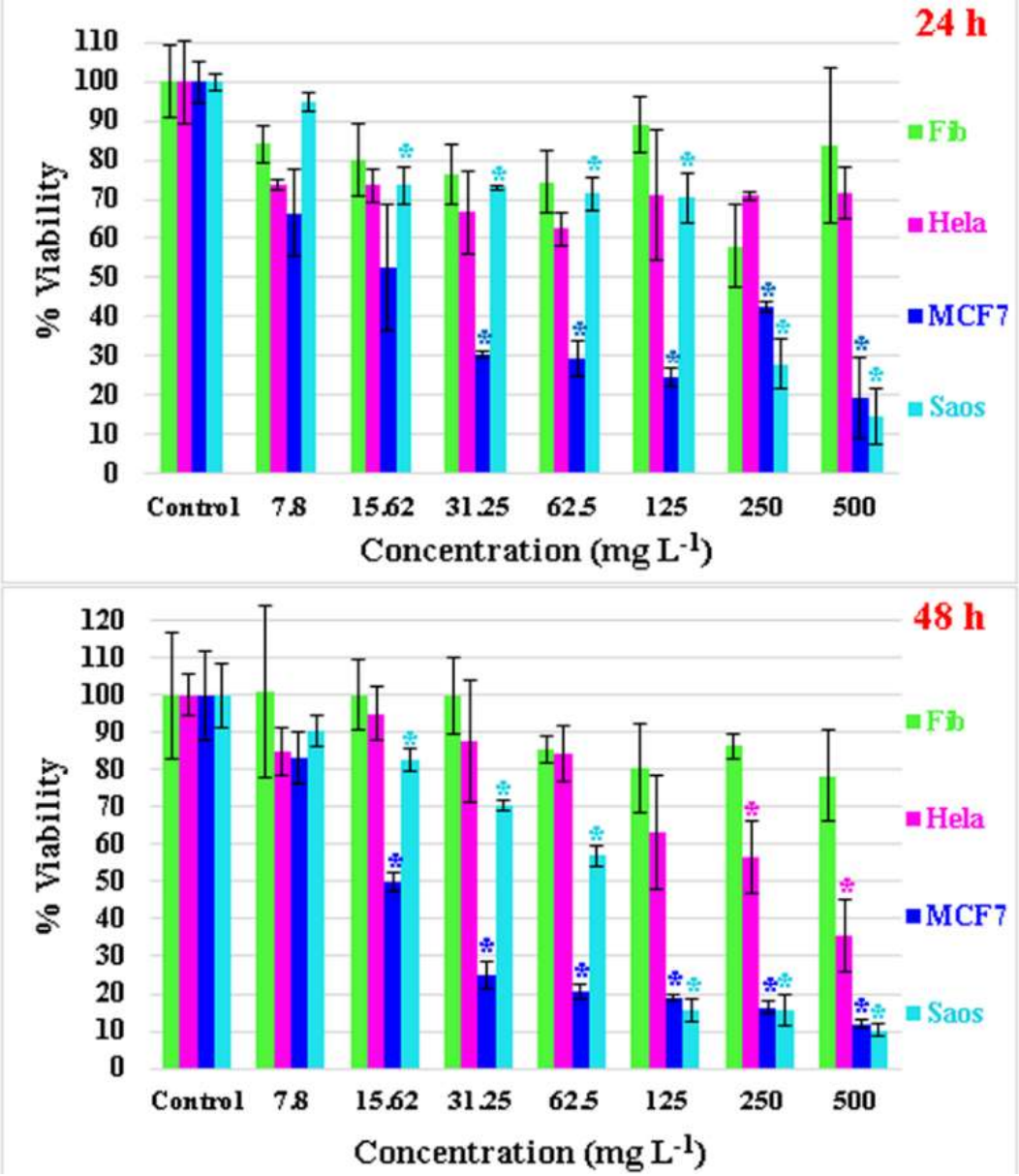


Figure 7. The cell viability of studied cell lines after exposure to increasing concentrations of the CD.COM.

CONCLUSIONS

In this manuscript, we investigated the antibacterial activity and cytotoxic effect of new triazole functionalized cyclodextrin (CD.Click) and cyclodextrin-triazole-titanium based nanocomposite (CD.COM). The antibacterial effects of CD.Click and CD.COM were explored against gram negative and gram positive bacteria. The antibacterial assay showed that the CD.Click due to the cyclodextrin and triazole rings, and the CD.COM because of the synergistic effects of cyclodextrin, TiO_2 NPs and triazole rings exhibited a broad variety of activity against both gram positive and negative bacteria. In addition, MTT assay assessed in vitro

the cytotoxic effect of CD.COM on cancer cell lines of HeLa, MCF-7, Saos and fibroblast. The CD.COM did not show toxicity in fibroblast cells at a range of 7.8 to 500 mg L^{-1} at 24 h, whereas at concentrations higher than 15 mg L^{-1}, it exhibited significant cytotoxicity against cancer cell lines of Saos and MCF-7. The toxicity was observed as concentration-dependent in all three cancer cell lines after 48 h, but, no significant change was detected in fibroblasts. These findings verify the promising properties of CD.COM against cancer cells and low toxicity to normal cells.

Acknowledgments: This research was partially supported by University of Mazandaran and research council of Shahrood University of Technology.

REFERENCES

1. Fallah Z, Nasr Isfahani H, Tajbakhsh M, Tashakkorian H and Amouei A. TiO_2-grafted cellulose via click reaction: an efficient heavy metal ions bioadsorbent from aqueous solutions. Cellulose. 2018;25(1):639-60. DOI:10.1007/s10570-017-1563-8
2. Seyednejhad S, Khalilzadeh MA, Zareyee D, Sadeghifar H and Venditti R. Cellulose nanocrystal supported palladium as a novel recyclable catalyst for Ullmann coupling reactions. Cellulose. 2019;26(8):5015-31. DOI:10.1007/s10570-019-02436-7
3. Fallah Z, Nasr Isfahani H and Tajbakhsh M. Removal of fluoride ion from aqueous solutions by titania-grafted β-cyclodextrin nanocomposite. Environ Sci Pollut Res. 2020;27:3281-94. DOI:10.1007/s11356-019-06948-4
4. Fallah Z, Nasr Isfahani H, Tajbakhsh M. Cyclodextrin-triazole-titanium based nanocomposite: Preparation, characterization and adsorption behavior investigation. Process Saf Environ Prot. 2019.124:251-65. DOI:10.1016/j.psep.2019.02.016
5. Amirsoleimani M, Khalilzadeh MA, Sadeghifar F and Sadeghifar H. Surface modification of nanostarch using nano silver: a potential antibacterial for food package coating. J Food Sci Technol. 2018;55(3):899-904. DOI:10.1007/s13197-017-2996-7
6. Chen Y, Liu Y. Cyclodextrin-based bioactive supramolecular assemblies. Chem Soc Rev. 2010;39(2):495-505. DOI:10.1039/B816354P
7. Li R, Dou J, Jiang Q, Li J, Xie Z, Liang J and et al. Preparation and antimicrobial activity of β-cyclodextrin derivative copolymers/cellulose acetate nanofibers. Chem Eng J. 2014;248:264-72. DOI:10.1016/j.cej.2014.03.042
8. Irie T and Uekama K. Pharmaceutical applications of cyclodextrins. III. Toxicological issues and safety evaluation. J Pharm Sci. 1997;86(2):147-62. DOI:10.1021/js960213f
9. Kilsdonk EP, Yancey PG, Stoudt GW, Bangerter FW, Johnson WJ, Phillips MC and et al. Cellular cholesterol efflux mediated by cyclodextrins. J Biol Chem. 1995;270(29):17250-6. DOI:10.1074/jbc.270.29.17250
10. Fu Y, Wang X, Zhang Y, Liu Z and Xue W. Effect of cyclodextrins on the structure and functions of blood components in vitro. J Bioact Compat Polym. 2015;30(5):541-54. DOI:10.1177/0883911515585184
11. Liu Y, Liu Y, Han K, Cai Y, Ma M, Tong Q and et al. Effect of nano-TiO_2 on the physical, mechanical and optical properties of pullulan film. Carbohydr Polym. 2019;218:95-102. DOI:10.1016/j.carbpol.2019.04.073
12. Ahmed DS, Mohammed MKA and Mohammad MR. Sol–gel synthesis of Ag-doped titania-coated carbon nanotubes and study their biomedical applications. Chem Pap. 2020;74:197-208. DOI:10.1007/s11696-019-00869-9
13. Ma S, Zhan S, Jia Y and Zhou Q. Superior antibacterial activity of Fe_3O_4-TiO_2 nanosheets under solar light. ACS Appl Mater Interfaces. 2015;7(39):21875-83. DOI:10.1021/acsami.5b06264
14. Lam SJ, Wong EHH, Boyer C and Qiao GG. Antimicrobial polymeric nanoparticles. Prog Polym Sci. 2018;76:40-64. DOI:10.1016/j.progpolymsci.2017.07.007
15. El-Sayed NS, El-Sakhawy M, Brun N, Hesemann P and Kamel S. New approach for immobilization of 3-aminopropyltrimethoxysilane and TiO_2 nanoparticles into cellulose for BJ1 skin cells proliferation. Carbohydr Polym. 2018;199:193-204. DOI:10.1016/j.carbpol.2018.07.004
16. Martina K, Baricco F, Berlier G, Caporaso M and Cravotto G. Efficient green protocols for preparation of highly functionalized β-cyclodextrin-grafted silica. ACS Sustainable Chem Eng. 2014;2(11):2595-603. DOI:10.1021/sc500546e
17. Fallah Z, Nasr Isfahani H, Tajbakhsh M, Mohseni M, Tashakkorian H, Amouei A and et al. Antibacterial and cytotoxicity effects of TiO_2-grafted cellulose nanocomposite. J Appl Chem Res. 2019;13(3):87-96.
18. Vatmurge NS, Hazra BG, Pore VS, Shirazi F, Chavan PS and Deshpande MV. Synthesis and antimicrobial activity of β-lactam–bile acid conjugates linked via triazole. Bioorganic Med Chem Lett. 2008;18(6):2043-7. DOI:10.1016/j.bmcl.2008.01.102

19. Kumbhare RM, Dadmal TL, Pamanji R, Kosurkar UB, Velatooru LR, Appalanaidu K and et al. Synthesis of novel fluoro 1,2,3-triazole tagged amino bis(benzothiazole) derivatives, their antimicrobial and anticancer activity. Med Chem Res. 2014;23(10):4404-13. DOI:10.1007/s00044-014-1006-0
20. Wang XL, Wan K and Zhou CH. Synthesis of novel sulfanilamide-derived 1,2,3-triazoles and their evaluation for antibacterial and antifungal activities. Eur J Med Chem. 2010;45(10):4631-9. DOI:10.1016/j.ejmech.2010.07.031
21. Marepu N, Yeturu S and Pal M. 1,2,3-Triazole fused with pyridine/pyrimidine as new template for antimicrobial agents: Regioselective synthesis and identification of potent N-heteroarenes. Bioorganic Med Chem Lett. 2018;28(20):3302-6. DOI:10.1016/j.bmcl.2018.09.021
22. Shafi S, Mahboob Alam M, Mulakayala N, Mulakayala C, Vanaja G, Kalle AM and et al. Synthesis of novel 2-mercapto benzothiazole and 1,2,3-triazole based bis-heterocycles: Their anti-inflammatory and anti-nociceptive activities. Eur J Med Chem. 2012;49:324-33. DOI:10.1016/j.ejmech.2012.01.032
23. He YW, Dong CZ, Zhao JY, Ma LL, Li YH and Aisa HA. 1,2,3-Triazole-containing derivatives of rupestonic acid: Click-chemical synthesis and antiviral activities against influenza viruses. Eur J Med Chem. 2014;76:245-55. DOI:10.1016/j.ejmech.2014.02.029
24. Rajabi M, Godugu K, Sudha T, Bharali DJ and Mousa SA. Triazole modified tetraiodothyroacetic acid conjugated to polyethylene glycol: High affinity thyrointegrin $\alpha_v\beta_3$ antagonist with potent anticancer activities in glioblastoma multiforme. Bioconjugate Chem. 2019;30(12):3087-97. DOI:10.1021/acs.bioconjchem.9b00742
25. Ghanaat J, Khalilzadeh MA, Zareyee D, Shokouhimehr M and Varma RS. Cell cycle inhibition, apoptosis, and molecular docking studies of the novel anticancer bioactive 1,2,4-triazole derivatives. Struct Chem. 2020;31(2):691-9. DOI:10.1007/s11224-019-01453-3
26. Ghanaat J, Khalilzadeh MA and Zareyee D. Molecular docking studies, biological evaluation and synthesis of novel 3-mercapto-1,2,4-triazole derivatives. Mol Divers. 2020. DOI:10.1007/s11030-020-10050-0
27. Tavakolinia F, Baghipour T, Hossaini Z, Zareyee D, Khalilzadeh MA and Rajabi M. Antiproliferative activity of novel thiopyran analogs on MCF-7 breast and HCT-15 colon cancer cells: Synthesis, cytotoxicity, cell cycle analysis, and DNA-binding. Nucleic Acid Ther. 2012;22(4):265-70. DOI:10.1089/nat.2012.0346
28. Rajabi M, Khalilzadeh MA and Mehrzad J. Antiproliferative activity of novel derivative of thiopyran on breast and colon cancer lines and DNA binding. DNA Cell Biol. 2012;31(1):128-34. DOI:10.1089/dna.2011.1291
29. Rajabi M, Khalilzadeh MA, Tavakolinia F, Signorelli P, Ghidoni R and Santaniello E. Naphthalene-fused (α-alkoxycarbonyl)methylene-γ-butyrolactones: Antiproliferative activity and binding to bovine serum albumin and DNA. DNA Cell Biol. 2012;31(5):783-9. DOI:10.1089/dna.2011.1433
30. Kuntala N, Telu JR, Banothu V, Nallapati SB, Anireddy JS and Pal S. Novel benzoxepine-1,2,3-triazole hybrids: synthesis and pharmacological evaluation as potential antibacterial and anticancer agents. Med Chem Comm. 2015;6(9):1612-9. DOI:10.1039/C5MD00224A
31. Neeraja P, Srinivas S, Banothu V, Sridhar B, Mukkanti K, Dubey PK and et al. Assembly of benzothiazine and triazole in a single molecular entity: Synthesis of -oxicam derived novel molecules as potential antibacterial/anti-cancer agents. Mini Rev Med Chem. 2020;20(10):929-40. DOI:10.2174/1389557520666200124091315
32. Rao VM, Rao AS, Kumar PR, Rani SS and Pal M. 2-Amino analogues of alkynyl substituted 1,4-dihydropyrimidine: Their synthesis and evaluation as potential antimicrobial/cytotoxic agents. Lett Drug Des Discov. 2018;15(10):1095-102. DOI:10.2174/1570180814666171121151921
33. Suryapeta S, Papigani N, Banothu V, Dubey PK, Mukkanti K and Pal S. Synthesis, biological evaluation, and docking study of a series of 1,4-disubstituted 1,2,3-triazole derivatives with an indole-triazole-peptide conjugate. J Heterocycl Chem. 2020. DOI:10.1002/jhet.4020
34. Xu J and Liu S. Synthesis of well-defined 7-arm and 21-arm poly(N-isopropylacrylamide) star polymers with β-cyclodextrin cores via click chemistry and their thermal phase transition behavior in aqueous solution. J Polym Sci part A: Pol Chem.2009;47(2):404-19. DOI:10.1002/pola.23157
35. Balouiri M, Sadiki M and Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016;6(2):71-9. DOI:10.1016/j.jpha.2015.11.005
36. Yi P, Wang Y, Zhang S, Zhan Y, Zhang Y, Sun Z and et al. Stimulative nanogels with enhanced thermosensitivity for therapeutic delivery via β-cyclodextrin-induced formation of inclusion complexes. Carbohydr Polym. 2017;166:219-27. DOI:10.1016/j.carbpol.2017.02.107
37. Aly MRES, Saad HA and Mohamed MAM. Click reaction based synthesis, antimicrobial, and cytotoxic activities of new 1,2,3-triazoles. Bioorganic Med Chem Lett. 2015;25(14):2824-30. DOI:10.1016/j.bmcl.2015.04.096
38. Abdolmaleki A, Mallakpour S, Mahmoudian M and Sabzalian MR. A new polyamide adjusted triazinyl-β-cyclodextrin side group embedded magnetic nanoparticles for bacterial capture. Chem Eng J. 2017; 309(Supplement C): 321-9. DOI:10.1016/j.cej.2016.10.063

39. San-Xiang T, Shao-Zao T, Jing-Xing C, Ying-Liang L and Ding-Sheng Y. Preparation and properties of antibacterial TiO_2@C/Ag core–shell composite. Sci Technol Adv Mater. 2009;10(4):045002. DOI:10.1088/1468-6996/10/4/045002
40. Zhang HM, Li Z, Uematsu K, Kobayashi T and Horikoshi K. Antibacterial activity of cyclodextrins against Bacillus strains. Arch Microbiol. 2008;190(5):605-9. DOI:10.1007/s00203-008-0415-1
41. Donova MV, Nikolayeva VM, Dovbnya DV, Gulevskaya SA and Suzina NE. Methyl-β-cyclodextrin alters growth, activity and cell envelope features of sterol-transforming mycobacteria. Microbiology. 2007;153(6):1981-92. DOI:10.1099/mic.0.2006/001636-0
42. Thirukovela NS, Kankala S, Kankala RK, Paidakula S, Gangula MR, Vasam CS and et al. Regioselective synthesis of some new 1,4-disubstituted sulfonyl-1,2,3-triazoles and their antibacterial activity studies. Med Chem Res. 2017;26(9):2190-5. DOI:10.1007/s00044-017-1926-6
43. Li B, Zhang Y, Yang Y, Qiu W, Wang X, Liu B and et al. Synthesis, characterization, and antibacterial activity of chitosan/TiO_2 nanocomposite against Xanthomonas oryzae pv. oryzae. Carbohydr Polym. 2016;152:825-31. DOI:10.1016/j.carbpol.2016.07.070
44. Simon-Deckers A, Loo S, Mayne-L'hermite M, Herlin-Boime N, Menguy N, Reynaud C and et al. Size-, composition- and shape-dependent toxicological impact of metal oxide nanoparticles and carbon nanotubes toward bacteria. Environ Sci Technol. 2009;43(21):8423-9. DOI:10.1021/es9016975
45. Anandgaonker P, Kulkarni G, Gaikwad S and Rajbhoj A. Synthesis of TiO_2 nanoparticles by electrochemical method and their antibacterial application. Arab J Chem. 2019;12(8):1815-22. DOI:10.1016/j.arabjc.2014.12.015
46. Jayaseelan C, Rahuman AA, Roopan SM, Kirthi AV, Venkatesan J, Kim SK and et al. Biological approach to synthesize TiO_2 nanoparticles using Aeromonas hydrophila and its antibacterial activity. Spectrochim Acta A: Mol Biomol Spectrosc. 2013;107:82-9. DOI:10.1016/j.saa.2012.12.083
47. Khan S, Ul-Islam M, Khattak WA, Ullah MW and Park JK. Bacterial cellulose-titanium dioxide nanocomposites: nanostructural characteristics, antibacterial mechanism, and biocompatibility. Cellulose. 2015;22(1):565-79. DOI:10.1007/s10570-014-0528-4
48. Mallemula VR, Sanghai NN, Himabindu V and Chakravarthy AK. Synthesis and characterization of antibacterial 2-(pyridin-3-yl)-1H-benzo[d]imidazoles and 2-(pyridin-3-yl)-3H-imidazo[4,5-b]pyridine derivatives. Res Chem Intermed. 2015;41(4):2125-38. DOI:10.1007/s11164-013-1335-5
49. Zhang L, Bai H, Liu L and Sun DD. Dimension induced intrinsic physio-electrical effects of nanostructured TiO_2 on its antibacterial properties. Chem Eng J. 2018;334:1309-15. DOI:10.1016/j.cej.2017.11.075
50. Kamal A, Shankaraiah N, Devaiah V, Laxma Reddy K, Juvekar A, Sen S and et al. Synthesis of 1,2,3-triazole-linked pyrrolobenzodiazepine conjugates employing 'click' chemistry: DNA-binding affinity and anticancer activity. Bioorganic Med Chem Lett. 2008;18(4):1468-73. DOI:10.1016/j.bmcl.2007.12.063
51. Li SM, Dong YY, Ma MG, Fu LH, Sun RC and Xu F. Hydrothermal synthesis, characterization, and bactericidal activities of hybrid from cellulose and TiO_2. Carbohydr Polym. 2013;96(1):15-20. DOI:10.1016/j.carbpol.2013.03.058
52. Tan W, Li Q, Wang H, Liu Y, Zhang J, Dong F and et al. Synthesis, characterization, and antibacterial property of novel starch derivatives with 1,2,3-triazole. Carbohydr Polym. 2016;142:1-7. DOI:10.1016/j.carbpol.2016.01.007
53. Yadav P, Lal K, Kumar A, Guru SK, Jaglan S and Bhushan S. Green synthesis and anticancer potential of chalcone linked-1,2,3-triazoles. Eur J Med Chem. 2017;126:944-53. DOI:10.1016/j.ejmech.2016.11.030