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EVALUATION OF ANTIMICROBIAL ACTIVITY OF THIN FILMS OF NICKEL-POLYPROPYLENE GLYCOL NANOCOMPOSITES

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Abstract- The present study concentrates on the synthesis of thin films of polymeric-metal nanocomposites in accordance with their increasing demand in medical, cosmetic, sensors and food industries, etc. Ni-Polypropylene nanocomposites were synthesized using chemical reduction method. The nanocomposites were characterized using techniques Fourier Transform Infrared (FTIR) spectroscopy, X-Ray Diffraction (XRD) analysis and Transmission Electron Microscopy (TEM). Transmission Electron Microscopy revealed that the nanocomposites synthesized were in the range of 24-30nm in size. Fourier Transform Infrared analysis shows the interaction between Nickel and Polypropylene glycol resulting in formation of Ni-PPG nanocomposites. X-Ray diffraction data reveals the amorphous nature of the nanocomposites. Synthesized Ni-PPG nanocomposites were subjected to formation of thin films using spin coating technique. The thin films of nanocomposites were evaluated for their antimicrobial activity against both bacterial strains Gram positive and Gram negative. Antimicrobial analysis study of Ni-PPG nanocomposites thin films demonstrated that they possess a very good inhibitory effect against both the bacterial strains showing percent inhibition of 52.3% against Staphylococcus aureus MTCC 1809, 54.5% against Escherichia coli MTCC 723 and 48.7% against fungus Neurospora crassa .

Keywords: antimicrobial analysis, FTIR, nanocomposites, TEM, Thin films, XRD

1. INTRODUCTION

Thin films of nanoparticles is emerging as a very efficient and interesting area of research as they can play a vital role in controlling the microbial growth over surfaces by their enhanced and unique antimicrobial properties. Prevention of microbial growth in food packaging systems has been an area of great interest because of increasing culture of use of packed food in mostly developed and developing countries. Coating of some antimicrobial substances on the packing layer can prove very helpful in control of microbial growth. Antimicrobial activity of nanocomposites can be exploited in food packaging industry because of their increased efficacy in control of microbial growth because of increase of surface to volume ratio. Many researchers have shown their keen interest in improving the properties of polymers by using the idea of nanocomposite formation[1-5]. Nanoscale composites play a very effective and vital role in food packing systems as antimicrobial agents, oxygen scavenger and biosensors [6-8]. The use of nanocomposites in food packaging coating industry has a huge potential because a very minute quantity of nanocomposites will be needed as compared to its micro part. Nanocomposites exhibit excellent properties as reported such as, high melting point, low density, low coefficient of thermal expansion, high thermal conductivity, good chemical stability and improved mechanical properties such as higher specific strength, better wear resistance and specific modulus and have good potential for different industrial fields [9-12].

There are many polymers that are frequently utilised for food packaging systems. Various grades of polyethylene (HDPE, LDPE etc.), polystyrene (PS), polyethylene terephthalate (PET), polyvinyl chloride (PVC) and polypropylene (PP).But as we can observe that there is no pure polymer that posses or acquire all the properties required for food packaging, so we need to blend them with some additive to form composites with desirable properties. The thermal stability and mechanical properties of polymers can be enhanced by incorporating nanoparticulate material into it [13]. By blending the desired properties of two substances forming nanocomposites, we can get the smart choice for coating food packaging systems [14]. Propylene glycol is been considered an important ingredient in food, pharmaceutical industries and cosmetics as it posses low human toxicity. Polypropylene glycol (PPG) is also well known to be used in food and beverage packaging [15, 16]. On the other hand, Nickle II coordination complexes have been widely studied for their antimicrobial activity [17]. Many researches have depicted that nickel (II) complexes exhibit antimicrobial activity by penetrating the cell of microbes and inactivating their enzymes [18-23]. Recommended antimicrobial activity in comparison with the ligands has been shown in some nickel compounds [24, 25]. Antibacterial activity of nickel compounds have been observed against Gram-positive (6 strains of Staphylococcus aureus, 4 of Bacillus subtilis

and 7 of Streptococcus pyogenes) and the - Gram-negative (5 strains of Escherichia coli, 2- of Klebsiella pneumoniae, 6-Pseudomonas aeruginosa and 3-Pseudomonas fluorescens) [26].

Nickel as discussed is well known for its antimicrobial activity and polypropylene glycol is very commonly used in packaging industries because of its very vital properties. So, In the present study, we have blended up the properties of PPG and nickel by synthesizing their nanocomposites by chemical reduction method to elaborate their properties to a wider range. Prepared Nickel –PPG nanocomposites were characterized using various techniques like FTIR, XRD, TEM. Thin films of nanocomposites were prepared using spin coating technique and analysed for their antibacterial and antifungal activity.

2. MATERIALS AND METHODS

2.1 Synthesis of Nickel-Polymeric Nanocomposites

Nickel-Polypropylene glycol nanocomposites were synthesized by chemical reduction method using NaOH as reducing agent. Nickel acetate (2M) solution was added into 10% (v/v) solution of polypropylene glycol in double deionised water preheated to 70°C. The solution was kept at constant stirring for 1 hour. Sodium hydroxide (0.5M) was added drop by drop under constant stirring and temperature maintained constant showing the formation of precipitates of nanoparticles. The solution mixture was again stirred for half an hour followed by the addition of thiourea as capping agent. An overnight stirring was given. The suspension solution was sonicated in water bath for 30 minutes. The suspended nanocomposites were centrifuged and deep freezed at -80°C for 24 hours and lyophilized. The dried powder of Nickel-Polypropylene glycol nanocomposites was obtained and used for further characteristic analysis studies.

2.2 Formation of Thin Films of Nanocomposites

Before coating of nanocomposites, the surface of glass substrate was cleaned using deionised water and then wiped with ethanol. Any chemical treatments on the surface are not required, as in case of the self assembly methods [27]. 50 µl of homogenised stock solution of Ni-PPG nanocomposites was applied over the glass substrate and spin coated at 4000 rpm for 30 seconds. The coated glass substrate was oven dried at 80°C for half an hour and then cooled down. The same process was repeated for three times to get the desired thickness of thin films. After that the coating on the glass substrate were annealed at 300°C to get final thin films of Ni-PPG nanocomposites.

2.3 Characterisation

The FTIR spectra of lyophilized Ni-Polypropylene glycol nanocomposites were recorded using KBr pellet technique in 1/100 weight ratio using FTIR spectrophotometer (Spectrum B×11, Perkin-Elmer) in the range of 4000-400cm^{-1} with resolution±4cm^{-1}.

XRD diffraction patterns of nanocomposites were obtained by using X-Ray diffraction analyser (RIGAKU-miniflex Desktop X-ray Diffraction) using CuK_{α} as radiation source (λ = 1.54184 Å) at 30KV over the range of 20°-80°.Crystallinity and purity of powder nanocomposites sample were analysed using XRD pattern.

The morphological analysis and size of nanocomposites was determined using TEM (Transmission Electron Microscopy) (TEM; Hitachi H7500). The lyophilized powder of nanocomposites was suspended in double distilled water, homogenized and ultrasonicated for 10 minutes for getting a well dispersed solution just before observation. A copper grid was used with a carbon film for loading the samples and observation.

Contact angle of the thin films deposited was studied using travelling microscope subordinated with angle measurement plate. Contact angle study was done to investigate the type of contact the packaging surface will make with the carrying content. Smaller the contact angle less than (<90°) means the surface in hydrophilic or wettable. On the other hand, contact angle more than (>90°) implies that the thin film surface is hydrophobic in nature.

Antibacterial activity of thin films of Ni-PPG nanocomposites was tested using agar plate method by noticing the zone of inhibition around coated slide against both Gram positive and Gram negative bacterial strains Staphylococcus aureus MTCC 1809 and Escherichia coli MTCC 723. For that, 20 ml of agar solution was poured in disposable petriplates and allowed to solidify. 20µl of fresh bacterial cultures were spread over solidified media using spreader and coated slides were placed in centre [28]. The petriplates were kept in incubator at 37˚C for 24 hours and then observed to examine the bacterial growth. All the experiments were performed in triplicates.

Antifungal activity of Ni-PPG nanocomposites was studied against Neurospora crassa a type of red bread mold. Potato dextrose agar was used as the culture media for observing the antifungal activity of nanocomposites thin films. Same procedure was followed as for antifungal study except the growth of fungus was observed after 48 hours.

3. RESULTS AND DISCUSSION

3.1 Ftir Spectra

The IR spectra of nanocomposites are represented in figure 1. The FTIR spectrum shows the characteristic peaks at 652.09 cm^{-1}, 2972.58 cm^{-1} and 3401.76 cm^{-1}.

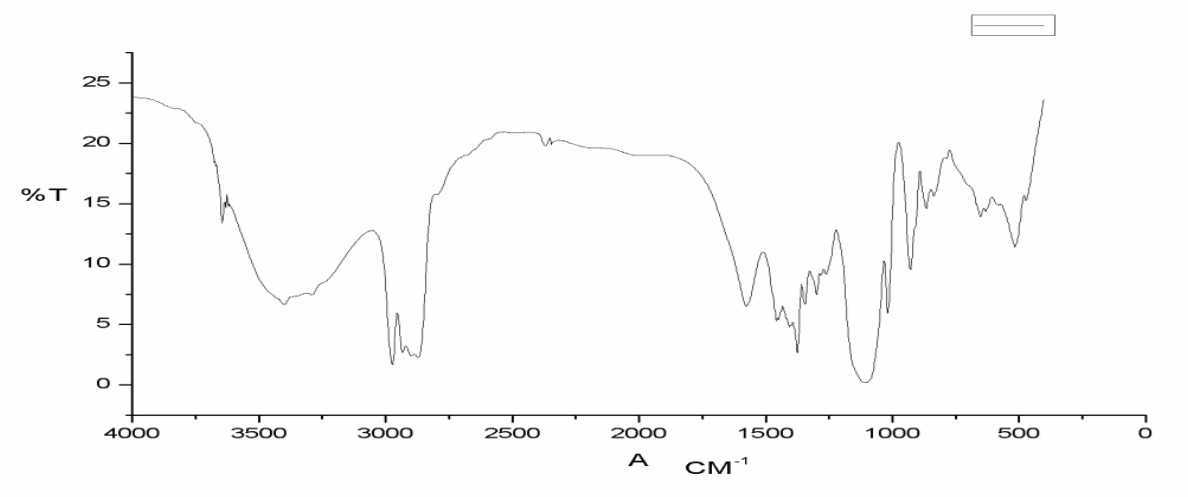


Fig. 3.1 FTIR Spectrum of Ni-PPG Nanocomposites

The peaks in the region 2900cm^{-1}, 1100cm^{-1} presents presence of PPG. There is slight deviation of peaks because of adsorbtion of Ni nanoparticles in polymer matrix. The symmetry of spectra decreases towards lower frequencies indicating the presence of OH group. Presence of Band at 1100 cm^{-1} represents single C-O bond stretching mode [29]. Peaks in spectral region around 517.68 cm^{-1} may be assumed to indicate Ni-O stretching vibrational mode [30-31].Stretching of OH group confirmed by the peak at 3644.68cm^{-1}. Peaks at 1375.07cm^{-1} and 27972.58 cm^{-1} shows methyl C-H asym./sym. bend. Presence of methyne C-H bend may be concluded by the peak at 1345.79cm^{-1}. Peak at 928.58cm^{-1} indicates the vinyl C-H out-of-plane bend. Bend in vinylidene C-H plane observed by the presence of peak at 1298.73cm^{-1} [32].

3.2 XRD Analysis

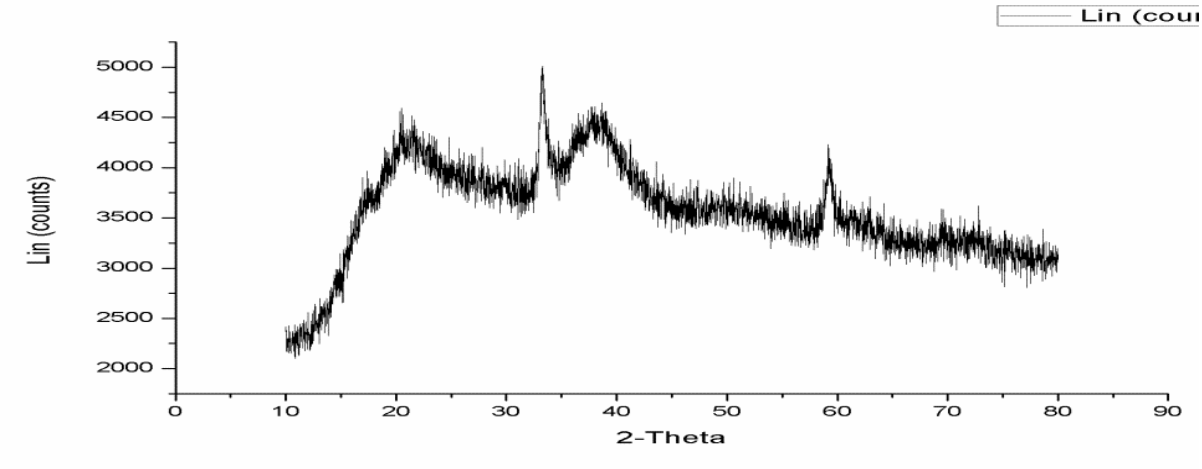


Fig. 3.2 XRD Spectrum of Ni-PPG Nanocomposites

Fig. 3.2 shows XRD analysis data of Ni-PPG nanocomposites. XRD works on the basis of Scherrer formula

$$d = 0.9\lambda/\beta\cos\theta$$

Where d= average particle size, β is full width at half maxima(FWHM), θ is the Bragg angle, λ is the wavelength of Cu K in radians.

XRD peaks appeared at 2θ value 34° and 59°. The shift of characteristic peaks of nickel from their usual value represented the formation of Ni-PPG nanocomposites. A broad band appears between 2θ=35°- 40°. Corresponding peaks of Ni and PPG to the reported data available confirms the formation of nanocomposites (JCPDS file data). The broadening of the peaks may be a sign of poor crystallization of the structure and/or

small particle size [33]. The XRD pattern indicated the "saw tooth" reflections shown by XRD pattern were typical of two dimensional turbostratic phases which have orientation disordered layers [34]. Presence of broad band and disappearance of sharp peaks indicate the amorphous nature of nanocomposites formed because of adsorption of metal particles in polymer matrix.

3.3 TEM

Fig. 3.3 depicts the size and structural morphology of Ni-PPG nanocomposites that Ni-PPG nanocomposites posses a well organised spherical shape with particle size in the range of 24-30 nm in size. The Ni nanoparticles were well dispersed and uniformly mixed in PPG matrix forming Ni-PPG nanocomposites.

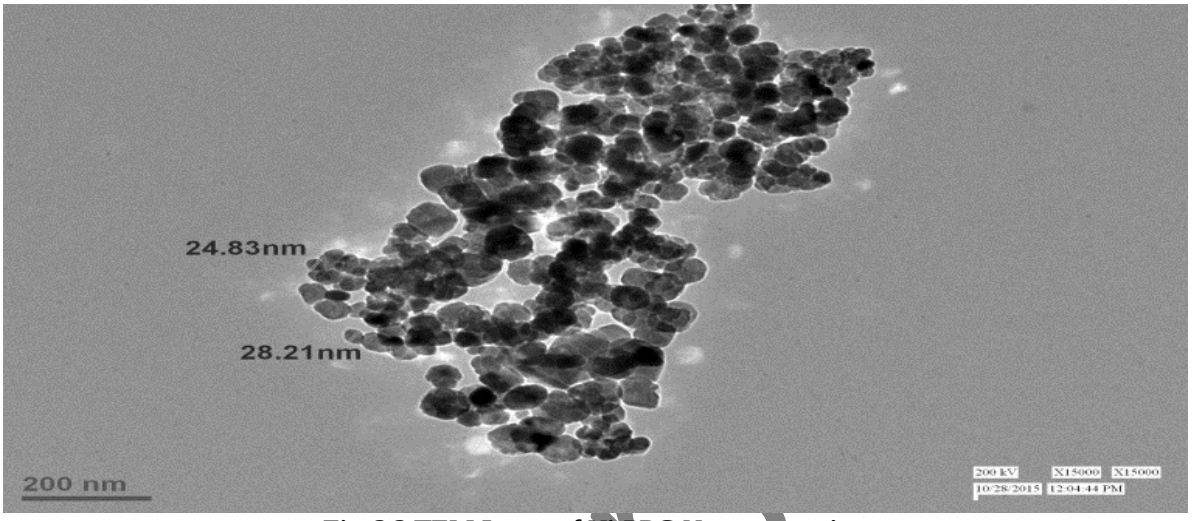


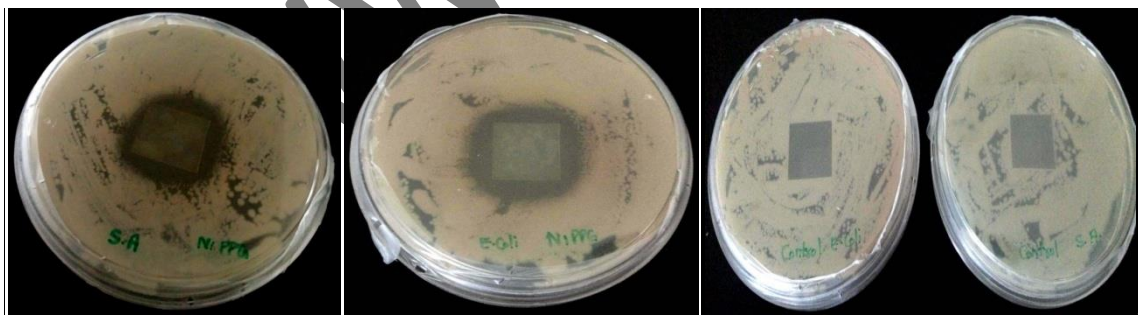
Fig. 3.3 TEM Image of Ni-PPG Nanocomposites

3.4 Contact Angle Measurement

The contact angle of the water droplet with Ni-PPG coated substrate was measured using travelling microscope subordinated with angle measurement plate. Contact angle between liquid drop and coated surface was measured directly from the angle formed at the contact between the liquid and the coated surface and it was found to be 93°±2 which implies a hydrophobic surface showing less wettability.

3.5 Antimicrobial Activity

3.5.1 Antibacterial Study



(a) (b) (c)

Fig. 3.4 Antibacterial images of Ni-PPG nanocomposites thin films against bacterial strains (a)Staphylococcus aureus MTCC 1809 (b) Escherichia coli MTCC 723 (c)uncoated glass against bacterial strains Staphylococcus aureus MTCC 1809 and Escherichia coli MTCC 723

The antibacterial activity of Ni-PPG nanocomposites is shown in fig. 3.4(a), 4(b). A clear zone of inhibition is shown against both Gram positive and Gram negative bacterial strains Staphylococcus aureus MTCC 1809 and Escherichia coli MTCC 723. The diameter of the clear zone of inhibition was measured and taken as to measure antibacterial activity of Ni-PPG nanocomposites. The glass substrate coated with thin films of Ni-PPG nanocomposites showed a zone of inhibition of diameter 4.2 cm against bacterial strain Staphylococcus aureus MTCC 1809 and a diameter of 4.4 cm against Escherichia coli MTCC 723. Glass substrate without any coating (figure 4c) were taken as negative control to observe the effective zone of inhibition. The percent inhibition was counted as to show the bacterial growth inhibition rate calculated by the formula:

$$\% \text{ inhibition} = \frac{(\text{treated surface diameter-control diameter})}{(\text{treated surface diameter})} \times 100$$

Table-3.1 Percent inhibition data of bacterial strains (1) Staphylococcus aureus MTCC 1809 and (2) Escherichia coli MTCC 723 (3) control against Staphylococcus aureus MTCC 1809 (4) control against Escherichia coli MTCC 723

S. No.	Bacterial strains	Diameter of Coated substrate/ negative control (cm)	Diameter of Zone of inhibition(cm)	Percent inhibition(%)
1	**Staphylococcus aureus MTCC 1809**	2	4.2	52.3
2	**Escherichia coli MTCC 723**	2	4.4	54.5
3	**Staphylococcus aureus MTCC 1809**	2	0	0
4	**Escherichia coli MTCC 723**	2	0	0

Table-3.1 shows the % inhibition data of both the bacterial strains and their controls. It can be clearly revealed from the results that thin films of Ni-PPG nanocomposites posses inhibitory activity against both the bacterial strains Staphylococcus aureus MTCC 1809 and Escherichia coli MTCC 732 and we can conclude that the thin films of Ni-PPG nanocomposites are effectively able to show the antibacterial activity.

3.5.2 Antifungal Study

(a) **(b)**

Fig. 3.5(a): Growth of Neurospora crassa against Ni-PPG nanocomposites coated thin films 3.5(b):Growth of Neurospora crassa against uncoated glass substrate

Fig. 3.5(a) shows the antifungal activity of Ni-PPG nanocomposites thin films against fungus Neurospora crassa. A clear zone of inhibition can be observed showing a zone of 3.9 cm and a percent inhibition of 48.7%. Fig. 3.5(b) shows a clear growth of fungus around and upon the uncoated glass substrate as negative control.

CONCLUSION

The present research deals with the successful synthesis of Ni-PPG nanocomposites using chemical reduction method. Characterization techniques FTIR, XRD and TEM confirmed the synthesis of Ni-PPG nanocomposites. The present study revealed that nanocomposites formed were well organised spherical shape with size 24-30 nm. Thin films of nanocomposites of Ni-PPG prepared using a very simple approach, spin coating method exhibited a very good % inhibition activity against both Gram positive and Gram negative bacterial strains, fungus and low wettability nature that can be well employed in packaging industry as a barrier between packed food and external environment. The effective antibacterial and antifungal activity of thin films can be enhanced by changing the parameters used for coating purpose. It can be foreseen that Ni-PPG thin films can be used as

an antimicrobial coating in food packaging industry. The nanocomposites prepared posses properties of both metal and polymer used, so they can be applied to broader range of applications as compared to individual part.

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