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Using mechanical technique for preparing antibacterial offset lithography ink with ZnO nanoparticles

Maryam Ataeefard [a,*], Fereshteh Mirjalili [b,1]

[a] Department of Printing Science and Technology, Institute for Color Science and Technology, P.O. Box 16765-654, Tehran, Iran
[b] Department of Polymer Engineering and Color Technology, Amirkabir University of Technology, P.O. Box 15875-4413, Tehran, Iran

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ABSTRACT

This paper presents a study on antibacterial performance of an offset lithography/ZnO nanocomposite ink prepared using a mechanical mixing technique. The hybrid ink exhibited very good antibacterial activity against Gram-negative *Escherichia coli* and Gram-positive Bacillus *Staphylococcus aureus* bacteria. The thermal properties of the pure and nanoparticle loaded ink films were evaluated using TGA and DSC techniques. Moreover, the quality of dispersion of ZnO nanoparticles within the ink matrix was characterized by means of Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and Fourier Transform Infrared Spectroscopy (FTIR).

1. Introduction

Offset lithography is the dominant method of printing in commercial use today. In this printing technique, there are coplanar image and non-image areas on a printing plate which are separated thorough differences in surface energy [1]. Application of the ink in this method as a film between 1 and 2 μm thick onto the substrate is done using a series of rubber-covered rollers. Offset lithographic inks are complex pastes in which a colored organic pigment is suspended in an oil based solvent and alkyd resin mixture [2]. After being applied, the ink film hardens thorough a two-phase process, including loss of solvent followed by cross-linking of the resin which then bind the pigment particles together [1,2].

As one of the most popular ways of creating printed matters, the most common applications of offset lithography include newspapers, magazines, brochures, stationery, and books [2,3]. Compared to other printing methods, offset lithography is best suited for cost-effectively producing large volumes of high quality prints in an economically sound manner that requires little maintenance [2]. In many of those applications, special properties like antibacterial performance are required.

In recent years, there have been a considerable number of studies dealing with the effect of nanostructured inorganic materials on the properties of organic–inorganic hybrid coatings [4–6]. In such hybrid coatings, the strong interfacial interaction between the nanofiller and the polymeric coating matrix, due to the nanometric size and large specific surface area of the nanofillers, results in coatings with particular properties [7].

In many industries such as pharmaceutical and food packaging industries, microbial contamination has been considered as a serious issue. In such industries, the development of antimicrobial agents, especially nanostructured coatings with antimicrobial properties has been attracting increasing attention in recent years [8,9].

Zinc Oxide (ZnO) is an environmentally friendly material with low level of toxicity, which is widely used as a dermatological active ingredient in creams, lotions and ointments due to its effective antibacterial activities [10]. In addition, ZnO nanoparticles have been shown to be useful antibacterial and antifungal agents when used as a surface coating on materials and textiles [8].

Moreover, incorporating nanostructured ZnO into polymeric matrices improves the mechanical strength of the resultant nanocomposite as a result of the strong interactions between the inorganic nanoparticles and the polymeric organic groups [11].

The bactericidal performance of ZnO has been demonstrated in both microscale and nanoscale formulations [12]. However, it has been shown that ZnO nanoparticles (10–50 nm) exhibit better antimicrobial properties than bulk ZnO (2 μm). The antibacterial activity of ZnO nanoparticles is due in part to their electrostatic interaction with cell surfaces as a result of releasing hydrogen peroxide (H_2O_2) [8,13]. It has been found that metal oxide nanoparticles also cause membrane damage [14]. In this regard, the

* Corresponding author. Tel.: +98 21 22969771; fax: +98 21 22969776.
E-mail address: ataeefard-m@icrc.ac.ir (M. Ataeefard).
[1] Tel.: +98 21 64542467; fax: +98 21 66469162.

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production of particle-induced Reactive-Oxygen-Species (ROS) production and oxidative injury inside bacterial cells has become an established paradigm underlying the ZnO antibacterial mechanism [15].

As an inorganic additive with selective toxicity to bacteria, but minimal effects on human cells, ZnO powders and nanoparticles have exhibited antimicrobial activity against both Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* bacteria [16].

The antimicrobial properties of surface coatings containing ZnO nanoparticles have been studied [17]. However, there are no comprehensive studies on the effect of ZnO nanoparticles on antibacterial properties of printing inks. Antimicrobial coating and ink are of great interest for protection of surfaces, since survival of microorganisms on surfaces in the environment can result in the spread of the diseases [18].

Therefore, the present study was carried out with an objective to prepare a hybrid nanoZnO-lithographic ink, and characterize the antibacterial properties of the resultant ink against the most prevalent species of Gram-positive and Gram-negative bacteria namely *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*), respectively. In order to prepare the nanoZnO containing inks, a mechanical technique using three roll mills as a feasible and common industrial method was employed. Furthermore, the thermal and morphological properties of the hybrid ink were evaluated by means of Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and FT-IR spectroscopy.

2. Experimental

2.1. Material

A nanozinc oxide (nZnO) powder with the average particle size of 20–30 nm, confirmed by High Resolution SEM results, was obtained from TECNAN Co. (Iran, Tehran). The bacterial species, *Staphylococcus aureus* (*S. aureus*, ATCC 6538) and *Escherichia coli* (E. coli, ATCC 8739) were used as Gram-positive and Gram-negative bacteria, respectively. The offset lithography ink (LI) was a web cold-set solvent-based cyan ink supplied by Persia Ink Co. (Iran, Tehran). Typical formulation of the used ink is as follow [2]:

Organic pigment (Phthalocyanines)	18.00
Quickset varnish (low solubility modified rosin ester cooked into linseed oil)	40.00
Gloss varnish (low melting point modified rosin ester high solubility and long oil alkyd)	15.00
Fast setting varnish (soluble modified rosin ester)	15.00
Polyethylene wax paste	5.00
Anti set-off paste	3.00
Cobalt/manganese driers	1.00
280–320 °C petroleum distillate (solvent)	3.0

The application of the ink was performed using a laboratory K-Printing Proofer on paper and glass with RK Print-Coat Instruments and K Hand Coater respectively (UK).

2.2. Preparation of LI–ZnO nano-ink

LI–nZnO hybrid inks were prepared by adding certain calculated weight fractions of nZnO powder (1, 3 and 5 wt.%) into the lithography ink. After an initial mixing, the nZnO–lithography ink mixtures were milled using three roll mills for five times. The prepared LI–nZnO hybrid inks are coded as LI0, LI1, LI3, and LI5, Which LI0 refers to the pure ink without any loads of nZnO.

2.3. Characterization of LI–ZnO nano-ink

The antimicrobial tests were performed using the agar-well diffusion method as a semi-quantitative antibacterial test technique, according to ISO 22196 [19]. As mentioned before, two types of bacterial strains, namely *Staphylococcus aureus* (*S. aureus*), ATCC 6538 and *Escherichia coli* (*E. coli*), ATCC 8739 were used to conduct the antimicrobial tests. For this purpose, the typical procedure was carried out as follows.

Bacterial cultures were grown overnight on a nutrient agar media. The cultures were then transferred into a flask containing nutrient broth and allowed to grow at 35 C for 16–20 h. At the beginning of the logarithmic phase, the cultures were centrifuged and washed twice with a saline solution to yield a final bacterial concentration of approximately 4×10^5 Colony-Forming Unit (CFU) ml^{-1}. The ink samples were placed in a vial containing saline in which the strain cells were then pipette into. All samples were allowed to grow at 35 °C for 24 h. At the end of the incubation period, the samples were gently rinsed three times in a sterile solution of NaCl 0.9% in order to eliminate the non-adherent bacteria. The number of viable bacteria was monitored with a colony counter by counting the number of Colony-Forming Units (CFUs) from the appropriate dilution on nutrient agar plates.

In order to determine the relative number of removed bacteria, the term "log Reduction" was calculated according to the following equation:

$$\log \text{Reduction} = \log A - \log B \quad (1)$$

In this equation, A and B is the average number of bacterial colony colonies in the untreated and treated samples, respectively [20–22]. The bacteria's reduction percentage was also calculated using the following equation:

$$R\% = \text{ReductionPercentage} = [(A - B/A] \times 100 \quad (2)$$

Thermal analysis was carried out using a Shimadzu DSC-60 differential scanning calorimeter (Kyoto, Japan). Nitrogen gas was used to provide an inert atmosphere. The sample and the reference pan were heated up to 150 C with a heating rate of 10 °C min^{-1}. The thermal stability of the samples was measured using a Shimadzu thermo gravimetric analyzer (Kyoto, Japan). The samples were heated from 25 to 600 C with a heating rate of 10 °C min^{-1} under nitrogen atmosphere.

The micrographs from the cross section of the samples were prepared using a Leo 1455VP Scanning Electron Microscope (SEM) (Oxford, UK). The quality of dispersion of ZnO nanoparticle was also monitored using Philips CM120 Transmission Electron Microscope (TEM), (Eindhoven, Netherlands). The preparation of samples for TEM analysis was carried out at the Institute of Biochemistry & Biophysics, Tehran University (Tehran, Iran). The samples were scanned by TEM at an accelerating voltage of 100 kV.

Fourier Transform Infrared (FTIR) spectra of samples were recorded by means of a Perkin–Elmer Spectrum One spectrometer. FTIR spectroscopy was carried out using KBr pellets prepared from LI–ZnO nanocomposites.

The color measurement of the samples was carried out using the Gretag Macbeth ColorEye 7000A spectrophotometer (Gretag Macbeth, Company USA). For this purpose, five replicates for each sample were prepared. The spectral reflectance factors of all samples were determined and then transformed into CIELAB color coordinates (L^*, a^* and b^*) by the instrument's software using CIE standard illuminant D65 and the CIE 1964 standard colorimetric