

Antimicrobial, selective antibiofilm, and antioxidant properties of plasticized PMMA/PVC and zinc oxide nano filler for biomedical applications

Muhammad Abid Zia¹, Muhammad Kaleem Khosa¹, Majid Muneer¹,
Khalid Mahmood Zia¹ and Muhammad Jawwad Saif²

¹Department of Chemistry, Government College University, Faisalabad, Pakistan

²Department of Applied Chemistry, Government College University, Faisalabad, Pakistan

Abstract: The PMMA/PVC/ZnO-nanocomposites with zinc oxide nanoparticle (particle size < 50nm) was synthesized by solution casting technique. Morphology of the synthesized nano composites have been investigated by FT-IR and XRD techniques. After characterization, synthesized composites were applied for antibacterial, selective antibiofilm and free radical scavenging screening. Antibacterial studies were measured against different bacterial strains. Antibiofilms activities were studied against those bacterial model pathogenic strains which showed highest and minimum sensitivity as a (~94 and ~88 at 160 µg/ml). Antioxidant activity of synthesized nanocomposites were measured by DPPH and showed scavenging capacity with IC₅₀, 110 to > 200 µg/mL. Thus PMMA/PVC/ZnO nanocomposite showed promising antimicrobial activity and antioxidant activity that can be used for biomedical applications.

Keywords: PMMA, PVC, nanocomposites, characterization.

INTRODUCTION

In recent years, the manufacturing of polymeric materials and nanocomposites has gained much attention as an emerging low-cost technology worldwide. Poly-(methyl methacrylate), (PMMA), is one of the most popular class of polymers which is used in composites industries. The PMMA is a long, soft chain and well-known brittle polymeric material that has been extensively used in different fields due to useful properties, such as low cost, good chemical stability, low density, non-toxicity and transparent optical properties (Wen *et al.*, 2019). Due to outstanding biocompatibility features of PMMA with oral tissues, it is extensively used in medical applications such as dentistry, however acrylic resin has very low impact, poor strength and fatigue resistance. Many attempts have been done to strength the thermal and mechanical properties of PMMA (Yaseen *et al.*, 2020). The most common method for increasing the toughness and thermal stability of Poly-(methyl methacrylate) is the blending of PMMA with polyvinylchloride (PVC) with high level of plasticizers and received much attention in polymeric electrolyte applications (Feng *et al.*, 2017; Ali *et al.*, 2015). Due to low-cost and hygienic properties, polyvinyl chloride (PVC) and oxygen-containing hydrophilic PMMA are extensively used thermoplastics. (Aziz *et al.*, 2019). PVC is used in microelectronic and plastics industries (for example, wires insulation and cables industries) because of its low combustibility, good abrasion resistance, corrosion as well as electrical insulation, and mechanical stability that arises as a result

of interaction between H and Cl atoms (Suresh *et al.*, 2017; Joseph *et al.*, 2018; Arunkumar *et al.*, 2017).

In the insulating polymers the electrically conductive networks can be increased effectively by addition of nano-fillers. Nanofillers, metal oxide and chloride like ZnO-Nps, MnO₂, CoCl₂ were mixed with polymers to the improvement the optical and thermal properties (Alghunaim *et al.*, 2018; Shakir *et al.*, 2019), among them zinc oxide as an inorganic nano-filler has an outstanding feature such as high thermal conductivity and high refractive indices, (Abdelghany *et al.*, 2019), promising antibacterial and UV-protection properties (Hammani *et al.*, 2018; Choudhary *et al.*, 2018). The design of new materials, PMMA/ZnO nanocomposites, is of extraordinary potential which has a wide range of applications in photocatalysis, electrical switches, gas sensing, cancer treatment and tissue engineering fields (Mauro *et al.*, 2017). Zinc oxide nanoparticles have been used as an antibacterial agent for biofilm preventions and water purification (Abdallah *et al.*, 2020), sunscreen lotion (Osmond *et al.*, 2010; Rasmussen *et al.*, 2010), wound dressing (Sudheesh *et al.*, 2012), anticancer (Guo *et al.*, 2011), antibacterial and antioxidant activities (Premanathan *et al.*, 2011; Das *et al.*, 2013). The antimicrobial activity of zinc oxide nanoparticles was investigated against different bacterial strains. During bactericidal investigation, it has been found that the solubility surface area and particle size of ZnO-Nps were remained unchanged by changing the others (Li and Guo 2020). In biological systems, oxidative stress which is a major cause of cardiovascular disease, cancer, arthritis and diabetes is due to release of reactive free radical

*Corresponding author: e-mail: mkhosapk@yahoo.com

species or their precursors, so the antioxidants play a key role in the functioning of all biosystems (Nash *et al.*, 2015; Safawo *et al.*, 2018).

The aims of current study are to tune the physical properties of polymethyl methacrylate (PMMA), 80%, and thermoplastic polyvinyl chloride (PVC), 20%, blends loaded with different weight ratios of ZnO-Nps by sonication methods followed by solution casting technique. The ZnO-Nps contents and influences of films thickness on the structure, morphology, antibacterial, selective antibiofilm formation and antioxidant properties were investigated and discussed the effect of ZnO-Nps PMMA/PVC blends performance.

MATERIALS AND METHODS

Chemicals

Polymethylmethacrylate (PMMA) (m.w. ~100,000, Degussa, Germany) and moisture free Polyvinyl chloride, (PVC) (Mw.48000 D), Zinc acetate, Hydrogenperoxide (H₂O₂), 1,1-diphenyl-2-picrylhydrazyl (DPPH), tetrahydrofuran (THF) and DMSO (Aldrich) were of analytical grade.

Instrumentation

Different techniques were applied to characterize the synthesized nanocomposites. Infrared spectra were recorded by FT-IR spectrophotometer (Bruker) in range of 4000 to 400 cm⁻¹. Structural morphology was studied using X-ray diffraction by 3040/60 X'Pert PRO diffractometer. Microplate Reader (ELx808™ Absorbance, Biotek, USA) was used to check the antibiofilm activity.

Preparation of ZnO-Nps

ZnO nanoparticles with particle size <50 nm were prepared by the reaction of Zn(CH₃COO)₂·2H₂O and H₂O₂ in alcoholic medium, typically, 5.0g of Zn(CH₃COO)₂·2H₂O was dissolved in 150ml methanol diluted with 100 ml of deionized water and solution was stirred for 3 hrs. at 50°C during stirring, 4 ml of hydrogen peroxide (H₂O₂, 47%) was added dropwise to make the solution clear. This solution was allowed to stand at ambient temperature for 24 hrs. and heated at 80°C. The resulted white powder was washed with distilled water and dried at 80°C in oven and then calcinated at 600 °C for one hour to get stable ZnO nanoparticles (Doddapaneni *et al.*, 2017).

Synthesis of plasticized PMMA/PVC/ZnO nanocomposites

Plasticized PMMA/PVC/ZnO nanocomposite was synthesized by solution casting technique followed by sonication (Mauro *et al.*, 2017). Typically, A solution of PMMA and PVC (80: 20 wt. %) was prepared in THF by string about 24 hrs at room temperature. ZnO-Nps with

different concentration with respect to concentration of PMMA/PVC matrix, 2.5%, 5.0%, 7.5% and 10% was mixed with PMMA and PVC blend by sonication of mixture about 30 mint. and heated gently to get the homogeneous mixture, The PMMA/PVC/ZnO-Nps mixture was casted on glass plates and solvent was evaporated in air. The obtained solid was placed in vacuum desiccator for further characterization.

Antibacterial Investigation

The antibacterial activity of synthesized PMMA/PVC/ZnO-nano composites was determine against *S. aureus* (ATCC 6538), *B. subtilis* (ATCC 6633) and *S. pyogenes* (ATCC12344), *P. aeruginosa* (ATCC 10145), *E. coli* (ATCC 15224) and *S. Typhi* (ATCC 14028) by “disc-diffusion method” (Annie *et al.*, 2002; Jamil *et al.*, 2012; Khosa *et al.*, 2015). The bacterial strains were cultured in agar-agar nutrient broth at 37°C for 24 hrs. The broth cultures of test organisms of approximately 10⁴-10⁶ (CFU/mL) were added to nutrient broth at 45°C in sterilized petri dishes and solidify. Solution of PMMA/PVC/ZnO-nanocomposite in DMSO (Five μL) was tipped on sterilized paper disks. These disks were placed on agar-agar nutrient broth and incubated for 24 hours at 37°C. Experiments were performed in triplicate and zone of inhibition was measured in mm using Cefixime (1 mg/mL) as a standard antibiotic drug.

Antibiofilm assay

Staphylococcus aureus (ATCC 6538) and *Staphylococcus typhi* (ATCC 14028) were selected as a model bacterial strains which showed a highest to least bacterial activity for PMMA/PVC matrix and PMMA/PVC/ZnO-Nps according to reported method (Abdallah *et al.*, 2020). Biofilm forming bacteria were incubated at 37°C for 24 hrs. in 10 ml of trypticsoy broth (TSB) containing 1% glucose solution. Each bacteria strain (200μL) was inoculated into 96μLplates of PMMA/PVC/ZnO-nanocomposites. Bacterial strains were further incubated for 24 hours at 37°C. After that, each well was washed with saline phosphate buffer solution of pH 7.2 and crystal violet solution was added (200μL of 0.1% w/v) and 100μL of 96% ethanol to all well and left for a further 30 min to extract the stained bound biofilm. Microplate Reader was used to note the absorbance of crystal violet solution at 490 nm. Ciprofloxacin, a standard drug, was used as a standard (20μg ml⁻¹). Reduction in size of biofilm was measured and compared with the positive control. The % of biofilm inhibition was calculated as:

$$\% \text{ inhibition} = 1 - \left(\frac{\text{optical density of composite}}{\text{optical density of negative control}} \right) \times 100 \quad (1)$$

Antioxidant activity

Free radicals scavenging activity of PMMA/PVC/ZnO-nanocomposites was investigated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) according to standard method

(Khoushika *et al.*, 2017). Methanolic solution of DPPH was prepared. Stock solution of composites (5mg/mL) was prepared in DMSO and diluted to different concentrations (5, 10, 20, 40, 100, 200 μ g/mL). Each concentration was mixed with freshly prepared DPPH solution in glass vials. These vials were sealed and placed in dark at 37°C for 30 minutes. When the colour of DPPH was changed from deep violet to light-yellow, the absorbance was noted at 517 nm on a spectrophotometer. The % scavenging activity was calculated as:

$$\% \text{ scavenging activity} = \frac{\text{absorbance of control} - \text{absorbance of test sample}}{\text{absorbance of control}} \times 100 \quad (2)$$

Ascorbic acid as a positive control was used to measure the antioxidant activity.

STATISTICAL ANALYSIS

All the experiments were conducted in triplicates and data obtained was analyzed statistically by one-way ANOVA test using Minitab Software.

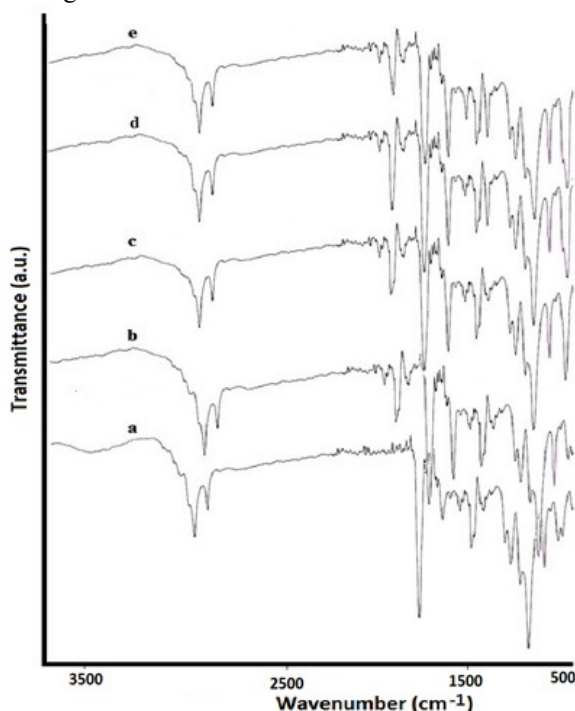


Fig. 1: Infrared spectra of ternary composites: (a; PMMA/PVC (80:20) blend, b; blend with: 2.5 %, c; 5%, d; 7.5% and e; 10% ZnO-Nps).

RESULTS

FT-IR investigation

Infrared spectra of PMMA/PVC with different contents of ZnO-Nano filler are shown in fig. 1. Characteristic absorption bands of PMMA (The bands at 1196, 1720, 1155 and 1050, 3455 cm^{-1} for $-\text{OCH}_3$, CO_{sym} , CO_{asym} , C-O-C and $-\text{OH}$ groups respectively) and PVC (2985, 2950 and 2810 cm^{-1}) were ascribed to $-\text{CH}$, $-\text{CH}_2$ functional groups (fig. 1-a). new bands at 1725, 610 cm^{-1} appeared in

PMMA/PVC with different contents of contents of ZnO-nanoparticles, while the $-\text{OH}$ band at 3455 cm^{-1} vanished.

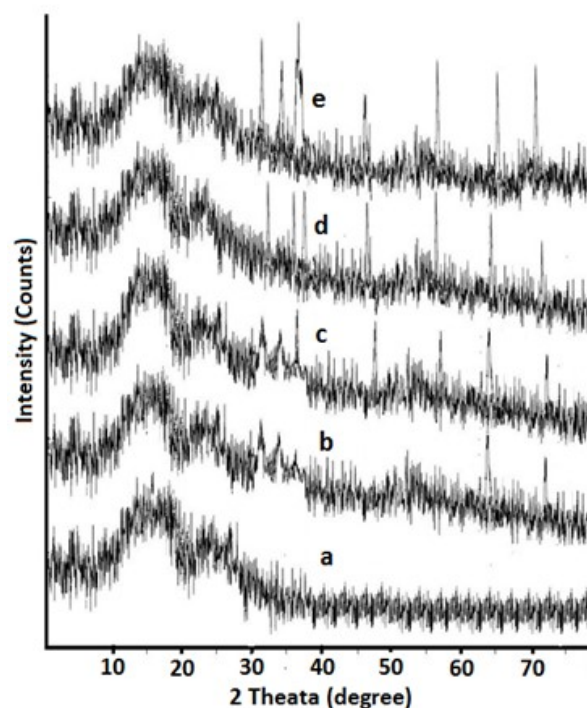


Fig. 2: XRD of PMMA / PVC / ZnO-Nps composites: (a; PMMA/PVC (80:20) blend, b-e blend with 2.5, 5, 7.5 and 10 % ZnO-Nps)

X-ray diffraction

The homogenous mixing and cross linkage between synthesized PMMA/PVC composites contained different contents of ZnO-Nps (2.5%, 5 %, 7.5 %, and 10 % w/w) was examined by wide-angle X-ray diffraction method. Fig. 2 showed the diffraction patterns for PMMA/PVC film and for PMMA/PVC/ZnO nanocomposites. two broad bands at $2\theta = 16.6^\circ$ and 24.5° related to amorphous PMMA/PVC matrix.

Biological Investigation

Antibacterial screening

The antibacterial activity of PMMA/PVC/ZnO-nanocomposites were determined and data is shown in table 1. The varying degree of antibacterial activity of PMMA/PVC blend and PMMA/PVC/ZnO-Nps was measured (mm) in term of zone of inhibition. Antibacterial activity of PMMA/PVC composite doped with ZnO-nanoparticles was compared with standard drug Cefixime.

Antibiofilm activity

Biofilm inhibition activity of PMMA/PVC blends loaded with different contents of ZnO-Nps which act as an inorganic filler was determined and results are shown in fig. 3 (A & B).

For this purpose, *S. aureus* (ATCC 6538) and *S. typhi* (ATCC 14028) were selected as model microorganisms because such strains shown highest and least antibacterial activity.

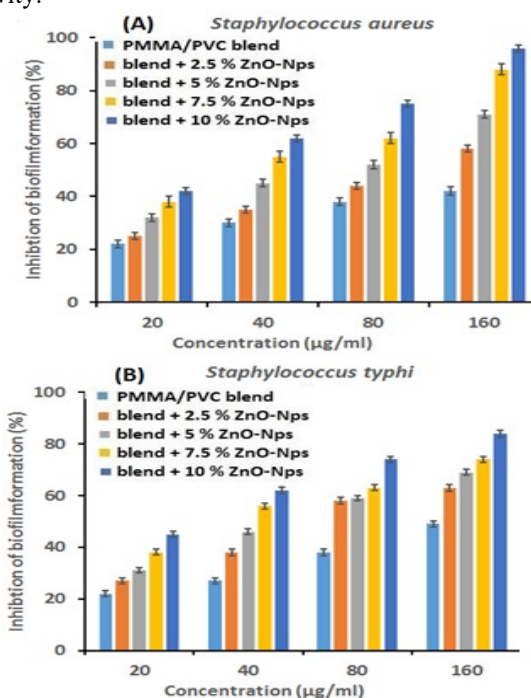


Fig. 3: (A &B) Antibiofilm activity of PMMA/PVC matrix and PMMA/PVC/ZnO nano-composites (P< 0.05 vs. control).

DISCUSSION

The structure identification of all prepared composites was confirmed from FT-IR spectrum. The characteristic absorption bands at respective wavelength as shown in fig.1 indicated the successful mixing between PMMA and PVC, whereas new band at 1725, 610 cm^{-1} appeared and decreased in intensity of band at 1720, 1155 cm^{-1} and band at 1480 cm^{-1} disappeared which confirmed the strong interaction between PMMA/PVC matrix with different contents of ZnO-Nps (fig. 1, b-e) (Adel *et al.*, 2020; Mauro *et al.*, 2017).

The XRD pattern of the PMMA/PVC matrix have two broad bands at $2\theta = 16.6^\circ$ and 24.5° related to the amorphous nature of the polymer matrix (fig. 2a). In PMMA/PVC/ZnO-nanocomposites has intense Bragg peaks corresponding to the planes (100), (002), (101), and (110), was increased with increasing ZnO-Nps contents inside the PMMA/PVC matrix (fig.2, b-e), while two broad bands are shifted to 2θ values of 17.7° and 24.8° . This means that the ZnO nano-particles were dispersed uniformly into PMMA/PVC and act as a reinforcement metal oxide, thus the new material is formed (Ilangoan *et al.*, 2017).

Such composites showed moderate to significant activity against all six tested bacterial strains. The results showed that *S. aureus* showed greater activity and *S. typhi* showed least sensitivity with 10% ZnO-nanoparticles which

Table 1: Antibacterial activity of PMMA /PVC blend and PMMA/PVC/ZnO-nanocomposites

Test compounds	*Zone of Inhibition of Sample (mm)					
	Gram (+Ve)			Gram (-Ve)		
	<i>S. aureus</i>	<i>S. pyogenes</i>	<i>B. subtilis</i>	<i>E. Coli</i>	<i>S. Typhi</i>	<i>P. aeruginosa</i>
PMMA/PVC (blend)	18 ± 1	15 ± 1	17 ± 1	14±1	18 ± 1	13 ± 1
ZnO-Nps	18 ± 1	22 ± 1	24 ± 1	21 ± 1	26 ± 1	23 ± 1
Blend + 2.5 percentage of ZnO-Nps	20 ± 1	19 ± 1	18 ± 1	19 ± 1	21 ± 1	16 ± 1
Blend + 5 percentage of ZnO-Nps	24 ± 1	23 ± 1	21 ± 1	23 ± 1	23 ± 1	21 ± 1
Blend + 7.5 percentage of ZnO-Nps	27 ± 1	24 ± 1	24 ± 1	25 ± 1	24 ± 1	23 ± 1
Blend + 10 percentage of ZnO-Nps	29 ± 1	27 ± 1	26± 1	26 ± 1	28 ± 1	27 ± 1
Cefixime	33 ± 1.5	31 ± 1	35 ± 1	29±0.5	36 ± 1	31 ± 2

± = SD, *Zone of inhibition: (5-10 mm = Activity present, 11-25 mm = Moderate activity, 26 - 40 mm = Strong activity)

Table 2: Antioxidant activity of PMMA / PVC blend and PMMA/PVC/ZnO-nanocomposites expressed as % of DPPH free radical scavenging.

Compound	% Scavenging ± sd Concentration µg / mL						IC ₅₀ (µg / mL)
	200	100	40	20	10	5	
PMMA/PVC (blend)	44 ± 1	39 ± 1	32 ± 1	26 ± 2	14 ± 1	8 ± 1	>200
ZnO-Nps	65± 15	51± 25	44 ± 1	33±15	21 ± 15	15 ± 25	125 ± 1
Blend + 2.5 percentage of ZnO-Nps	49 ± 1	43 ± 1	36 ± 1	29 ± 1	18 ± 1	10 ± 1	180 ± 1
Blend + 5 percentage of ZnO-Nps	58 ± 1	51 ± 2	40 ± 2	31 ± 2	21 ± 1	13 ± 1	150 ± 1
Blend + 7.5 percentage of ZnO-Nps	66 ± 1	59 ± 2	43 ± 1	33 ± 1	24 ± 1	15 ± 1	130 ± 1
Blend + 10 percentage of ZnO-Nps	72 ± 2	64 ± 1	47 ± 1	39 ± 1	26 ± 2	17 ± 1	110 ± 1
Ascorbic acid	87 ± 1	84 ± 1	80 ± 1	70 ± 1	56 ± 1	35 ± 1	8.75±1

explained that with increased contents of ZnO-nanoparticles due to uniform mixing of nanoparticles in polymeric blend and have a greater effect on them *in vivo* bioactivity as reported in literature (Methew *et al.*, 2019). PMMA/PVC blends containing different contents of ZnO-Nps showed significant biofilm inhibition activity as compared to PMMA/PVC blend. As the biofilm inhibition effect is concentration dependent and showed significant effect as the concentration of PMMA/PVC/ZnO-Nps increased. The inhibition % of biofilm formation for *S. aureus* and *S. typhi* was found to ~ 96 and ~ 84, respectively at 160 µg/ml concentration (Thaya *et al.*, 2016).

Antioxidant activity data showed that as the concentration of ZnO-Nps contents increased in PMMA/PVC blend, the % scavenging activity also increased. On comparison of IC₅₀ values of the PMMA/PVC blends and ZnO-Nps with IC₅₀ >200µg/ml and 125µg/ml respectively, it is found that synthesized nanocomposites are more active free radical scavenging activity than PMMA/PVC blend as well as ZnO-Nps (Joan *et al.*, 2017).

CONCLUSION

PMMA/PVC/ZnO-nanocomposite materials were successfully prepared by solution casting technique. Their structure was investigated by FT-IR and XRD. The interaction between the PMMA/PVC matrix with ZnO-Nps was confirmed by the shifting of absorption bands in IR spectra. The degree of crystallinity and area under peaks was determined by XRD which indicated that as the contents of zinc oxide nanoparticles increased in PMMA/PVC blend may affect the crystallinity and produce defects in polymeric matrix. Antibacterial, selective antibiofilm activity, and antioxidant screening showed the promising activity of the synthesized PMMA/PVC/ZnO nanocomposites. On the basis of biological screening, it is suggested that the synthesized PMMA/PVC/ZnO nanocomposites may have some excellent biomedical applications.

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