

PREPARATION, CHARACTERIZATION AND ANTIBACTERIAL PROPERTIES OF POLYCAPROLACTONE/ZnO MICROCOMPOSITES

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The micro scale zinc oxides in polycaprolactone microcomposites (PCL/ZnO-MCs) have prepared via solution casting method. The properties of the PCL/ZnO-MCs characterized by the Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), transmission electron microscope (TEM), thermogravimetric analysis (TGA), scanning electron microscope (SEM). According to the XRD patterns, the crystallinity of PCL was more pronounced after addition of ZnO microparticles (MPs), whereas the intensity of the bands was reduced by addition of ZnO-MPs. The FT-IR characteristic bands of PCL were found to shift to higher or lower wave number in PCL/ZnO-MCs due to formation of covalent bonding. TGA results exhibited that the thermal stability of the PCL/ZnO-MCs is improved with regard to that of pure PCL. The SEM images indicated that the interface adhesion between the ZnO-MPs and the PCL matrix can be enhanced by the surface modifications of ZnO-MPs by octadecylamine. TEM showed that the synthesis of PCL/ZnO-MCs was increased in interfacial interaction between ZnO and PCL matrix, which was obtained by modifying the surface of ZnO-MPs. The antibacterial activities of the PCL/ZnO-MCs films were examined against *Salmonella choleraesuis* as gram-negative bacterium and *Bacillus Subtilis* as a gram-positive bacterium by agar disc diffusion method. The antibacterial effects of the PCL/ZnO-MCs revealed that the antibacterial activity was enhanced with the enhancing of ZnO content.

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1. Introduction

The interest in polymer materials with enhanced properties that exhibit antimicrobial activity is continuously increasing because of the growing demand for healthy living. The potential fields of application for these materials include, for example, textiles, food packaging or medical devices to prevent infection. The use of inorganic antibacterial nano and microparticles has several advantages over organic agents, including thermal resistance, chemical stability, safety and a longer active period [1].

Zinc oxide (ZnO) is a unique material with a wide band gap of 3.37 eV and a large excitation binding energy (60 meV). Various chemical and physical processes can be used to synthesize ZnO microparticles such as sol gel, hydrothermal process and thermal evaporation [2]. It is widely used in various cosmetic applications including sunscreens, and skin care products for protection against skin irritation and enhanced healing of surgical wounds [3]. Furthermore, ZnO-MCs have been incorporated into a number of different polymers, including low density

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polyethylene, isotactic polypropylene, polyvinyl acetate, polyamide and cotton woven fabric, for more than antimicrobial purposes [1].

PCL is a semicrystalline polymer with a low melting point of around 60°C and a glass transition temperature of about -60 °C [4]. Polycaprolactone (PCL) is non-toxic synthetic aliphatic polyesters in biomedical applications are especially used as drug delivery devices, because when inside of the body they are completely degradable [5]. As a type of polymer, PCL is susceptible to be degraded by many types of bacteria [6]. Micro-sized additives are used as an effective strategy to alter and enhance the properties of PCL [7]. Various types of filler, like clay [8], carbon nanotubes [9], silica [10], hydroxyapatite [11] and TiO₂ [12] have been incorporated into PCL to prepare MPs. The results demonstrated that homogeneous dispersion of fillers in PCL matrix significantly improved the performance of the microcomposites. To our knowledge, no work has been reported about the preparation of PCL modified by ZnO-MPs. The ZnO-MPs can be widely used as polymer additives to make functional PCL/ZnO-MCs because of their high aspect ratio and antibacterial property. Herein, we report preparation and characterization of PCL/ZnO-MCs.

In this study, ZnO-MPs modified with ODA to improve the bonding between ZnO and the PCL matrix by solution casting method. Property of PCL/ZnO-MCs such as the antibacterial effect against *Bacillus Subtilis* (*B. subtilis*) and *Salmonella choleraesuis* (*S. choleraesuis*) were influenced by the content of ZnO.

2. Experimental

2.1 Materials

Poly (ϵ -caprolactone) (PCL) and Chloroform (CHCl₃) were purchased from Solvay caprolactone, England, Laboratory & Scientific Enterprise, Chinese, respectively. Microsize-ZnO with the particle size in 192 nm was purchased from Merck (Germany). Octadecylamine (ODA) was purchased as analytical grade reagent from Acros. (New Jersey, USA).

2.2 Preparation of ZnO-MPs modified

Octadecylamine was used as an agent of surface modification of ZnO-MPs. This combination was very useful in obtaining of stable colloidal solution of ZnO-MPs in chloroform. The octadecylamine (ODA) was dissolved in chloroform (100 ml). This step was repeated with the addition of a required quantity (3, 5, 7, 10, 15 wt.%) of ZnO-MPs to the solution of octadecylamine. The precipitated solid was collected by centrifugation and washed as solid to remove the extra solution of octadecylamine five times with ethyl acetate. The wet precipitated solid was next placed in (100 ml) chloroform. The colloidal solution in which surface modified ZnO-MPs were well dispersed was obtained.

2.3 Preparation of PCL/ZnO-MPs films

A required quantity (8.5, 9.0, 9.3, 9.5, 9.7 wt.%) of PCL was dissolved in (100 ml) of chloroform to form PCL polymer solution. The contents of surface modified ZnO-MPs were controlled by changing PCL/ZnO-MCs weight ratio from 3 to 15 weight percent. To prepare the PCL/ZnO-MCs (3, 5, 7, 10 and 15 wt.%) PCL films, the colloidal solution of ZnO were added to the PCL polymer solution, respectively. Both solutions were mixed by stirring until it became homogeneous under room temperature. Every solution was cast onto the Petri dishes, respectively, and dried in evacuated desiccators for a week to remove the solvent. After the solvent was removed, free-standing films were peeled from Petri dishes.

2.4 Characterization

FT-IR absorption spectra were carried out using a single beam Fourier transform-infrared spectrometer (FT-IR spectrum 100 series, USA). FT-IR spectra of the samples were obtained in the spectral range 4000–400 cm⁻¹. The X-ray diffraction scans (XRD) were obtained using PW 3040 MPD PANalytical PH14PS equipped using Cu-K α radiation ($\lambda=1.540562 \text{ \AA}$, the tube operated at 40kV, Bragg's angle (2θ) in the range 4-80°). The morphology of the films was

characterized by scanning electron microscope using (LEO1455 VPSEM, Germany), operating at 200 kV accelerating voltage. The surface of the samples was coated with a thin layer of gold by the vacuum evaporation technique to minimize sample charging effects due to the electron beam. Transmission electron microscopy (TEM) was performed on a (Hitachi H-7100, Japan) transmission electron with an accelerating voltage of 200kV. TEM samples were deposited on thin amorphous carbon films supported on copper grids ultrasonically and used to observe the morphology of the microcomposites. A drop of the solution was placed on a copper grid that was left to dry before transferring into the TEM sample chamber. TGA experiments were conducted using a (TGA/SDTAB51^e, METTLER TOLEDO, Switzerland) equipment at different heating speeds from room temperature to (800 °C) under a nitrogen atmosphere at heating rate of 10 °C/min. The weight loss of the samples during heating was automatically recorded and plotted as a function of temperature.

2.5 Antimicrobial activity testing

The aim of the tests described below was to examine the antibacterial activity of ZnO particles toward both types of bacteria, *S. choleraesuis* as a gram-negative and *B. subtilis* as a gram-positive bacteria.

The test was carried out by placing 10mm diameter of each sample (sheets form sample) onto a plate (MHA) in which microbes were growing. Microbes used were *S. choleraesuis* (ATCC 1260) and *B. subtilis* (ATCC 6633). For liquid sample, the test was done by impregnating a known volume of sample onto 6mm paper disc (approximately). Then, the paper disc was placed onto a plate in which microbes were growing. The microbe culture was standardized to 0.5 McFarland standards which are approximately 1.5×10^8 cells. Not more than 4 discs should be placed on the same agar plate. Ampicillin used for *S. choleraesuis* and penicillin used for *Bacillus subtilis*. The test must be done in duplicate according to protocol and quality. The plates were inverted and incubated at 37 °C for 24 hours, until sufficient growth occurred. After incubation, the diameters of the zones of the complete inhibition (as judged by the unaided eye) were measured including the diameter of the disc. Zones were measured with ruler diameter zone for *B. subtilis* was 15 mm and *S. choleraesuis* was 12 mm according to CLSI 2012.

3. Results and Discussion

3.1 Infrared spectroscopy

The FT-IR method was used to characterize poly (ϵ -caprolactone) (PCL), ZnO-MPs and PCL/ZnO-MCs. Figure 1 shows the FT-IR pattern of ZnO-MPs, pure PCL and PCL/ZnO-MCs. The broad peaks at 400-700 cm^{-1} region are attributed to the stretching of ZnO [13]. The characteristic absorption peaks are also given of PCL in Table 1. The peaks' assignments and corresponding positions of pure PCL are given as follows: (C-H) stretch (2942, 2865 cm^{-1}), (C=O) stretching (1721 cm^{-1}), (CH₂) deformation (1165-1468 cm^{-1}) [14]. The PCL/ZnO-MCs (15 wt.%) show the same characteristic peaks. Nonetheless, a shift evidentially occurs in the peak by the addition of ZnO-MPs to the PCL. These displacements consist of (C-H) stretch 2942–2940, 2865–2865, 1721–1722, 1468–1466 and 1165-1168 cm^{-1} . Moreover, in PCL/ZnO-MCs (15 wt.%), a broad peak was observed in 3440 cm^{-1} which can be associated with the interaction between ZnO-MPs and PCL by forming covalent bonding between C=O and amine group of ZnO. Thus, the peak shift observed in FT-IR spectra can be ascribed to the formation of covalent bonding between ZnO and C=O group of PCL on the surface of the ZnO-MPs [15].

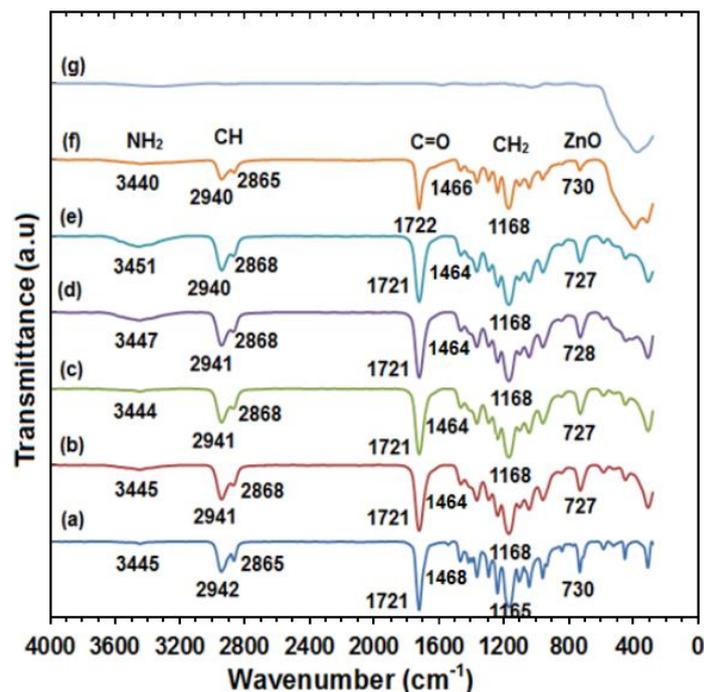


Fig. 1. FT-IR spectra of PCL (a), PCL/modified ZnO-MCs at 3, 5, 7, 10 and 15 wt.% (b-f) and pure ZnO.

Table 1. Band assignment of FT-IR spectra of PCL.

Wavenumber (cm ⁻¹)	Band Assignment
2942, 2865	Stretching C-H
1721	C=O
1165-1468	Deformation CH ₂

3.2 Crystalline structure

The XRD pattern exhibits a broad peak of PCL in the range of 15–30° (2θ) and several sharp and increased diffraction peaks for the PCL/ZnO-MCs films with relatively high ZnO content in Figure 2. These sharp peaks in the range of 30–70° (2θ) of ZnO-MPs is observed at 2θ angles of 31.82°, 34.49°, 36.30°, 47.59°, 56.65°, 62.90° and 67.98° [16]. The PCL/ZnO (15 wt.%) MCs also displays eight corresponding crystalline characteristic peaks at 2θ angles of 31.73°, 34.39°, 36.25°, 47.49°, 56.60°, 62.85° and 67.91°, similar to the main characteristic peaks of ZnO-MPs, showing that the crystal structure of ZnO was not changed by the presence of PCL. The XRD results indicate that ZnO-MPs remain in the PCL/ZnO-MCs. However, compared with ZnO-MPs, all the characteristic peak locations in the PCL/ZnO-MCs were shifted to slightly lower angles. In the PCL/ZnO-MCs, some slight interactions between ZnO and PCL may occur, which because a slight shift in the peak locations [17]. It can be seen that the intensity and the area under the peak at 2θ=21.3° and 23.8° decreased with the increase in the concentrations of ZnO-MPs. This implies that the decrease in degree of crystallization increases the amorphous region, which in turn increases the conductivity of the PCL/ZnO-MCs films. This behaviour demonstrates that complexation between the ZnO and PCL polymeric matrix takes place in the amorphous region. The crystallite size of microcrystalline ZnO-MPs was calculated by Scherer's formula ($D = 0.9\lambda/\beta \cos \theta$, where D is crystallite size, λ the wavelength of the X-rays, β the full-width at a half maximum intensity of the peak and θ the diffraction angle).

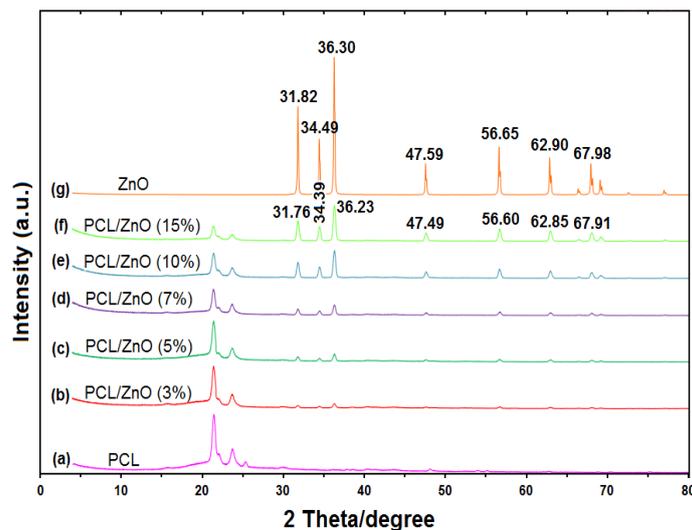


Fig. 2. XRD Patterns of PCL (a), PCL/modified ZnO-MCs at 3, 5, 7, 10 and 15 wt.% (b-f) and ZnO-MPs (g)

3.3 Thermogravimetric analysis

As an analytical technique, thermogravimetric analysis (TGA) is used to monitor and determine a volatile component weight change which occurs when a sample is heated. TGA thermograms of weight loss as a function of temperature for pure PCL and their complexes at the heating rate of 10 °C/min in the temperature ranging from room temperature to 800 °C are shown in Figure 3 [18]. In this figure, the TGA curve indicates that ZnO-MPs are extremely stable in nitrogen and any decomposition occurred in the 20-800 [19]. The TGA curves of the PCL/ZnO-MCs films and pure PCL are depicted in (Figure 3) and the results are summarized in Table 2. All the PCL/ZnO-MCs films and pure PCL displayed two step degradation processes, and the degree of thermal stability is related to ZnO content. As described in several earlier studies, the thermal stability generally increases in pure polymer films due to the presence of microparticles.

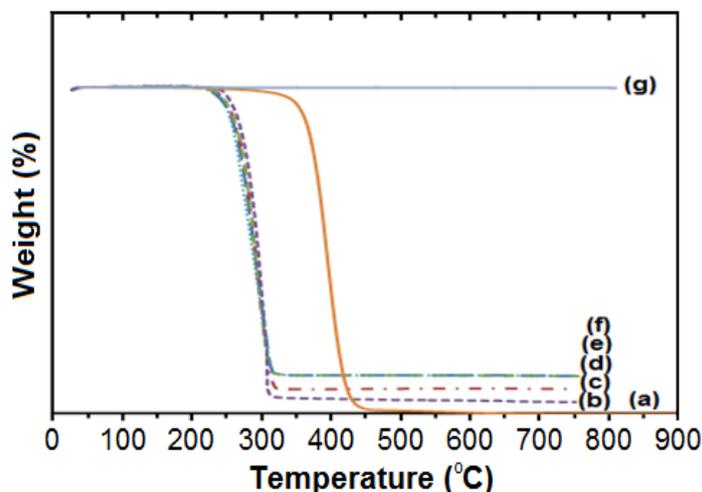


Fig. 3. TGA thermograms of PCL (a), PCL/modified ZnO-MCs at 3, 5, 7, 10 and 15 wt.% (b-f) and pure ZnO-MPs.

The first decomposition temperature and weight loss temperature of the PCL/ZnO-MCs and pure PCL are attributed to desorption of the water [20]. The second decomposition temperature and weight loss temperature (5 and 10wt.%) of the PCL/ZnO-MCs films extremely decreased. This indicates that PCL/ZnO-MCs films have less thermal stability than pure PCL film. The decrease in thermal stability can be ascribed to lower thermal stability of ZnO-MPs which have been modified with a silane coupling agent, compared to a matrix PCL polymer; that is to say, incorporating ZnO into the PCL decreases the thermal stability [21, 22].

Table 2. Effect of ZnO content on thermal stability of PCL/ZnO-MCs

Sample	ZnO Content		
	% (w/w)	T _{5wt%}	T _{10wt%}
PCL	0	351	362
PCL/ZnO	3	257	269
PCL/ZnO	5	251	262
PCL/ZnO	7	251	262
PCL/ZnO	10	248	257
PCL/ZnO	15	248	254

3.4 Surface morphology

The SEM images of ZnO and the PCL/ZnO-MCs filled with 3, and 5 wt.% ZnO are respectively shown in Figures 4(a-c). SEM was used to examine the morphology of fracture surfaces of specimens of the PCL/ZnO-MCs containing coupling agent [23]. In comparison with the matrix, the morphology of ZnO was easy to identify. The white dots in the images correspond to ZnO on the fractured surfaces of the PCL/ZnO-MCs [24]. In Figures 4(b-c) showed that after the surface modifications, there is no indication on the present of ZnO-MPs, but the fracture surfaces of the MPs. Most of them are smooth, and no cracks are created between the ZnO-MPs and the polymer matrix. It indicates that the surface modification of ZnO by ODA improves the interface adhesion between ZnO-MPs and the polymer matrix and this confirms that ZnO increases the PCL matrix through the microparticles matrix mechanism [25]. According to the Figure 4, it demonstrates that the system coupling agent (ODA) shows well-dispersed ZnO-MCs embedded in matrix. Meanwhile, Figures 4 (b-c) indicates not only well-dispersed ZnO but also aggregates in the composites with higher ZnO-MPs contents [26, 27].

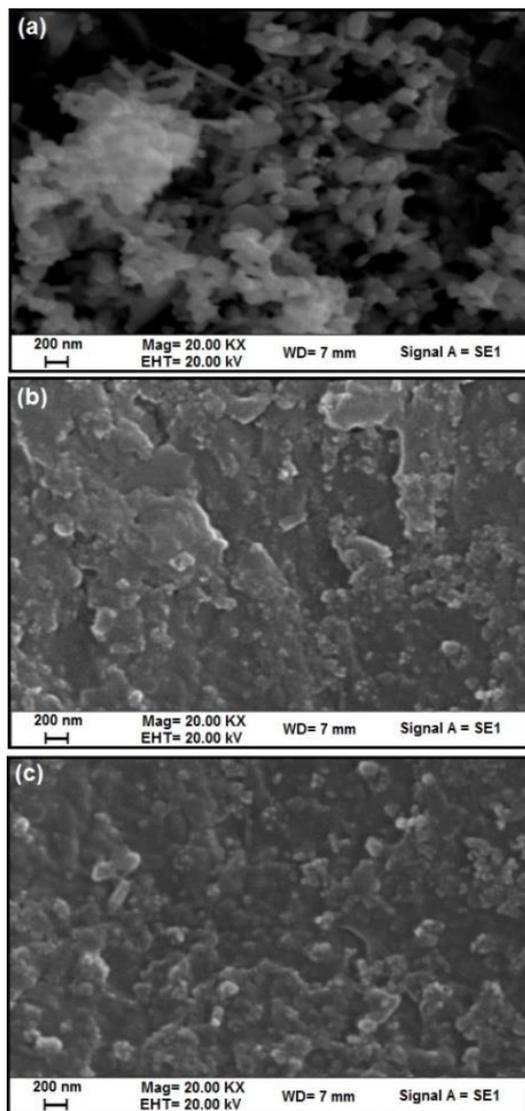


Fig. 4. SEM micrograph for pure ZnO (a) and PCL/ZnO-MCs 3 and 5 wt.% (b, c).

Fig. 5(a-c) indicate the TEM images of ZnO and PCL/ZnO filled with 3 and 5 wt.% ZnO, respectively. Micrometer scale and elongated shape were also observed in most of ZnO-MPs. Some irregularly shaped ZnO-MPs could be found as well. The dispersion states of the ZnO-MPs are shown in Figures 5(b-c). Well dispersion was also seen for most ZnO MPs in the PCL matrix. The organic nature of MPs surfaces is known as a main factor in promoting microparticles dispersion in the matrix. Therefore, the surface modification of ZnO-MPs is treated by organic ODA molecules which adhere to the surface of the ZnO-MPs, changing the surface properties from inorganic into organic and reducing the particle surface tension and helps increasing the compatibility between ZnO and polymer matrix [28]. The hydrophobic alkyl groups of ODA have a good compatibility with the hydrophobic PCL matrix and are able to increase the interfacial contact at the PCL/ZnO interface that lead to formation of inorganic/polymer MCs. Furthermore, the PCL chains anchored on the surface promote dispersion stability of the ZnO-MPs in the matrix. The diameter of the ZnO-MPs in the PCL matrix is about $0.192 \mu\text{m}$ [29].

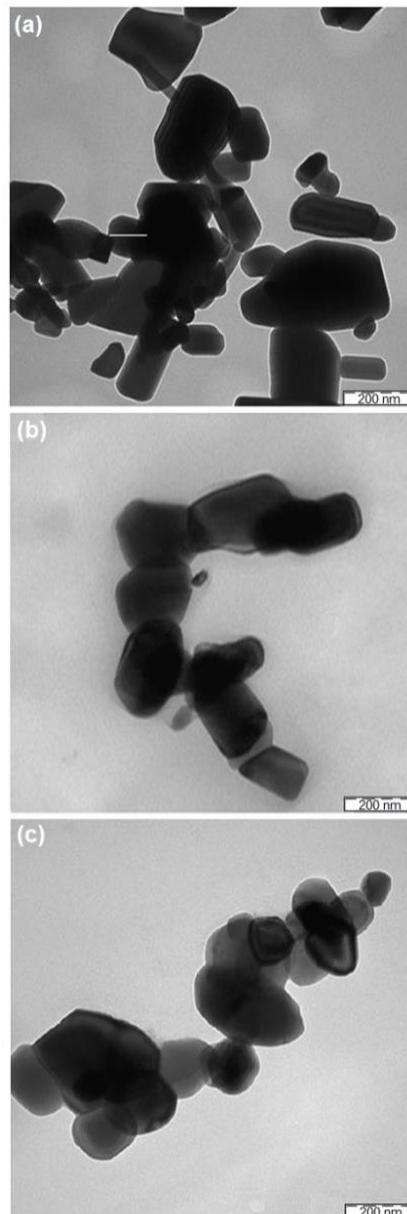


Fig. 5. Transmission electron micrographs for pure ZnO (a) PCL/ZnO-MCs at 3 and 5 wt.% (b, c).

3.5 Antibacterial activity

The antibacterial activities of PCL film, unmodified and modified ZnO in polymer films were tested using the *B. subtilis* as gram-positive bacterium and *S. choleraesuis* as gram-negative bacterium [Fig. 6 (a-d)]. Figure 6 shows the results of antibacterial experiments, indicating that the modified microcomposites films possess excellent antibacterial property compare that unmodified microcomposites films in the same percentage of ZnO (15 wt.%). On the other hand, according to achievement can also find that ZnO incorporated PCL films inhibit much more effective activity for *B. subtilis* and is suggested to have a strong affinity to the cells membranes of *B. subtilis*. However, the influence for *B. subtilis* more than that for *S. choleraesuis* [Figure 6 (c-d)]. The difference in activity against these two types of bacteria can be attributed to different organization of the cell wall. Gram-positive bacteria typically have one cytoplasmic membrane and thick wall composed of multilayers of peptidoglycan [30-32].

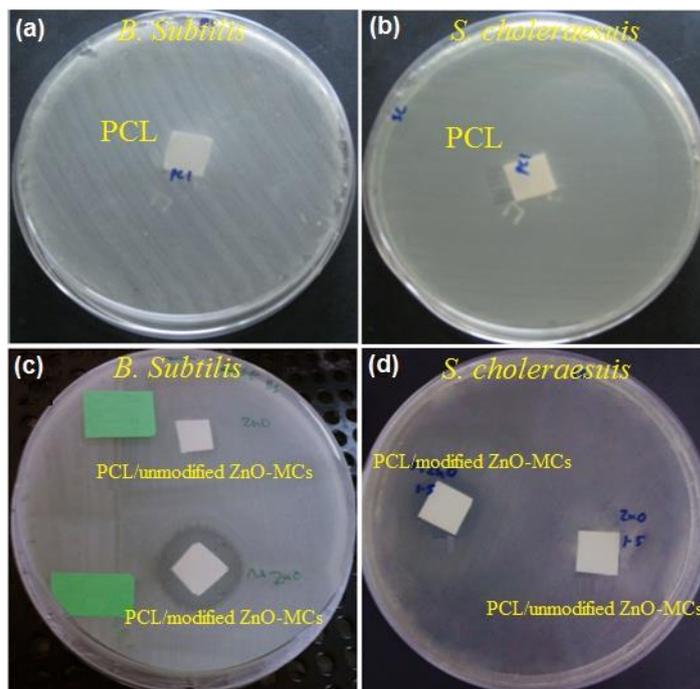


Fig. 6. Comparison of inhibition zones test between Gram-negative and Gram-positive bacteria (*B. Subtilis* and *S. choleraesuis*) for PCL (a-b), PCL/modified ZnO-MCs and PCL/unmodified ZnO-MCs for 15 wt.% of ZnO (c-d), respectively.

Thus, the cell membrane of gram-positive bacteria can be damaged more easily. In both cases, antibacterial activity of ZnO can be attributed to the damage of cell membranes, which leads to leakage of cell contents and cell death. However, exact cause of the membrane damage requires further study [33, 34]. Table 3 show typical results of the test which was carried out with the purpose of two qualitative evaluations for *B. Subtilis* and *S. choleraesuis*. The antibacterial activity is an evidence of bacteria-growth inhibition zone for ZnO and PCL/ZnO-MCs (10 and 15 wt.%) films but the inhibition zone is not present around the PCL film.

Table 3. Average of inhibition zones for PCL, and PCL/ZnO-MCs.

Sample	<i>B. subtilis</i> (mM)	<i>S. choleraesuis</i> (mM)
PCL	–	–
PCL/ZnO (10 wt.%)	14	11
PCL/ZnO (15 wt.%)	15	12

4. Conclusion

In this study, PCL/ZnO-MCs were effectively prepared through solution casting method. The results of spectroscopic methods such as XRD, FT-IR, TEM, SEM and TGA display that PCL/ZnO-MCs is successfully prepared in this research. According to FT-IR result, the interaction between ZnO and PCL is based on the formation of covalent bonding which is significant in the formation of the PCL/ZnO-MCs. The XRD results indicate that in the PCL/ZnO-MCs, some slight interactions between ZnO and PCL may occur, which causes a slight shift in the peak locations. As indicated in SEM images, after the ZnO-MPs who modified by ODA, the interface adhesion between the ZnO and the PCL matrix enhance, the aggregation of ZnO-MPs is decreased and the disparity is improved. TEM images revealed that after surface modification of ZnO by ODA, the PCL/ZnO-MCs film material promote well dispersed of the ZnO-MPs into PCL. The ZnO content showed significantly affected on the mechanical properties of the PCL/ZnO-MCs. The TGA

showed that by incorporating ZnO-MPs into PCL, the thermal stability of the PCL/ZnO-MCs was improved. The antibacterial activity of PCL/ZnO-MCs films established a strong antibacterial activity against *B. Subtilis* as gram positive bacterium.

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