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Comparison between Antimicrobial Assays of Catechol-based Polyurethane Foam Polymer and Catechol-modified Polyurethane Foam Polymer with Zinc Oxide Nanoparticles

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Abstract

Nano polymers have attracted a lot of attention in developing new antimicrobial agents due to their significant advantages. Nanocomposite materials with antimicrobial activity have been widely used in food packaging. The current work introduces efficient nanocomposite; catechol polyurethane foam polymer modified with zinc oxide nanoparticles (PCPU/ZnO) and its application for antimicrobial activity. Catechol polyurethane foam/zinc oxide was synthesized by coupling zinc oxide nanoparticles (ZnONPs) and catechol polyurethane foam polymer (PCPU). The main modifications on the surface of pyrocatechol polyurethane foam/zinc oxide nanocomposite were discussed. The new composite was characterized by ultraviolet-visible spectra (UV-Vis), Fourier transform infrared spectra (FTIR), pH of zero-point charge (PHZPC) and acidic/basic sites. The antimicrobial activity of PCPU/ZnO was tested against G+ve bacterium (Bacillus cereus), Gram-ve bacterium (Escherichia coli) and fungus; A. niger. In vitro agar well-diffusion method showed good inhibition zones of PCPU/ZnO against Escherichia coli (27 ± 0.11 mm), Bacillus cereus (16 ± 0.08 mm) and Aspergillus niger (23 ± 0.14 mm). While PCPU showed a significant antimicrobial effects; with lower inhibition zones against Escherichia coli (20 ± 0.21 mm), Aspergillus niger (15 ± 0.06 mm) and higher inhibition zone against Bacillus cereus (23 ± 0.06 mm).

Keywords: catechol polyurethane foam; zinc oxide nanoparticles; Bacillus cereus; Escherichia coli; Aspergillus niger.

Introduction

Nanocomposites have gained a lot of attraction due to their significant benefits for example lightweight, flexibility, ease of preparation, low cost and eco-friendliness. Nanocomposite materials, which possess antimicrobial properties, have been widely used in packaging and coating applications of food (Shankar & Rhim, 2016). Nanoparticles (NPs) have an extreme surface area to volume ratio owing to their nano-size which permits increasing of surface contact, reactivity and solubility (Khan,

Saeed, & Khan, 2019). The microbicide properties of NPs arise from direct interaction with the microbial cell wall and do not require cell penetration (Fernando, Gunasekara, & Holton, 2018). Metal oxide NPs such as zinc oxide nanoparticles (ZnO NPs) have been used over a large scale for many industrial applications (Chavali & Nikolova, 2019).

Strong antibacterial activity has been found for ZnO NPs due to their distinctive features like biocompatibility and high selectivity (Erazo, Mosquera, & Rodríguez-Paéz, 2019; Leung et al., 2016; Reddy, Nisha, Joice, & Shilpa, 2014). ZnO NPs also enable the enhancement of packaging characteristics, including mechanical robustness, barrier qualities, and stability (Espitia, Otoni, & Soares, 2016). ZnO NPs can interact with bacteria or cells, generation reactive oxygen species (ROS) and eventually cell death (Bisht & Rayamajhi, 2016; Mousa, Moawad, Abdallah, Abdel-Rasheed, & Zaher, 2023).

The most significant group of both natural and manufactured chain-breaking antioxidants are likely polyphenols (Viglianisi, Menichetti, Morelli, Baschieri, & Amorati, 2018). Catechol and its derivatives have widely attracted scientists since decades due to their antioxidant and chelating properties (Faure et al., 2013). Catechol, also known as pyrocatechol (PC) is an isomeric benzene diols, with the molecular formula C_6H_4 $(OH)_2$. Catechol occurs along with the polyphenol oxidase enzyme. Additionally, catechol is frequently used to create antioxidants, hair colours, and food additives (Huang et al., 2014; Suresh, Srivastava, & Mishra, 2012).

Polyurethane foam (PUF) represents the main polymeric materials that are present in widespread use in the industrial and medical fields (Howard, 2002; Sienkiewicz & Członka, 2022; Yang, Yu, Song, Maluk, & Wang, 2019). PUF is considered food safe and mainly used for a variety of applications, which involve food processing, packaging and safe transport. PUF is also used as insulation materials for sound and cold and it can find it as part of refrigerators, freezers, food dryers, window frames and furniture (Qiao et al., 2017). Polyurethane foam with a high surface area and low coast should be resistant to viruses, bacteria and fungi (Sienkiewicz & Członka, 2022).

All over the world, illnesses of hospitalizations and even deaths have been occurred due to foodborne contaminations (Espitia et al., 2016). Foodborne illness is mainly caused by microbial infection such as viruses, bacteria, and fungi. Foodborne sickness signs and symptoms include fever, nausea, vomiting, diarrhoea, and stomach aches and cramps (Switaj, Winter, & Christensen, 2015; Vats & Nigam, 2022). Food contamination can occur with its exposure to the environment during processing, and packaging. Microorganisms have been managed or inhibited using antimicrobial agents. Reduce the danger of pathogen contamination and increase food shelf life with the use of antimicrobial packaging, a type of active packaging (Malhotra, Keshwani, & Kharkwal, 2015).

A variety of bioassays, including well diffusion and broth or agar dilution, are frequently employed to assess or test a compound's *in vitro* antimicrobial activity (Balouiri, Sadiki, & Ibnsouda, 2016).

Food pathogens are harmful and cause food poisoning, which upon consumption, results in several health complications. It can lead to abdominal cramping, diarrhea, fatigue, fever and vomiting (Ma et al., 2023). *Bacillus cereus*, *Escherichia coli* and *Aspergillus niger* are counted among food pathogens, that can cause serious infections. *Bacillus cereus* (*B. cereus*) is a spore-forming bacterium which widely spread in environments and produce enterotoxins that cause food poisoning (Krzepiłko, Matyszczuk, & Święciło, 2023; Na, Kim, Rhee, & Oh, 2018). *E.coli* is normally found in the environment and it cause severe diseases when transmitted to humans through consumption of contaminated foods (Ma et al., 2023; Na et al., 2018). *Aspergillus niger* (*A. niger*) is a fungus, which produce toxin such as mycotoxins and aflatoxins only under certain conditions. Moreover it can produce spores that result in a serious lung infection called aspergillosis which badly affects the respiratory system (Navale, Vamkudoth, Ajmera, & Dhuri, 2021; Sheikh-Ali et al., 2014).

The present study demonstrates the synthesis of catechol polyurethane foam modified with ZnO NPs (PCPU/ZnO). The antimicrobial activity of catechol polyurethane foam (PCPU) and catechol polyurethane foam/ZnONPs (PCPU/ZnO) was tested against *E. coli*, *B. cereus* and *A. niger* as a model for bacterial and fungal infections.

Materials and Methods

Materials and Reagents

PCPU/ZnO: Commercial flexible PUF sheets (d = 12 kg/m3) were purchased from Foamex Company for foam production, New Damietta, Egypt.

Reference microbial strains: American Type Culture Collection (ATCC) bacterial strains including *E. coli* (ATCC25922), *B. cereus* (ATCC9634) and fungal strain; *A. niger* (van Tieghem 1867) were gained from the Microbiology laboratory, Faculty of Science, Damietta University. The microbial strains were sub-cultured on nutrient agar, nutrient broth and Dox agar (Oxoid, UK). Penicillin (standard antibacterial) and Fluconazole (standard antifungal) were purchased from Pfizer Co., Ltd. Dimethyl formamide (DMF) was purchased from Sigma, USA.

Apparatus: A KBr disc was used to analyze the Fourier transform infrared spectra (FTIR) using a JASCO FTIR-410 spectrometer (Germany). A JASCO UV/VIS Spectrometer V-630 (Japan) was used for all UV/Vis absorbance measurements.

Methods

Preparation and characterization of TPU/ZnO

ZnO NPs were prepared by reduction of $ZnSO_4.7H_2O$ solution using ammonium bicarbonate ($NaHCO_3$) in the presence of ammonia (NH_4OH) solution at 60°C for 30 minutes. The formed colloid was filtered, washed, dried at 100 °C and calcined at 400 °C for 2 h (Cao, Zhang, Wang, & Wang, 2009). PUF cubes were boiled in HCl (1 mol/L) for 3 h and washed with distilled water. Then PUF were rinsed in HCl (0.1 mol/L) for 30 minutes in ice bath and $NaNO_2$ solution were added drop wise with stirring to form the diazonium salt. A mix of 1M pyrocatechol/1M NaOH were added drop wise with stirring to form catechol polyurethane foam. PCPU were soaked overnight, then washed and dried in the next day. PCPU was refluxed with ZnO NPs in 200 mL ethanol at 60 °C for 2 h. The PCPU/ZnO was washed, dried overnight and blended.

FTIR spectroscopy of PCPU and PCPU/ZnO were measured in the range of 400 to 4000 cm^{-1}. UV/Vis spectra of PCPU and PCPU/ZnO were studied in the range of 200–900 nm .The bandgap of PCPU and PCPU/ZnO was determined from UV/Vis measurements. The total acidic and basic groups of PCPU and PCPU/ZnO were determined by Boehm's titration. The surface charge at different pH and pH at zero charge point (pH_{PZC}) of PCPU and PCPU/ZnO were evaluated over initial pH range of 2-14. Magnetic susceptibility of PCPU and PCPU/ZnO were estimated. The chemical stability of of PCPU and PCPU/ZnO were investigated in different buffer solutions and organic solvents.

Antimicrobial activity evaluation

The Antimicrobial activity of PCPU and PCPU/ZnO was studied against G+ve *B. cereus*, G-ve *E. coli* and fungus; *A. niger* using agar well-diffusion method. Clinical and Laboratory Standards Institute recommendations were followed for agar well-diffusion (Balouiri et al., 2016). The culture media (Nutrient agar and Dox agar) were prepared, sterilized (121°C, 15 min) and coaled at 47°C. Microbial cultures were adjusted to 0.5 McFarland standards ($1–2\times10^8$ CFU/mL). Then 100 µl of each microbial culture was inoculated specifically into the agar media. After that, triplicates of the inoculated agar media were added to sterile Petri dishes. After solidification, a sterile corkborer was used to make small (5 mm) wells. A 300 µg/mL of PCPU, PCPU/ZnO, Penicillin (antibacterial) and Fluconazole (antifungal) in DMF were added separately into the wells. Dox agar plates were incubated at 30 °C for 5 days whereas inoculated nutrient agar plates were incubated at 37 °C for 24 hours. Zones of inhibition were measured in millimeters (mm) following the incubation period. Standard deviation (SD) and the mean are used to present data. Inhibition zones of PCPU and PCPU/ZnO were evaluated.

Results

Characterization of PCPU/ZnO

FTIR spectroscopy identified PCPU and PCPU/ZnO specific functional groups (Figure 1). The characteristic peaks of PCPU were detected at 3771-3697, 3695-3075, 2934, 2870, 2346, 1690, 1627, 1506, 1386, 1201 and 1059 cm^{-1}. Sharp band of phenolic –OH was

observed at 3771-3697 cm^{-1} and broadband of carboxylic –OH and –NH was observed at 3695-3075 cm^{-1}. Several peaks were observed at 2934, 2870 (–CH aliphatic and aromatic), 2346 (O=C=O), 1690 (carboxylic C=O), 1627 (conjugated C=C), 1506 (N–O), 1386 (C–H bending), 1201 and 1059 (C–N) cm^{-1}. Zn-O's distinctive peaks, which were seen at 418 cm^{-1}, are the result of the coupling of PCPU and ZnONPs.

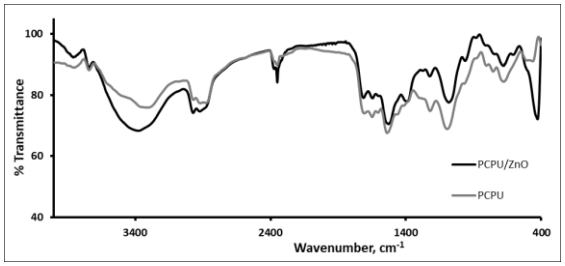

Figure 2. IR spectra of PCPU/ZnO and PCPU

The bandgap energy (E_g) of PCPU and PCPU/ZnO was determined from UV-Vis measurements using Tauc equation; $(\alpha h\upsilon)^2 = C\,(Eg - h\upsilon)$. Where C is a constant, α is the absorption coefficient ($\alpha = 2.303\ A/t$), A is the absorbance and t is the thickness. The energy hυ (in eV) were calculated ($h\upsilon = 1240/\lambda$), where λ is the wavelength in nm. The $(\alpha h\upsilon)^2$ were plotted against hυ and the energy gap can be approximated from the straight portion of the hυ axis at hυ = 0. The bandgap energy of PCPU and PCPU/ZnO was estimated at 2.98 and 2.92 eV (Figure 2). Lower energy gap of PCPU/ZnO than PCPU refers to an increase in particle size (Jacobsson, 2010), confirming the coupling between PCPU and ZnO NPs.

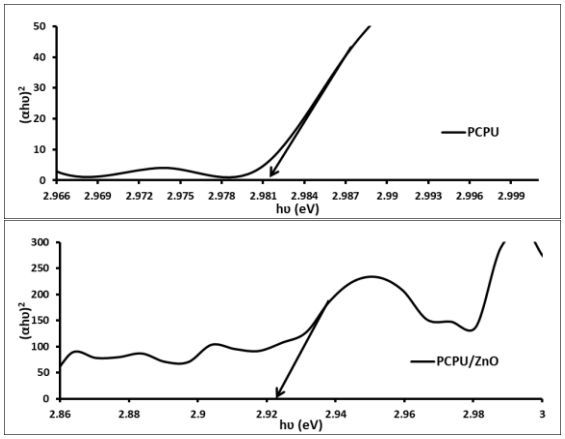

Figure 2. Bandgap energy of PCPU/ZnO and PCPU

The pH of zero point charge (pH_{PZC}) helps in predicting the surface behaviour of sorbent (Boumediene, Benaïssa, George, Molina, & Merlin, 2018). The surface charge at different pH and pH at zero charge point (pHPZC) of PCPU and PCPU/ZnO were evaluated over initial pH range of 2-14. The differences between the initial and final pH values ($\Delta pH = pH_f - pH_i$) were plotted against the initial pH. The pH_{PZC} of PCPU and PCPU/ZnO was found 5.4.and 5.6 (Figure 3). The surface of PCPU or PCPU/ZnO will be positively charged at pH< pH_{PZC} and negatively charged at pH > pH_{PZC}.

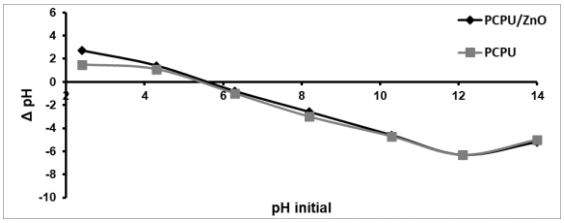

Figure 3. pH_{PZC} of PCPU/ZnO and PCPU

The total acidic and basic groups of PCPU and PCPU/ZnO were determined by Boehm's titration using $NaHCO_3$, Na_2CO_3, NaOH and HCl (0.05 M). The acidic sites of PCPU and PCPU/ZnO were 61 and 113 mmol/g while basic sites were negligible. The surface charge of PCPU/ZnO was mainly more acidic than PCPU.

Magnetic susceptibility of PCPU and PCPU/ZnO were calculated from the readings of Evans balance according to the following equation; $\chi_g = CL\,(R - R_0)/10^9(M - M_0)$. Where: C is the calibration constant of the balance (1.35 cm), L is the sample height in cm, R is the balance reading for the sample in a tube, R_o is the balance reading for the empty tube, M is the mass of sample in gm and M_0 is the mass of sample in gm. The magnetic susceptibility PCPU and PCPU/ZnO were -1.14×10^{-6} and -0.31×10^{-6} cm^3/mol, which is small and negative. PCPU and PCPU/ZnO were a diamagnetic material (Table 1).

Table 1. Magnetic susceptibility of PCPU and PCPU/ZnO

Sorbent	C	L	R_s	R_0	M_s	M_0	χ_g
PCPU	1.35	2	-31	-28	0.757	0.7499	-1.14×10^{-6}
PCPU/ZnO	1.35	1.5	-33	-28	0.782	0.7498	-0.31×10^{-6}

In diverse buffer solutions (pH: 2-14) and various organic solvents (such as CH_3OH, CH_3COCH_3, C_6H_6, $C_6H_5CH_3$, DMF, and DMSO), the chemical stability of PCPU and PCPU/ZnO was evaluated. The fact that the weights of PCPU and PCPU/ZnO were

unaffected by the investigated solutions and solvents is evidence of their chemical stability.

Antimicrobial activity evaluation

The antimicrobial potential of PCPU and PCPU/ZnO against G+ve *B. cereus*, G-ve *E. coli*, and *A. niger*, was examined using the agar well-diffusion method. PCPU and PCPU/ZnO inhibited *B. cereus*, *E. coli*, and *A. niger* and zones of inhibition (ZOI) were calculated (Figure 4 and Table 2).

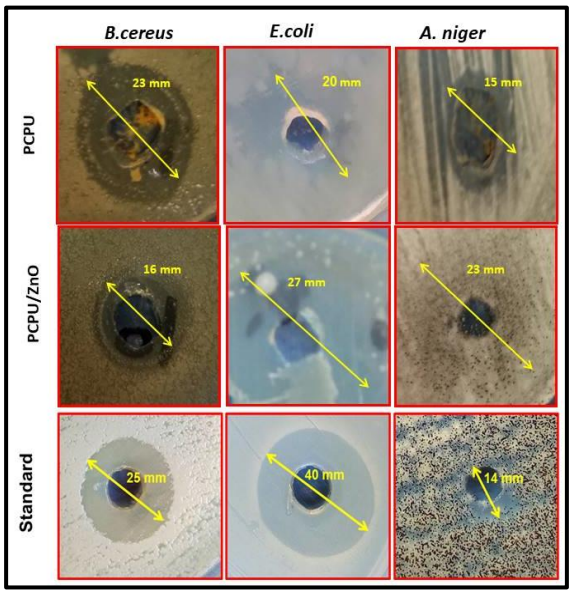

Figure 4. Antimicrobial activity of PCPU and PCPU/ZnO in comparison with Penicillin (standard antibacterial) and Fluconazole (standard antifungal) using agar well diffusion method against *B. cereus*, *E. coli*, and *A. niger*. Arrows denote the diameter of inhibition zones (mm).

Table 2. Zones of inhibition (ZOI) of PCPU and PCPU/ZnO in comparison with Penicillin (standard antibacterial) and Fluconazole (standard antifungal)

Compound	**Zones of inhibition (mm ± SE)**		
	E. coli	***B. cereus***	***A. niger***
PCPU	20 ± 0.21	23 ± 0.06	15 ± 0.06
PCPU/ZnO	27 ± 0.11	16 ± 0.08	23 ± 0.14
Penicillin	40 ± 0	25 ± 0.03	-
Fluconazole	-	-	14 ± 0.14

Discussion

The current study provided a promising method for the fabrication of NPs combined with polymers using simple fast approach. PCPU/ZnO showed a higher antibacterial action against Gram-negative *E. coli* than PCPU with inhibition zones of 27 ± 0.11 and 20 ± 0.21 mm, respectively. While PCPU showed a higher antibacterial action against Gram-positive *B. cereus* than PCPU/ZnO with inhibition zones of 23 ± 0.06 and 20 ± 0.21 mm, respectively. Penicillin showed a stronger antibacterial effect; with inhibition zones of 40 ± 0 mm against *E. coli* and 25 ± 0.03 mm against *B. cereus*. Also, PCPU/ZnO displayed a higher antifungal action against *A. niger* (23 ± 0.14 mm) than PCPU (15 ± 0.06 mm). Fluconazole showed a lower antifungal effect; with inhibition zone of 14 ± 0.14 mm against *A. niger*.

The mechanism of the antimicrobial activity of PCPU and PCPU/ZnO was evaluated in terms of the surface charging behaviour. Bacterial cell walls have a negative charge due to the presence of acidic compounds such as peptidoglycan. Gram-positive bacteria (*B. cereus*) contain thicker layers from peptidoglycan than Gram-negative bacteria (*E. coli*) (Schleifer & Kandler, 1972).

The surface charge of PCPU/ZnO and PCPU was mainly acidic as the characterization results were approved. PCPU/ZnO and PCPU negative charge might produce a repulsion force between it and the bacterial cells which might decrease its antibacterial effect compared to the standard antibacterial agent, Penicillin. Gram-positive bacteria (high peptidoglycan content) had a greater repulsion force than Gram-negative bacteria, which may reduce their antibacterial activity (Isticato & Ricca, 2016).

Also, the surface charge of PCPU/ZnO was mainly more acidic than PCPU as the characterization results were approved. In case of *B. cereus* (high negative charge of peptidoglycan content), the antibacterial effect of PCPU (lower negative charge) was higher than PCPU/ZnO (higher negative charge) due to lower repulsion forces. The main mode of action against G+ve bacteria is cell wall breakdown. G+ve bacteria have greater peptidoglycan coatings, which results in higher -ve charge values in their cell walls. The development of oxidative stress in microbial cells is one of the most commonly cited processes of cell wall disruption. Reactive oxygen species (ROS) can be produced inside of cells, causing stress in microbial cells, damaging cellular structures, as well as the oxidative breakdown of proteins and lipids, which can result in cell death (Metryka, Wasilkowski, & Mrozik, 2021).

While in case of *E. coli* (low negative

charge of peptidoglycan content), the antibacterial effect of PCPU/ZnO (higher negative charge) was higher than PCPU (lower negative charge). The principal mechanism of action for G-ve bacteria is a change in membrane permeability. The *E. coli* cells' low-density area in the cytoplasm shows that the cytoplasmic membrane's permeability can be altered, and Zn^{2+} ions can enter the cytoplasm to disrupt cellular activity. The permeability of the cell membrane could be harmed by the interaction between ZnO NPs and the bacterial cell wall (Huang, Bao, Liu, Wang, & Hu, 2017).

Due to the numerous phenolic hydroxyl groups included in polyphenols, including catechol, which can harm bacterial cell walls and denature bacterial proteins, these substances have antibacterial capabilities. ROS, which are extremely reactive molecules and free radicals formed from molecule oxygen, are produced in different ways by catechol. ROS may be used in antimicrobial applications since it can kill cells by attacking and damaging proteins, lipids, and DNA (Razaviamri, Wang, Liu, & Lee, 2021). Furthermore, catechol can function the ZnO NPs on the surface of the catechol-containing polymer by reducing soluble metal ions, such as Zn (Huang et al., 2017).

The microbicidal activity of ZnO NPs can be explained in terms of the generation of ROS (Shi et al., 2014; Venkatasubbu, Baskar, Anusuya, Seshan, & Chelliah, 2016). Strongly oxidizing ZnO acquires this property, and oxidizing sites are developed as the channels for microbicidal activity. The production of ROS can cause oxidative stress in microbial cells, which inhibits DNA replication and protein synthesis. By releasing Zn^{2+}, which inhibits the glycolytic enzyme by oxidizing the thiol group due to its special affinity for the sulfur group, ZnO can also harm the cytoplasmic membrane (Mendes et al., 2022).

Conclusion

Catechol polyurethane foam (PCPU/ZnO) was synthesized by immobilization of ZnONPs within catechol polyurethane foam. PCPU/ZnO was characterized by IR, UV/Vis, bandgap energy, magnetic susceptibility, chemical stability and pH_{PZC}. *In vitro* antimicrobial activity PCPU/ZnO and PCPU was studied against *E. coli*, *B. cereus* and *A. niger*. Successfully inhibiting the growth of tested microbial strains can be proven PCPU/ZnO as an effective antimicrobial agent.

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الملخص العربي

عنوان البحث: مقارنة بين القدرة المضادة للميكروبات لبوليمر البولي يوريثان القائم على الكاتيكول وبوليمر البولي يوريثان القائم على الكاتيكول مع جسيمات أكسيد الزنك النانومترية

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لقد حازت البوليمرات النانومترية على الكثير من الاهتمام في تطويرها كعوامل واعدة مضادة للميكروبات وذلك بسبب مزاياها العديدة. هذا وقد تم استخدام المواد المركبة النانومترية ذات النشاط المضاد للميكروبات على نطاق واسع في تغليف المواد الغذائية. تقدم الدراسة الحالية طريقة تصنيع بوليمر البولي يوريثان القائم على الكاتيكول والمدعم بجسيمات أكسيد الزنك النانومترية كمركب نانومتري فعال بالإضافة الى اختبار قدرته في النشاط المضاد للميكروبات. تم تصنيع البولي يوريثان الكاتيكول / أكسيد الزنك عن طريق اقتران الجسيمات النانومترية لأكسيد الزنك وبوليمر البولي يوريثان الكاتيكول. تم توصيف المركب الجديد بواسطة طيف الأشعة فوق البنفسجية والمرئية، طيف الأشعة تحت الحمراء، درجة الحموضة لشحنة نقطة الصفر والمواقع الحمضية/القاعدية. تم اختبار النشاط المضاد للميكروبات للبوليمر المصنع ضد البكتيريا الموجبة لصبغة جرام باسيلس سيريس، البكتيريا السالبة لصبغة جرام ايشريشيا كولاي فطر أسبرجيلس نيجر. أظهر اختبار انتشار المواد خلال الأجار معمليا مناطق تثبيط جيدة نتيجة تطبيق بوليمر البولي يوريثان الكاتيكول / أكسيد الزنك ضد ايشريشيا كولاي (٢٧ ± ٠,١١ مم)، باسيلس سيريس (١٦ ± ٠,٠٨ مم) و أسبرجيلس نيجر (٢٣ ± ٠,١٤ مم). في حين أظهر بوليمر البولي يوريثان الكاتيكول تأثيرات مضادة للميكروبات ممثلا في مناطق تثبيط أقل من البوليمر المدعم بجسيمات النانو ضد ايشريشيا كولاي (٢٠ ± ٠,٢١ مم) وأسبرجيلس نيجر (١٥ ± ٠,٠٦ مم) مقارنة بمنطقة تثبيط أعلى ضد باسيلس سيريس (٢٣ ± ٠,٠٦ مم).