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Antibacterial flexographic ink containing silver nanoparticles

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ABSTRACT

The current work deals with the effects of incorporation of silver nanoparticles on the antibacterial and the thermal properties of a flexographic ink. The stable and uniform dispersion of silver nanoparticles in the ink were confirmed by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The thermal properties of the pure and nanoparticle loaded ink films were also evaluated using TGA and DSC techniques. The results from this study proved acceptable dispersion characteristics, wherein, the flexographic ink showed a significant antibacterial activity against Gram-positive and Gram-negative bacteria

1. Introduction

Roll-to-roll printing techniques such as gravure or flexographic printing have traditionally been employed to print newspapers, magazines and packages [1]. The flexographic printing is a two-step method in which the ink is, first, transferred to a roll covered with a patterned flexography rubber. Then the rubber transfers the pattern onto a printable substrate [2].

Flexographic printing technique is convenient for its guaranteed high quality printing on substrates while keeping the manufacturing costs reasonably low. Besides, flexographic printing technique is one of the simplest methods of printing used in decorating or packaging applications. Correspondingly, the flexographic printing technique has been growing and being considered in the recent years and it is still progressing [3].

Flexographic printing is a standardized method and offers many advantages comparing to other techniques to both academic and industrial users. Beside the continuous process improvements, innovations have strengthened the technique for high-quality printing [4]. A functional flexographic ink must show several qualities by: (1) producing colors or other visual effects, (2) adhering well to the substrate, (3) withstanding chemicals attacks, abrasion, and extreme temperatures condition in practice, and (4) producing consistent finishes [3]. In this respect many works have been done to improve the quality of the printing ink. Using nanoparticle is one of the common improvements that have been done in the printing ink and coating.

Employing inorganic nanomaterials as effective additives to improve printing has been successful. Consequently, different kinds of nanoparticles have been proposed and tested in order to produce organic/inorganic nanocomposite printing ink [5,6]. Such systems could claim property improvements of an organic polymer including rheology, optics, dielectrical and so on, with incomparable advantages of nanoparticles [7].

Several studies have been devoted to demonstrate the variety of characteristics of this particular class of organic/inorganic hybrid nanocomposites. The researches have studied the morphological [8], mechanical [9], thermal [10] and antimicrobial [11] properties of these systems to improve the quality of prepared coatings and ink; however, researchers have not been involved with the development of organic/inorganic nanocomposites concerning the antibacterial applications.

Recent studies suggest that silver (Ag) nanoparticles draw many interests for their unique and powerful antibacterial activities against a wide spectrum of bacteria [11,12]. It has been generally agreed that the antimicrobial materials, while constituting silver nanoparticles, are more significant concerning antibacterial in comparison with various types of metallic nanoparticles, e.g. copper, mercury, tin, chromium, and lead [13–15]. In recent years the impression of silver nanoparticles toward antibacterial characteristics has been successfully achieved in the variety of medical applications, from wound dressings to urinary catheters [16].

The degree of activity of the Ag^+ ions strongly bounds to electron donor groups in a biological molecule, e.g. sulfur, oxygen or nitrogen, which severely determines the antibacterial characteristics of

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silver. On the other hand, the cytotoxicity effect, as a consequence of the excess amount of silver released from the coating, should be considered. Thus, the content of silver nanoparticle is required to be optimized, in order to represent an ink with controlled antibacterial properties [17].

The exact antimicrobial mechanism of silver is not known yet; however, several mechanisms have been postulated for the antimicrobial activity of silver: (1) adhesion of nanoparticles at the surface causes deformation of the membrane which leads to an increase in the membrane permeability, (2) nano Ag particle penetration into the bacteria cell results in DNA damages, (3) dissolution of nano Ag particles releases antimicrobial Ag ions. It has been cleared that the free silver ion combining thiol (SH) groups of cellular components, such as protein causes inactivation of bacteria [12,15].

In the past decade, flexographic printing has successfully penetrated new printing markets and has grown substantially. Several factors are important in this respect. One is the increase use of the flexible packaging [3,4]. Since the printed substrates are normally in close contact with human, the use of antibacterial packaging becomes common for many years [18], while the idea of using antibacterial ink is yet a novel. In this study, the attempt was made to exploit silver nanoparticles in a flexographic printing ink as an antimicrobial agent; subsequently the antibacterial flexographic ink was used in the packaging industry. However, the nanosilver/flexography ink nanocomposite had been prepared to increase conductivity of the ink [19]. With this view, different amounts of a silver nanoparticle were incorporated into the flexographic ink. Thermal, morphological and antimicrobial properties of the resultant nanocomposite were studied using TGA, DSC, SEM, TEM, and FTIR techniques.

2. Experimental

2.1. Materials

A spherical nanosilver (nAg) dispersion in mono ethyl glycol (3780 ppm) confirmed by ICP results, with an average diameter of 50 nm was obtained from the Sharif Nano Pigment Co (Tehran, Iran). 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. Flexographic ink (FI) was a cold-set solvent-based cyan ink purchased from the Persia Ink Co. (Tehran, Iran). A typical formulation of the used ink contains about 20% of Organic pigment (Phthalocyanines), 16% of maleic resin varnish, 38% of nitrocellulose varnish, 38% of wax compound, 4% of plastisizer, 11% of methylated spirits, and 7% of isopropyl acetate.

2.2. Preparation of FI–nAg nano-inks

FI-nAg nano-inks were prepared by adding certain calculated amounts of silver nano particle suspension (0,100, 200, 300 ppm ml^{-1}) into FI. The four prepared FI–nAg nano-inks were coded as FI0, FI1, FI2, and FI3, respectively. The mixtures were then sonicated for 15 min with a sonication frequency of 0.5 kHz and the power of 70 W ml^{-1}. The application of the nano-ink was performed with the K Hand Coater (UK) on the paper and glass. The nano-inks were then allowed to remain for one week at room temperature.

2.3. Characterization of FI–nAg nano-ink

The agar-well diffusion method was employed to determine the antimicrobial activities of FI–nAg in accordance with ISO 22196 [20]. As mentioned before, two types of bacterial strains, namely *S. aureus*; ATCC 6538 and *E. coli*; ATCC 8739 was used to conduct the antimicrobial tests.

The typical procedures to evaluate the antibacterial activity of FI–nAg were as below. The bacterial cultures were grown overnight in a nutrient agar medium. The grown cultures were then transferred to a flask containing a nutrient broth in which they were allowed to grow at the 35 °C for 16 to 20 h. At the beginning of the logarithmic phase, they were centrifuged and washed twice with a saline solution to yield a final bacterial concentration of approximately 4×10^5 CFU (colony-forming unit) ml^{-1}. The samples were placed into a vial containing saline solution, and the bacteria cells were then pipette into the vial. The samples were then allowed to grow at the 35 °C for 24 h, after which they were counted to determine the number of viable bacteria. At the end of the incubation period, the samples were gently rinsed three times in a sterile solution of NaCl (0.9%) to eliminate the non-adherent bacteria.

Three samples were prepared for each antibacterial test, and each test was performed at least two times to ensure reproducibility for all experiments. 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 Eq. (1):

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

where A and B is the average number of bacterial colony colonies on the untreated and treated samples, respectively [21–23]. The bacteria's reduction percentage was also calculated using Eq. (2):

$$R\% = \text{Reduction percentage} = \left[\frac{(A-B)}{A}\right] \times 100 \quad (2)$$

Thermal analysis was carried out using a Shimadzu differential scanning calorimeter, DSC–60 (Kyoto, Japan). Nitrogen gas was used to provide an inert atmosphere. The samples 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 determined using a Shimadzu thermogravimetric 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 morphology of the nanocomposites was characterized from the fractured cross sections of the nano particle loaded ink thin films using a Leo 1455VP scanning electron microscope (SEM), (Cambridge, UK). In addition, the quality of silver nanoparticles dispersion in the ink was evaluated with the aid of a Philips CM120 transmission electron microscope (TEM), (Eindho-ven, Netherlands). The samples were scanned by TEM at an accelerating voltage of 100 kV.

Fourier transform infrared (FTIR) spectra from the samples were recorded by a Perkin-Elmer Spectrum One spectrometer. FTIR spectroscopy was carried out using KBr pellets prepared from FI–Ag nanocomposites.

The color measurements for the samples were carried out using the Gretag Macbeth ColorEye 7000A spectrophotometer (Gretag Macbeth Company, USA). For this purpose, five replicates for each sample was done. 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 observer. The color measurement for each sample was performed on three different points from the sample.

The total changes in color of the samples (ΔE_{Lab}) were calculated using the CIE 1976 color difference equation (Eq. (3)).

$$\Delta E_{Lab} = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2} \quad (3)$$

where ΔL^*, Δa^*, and Δb^* represent the differences between the initial and final values of L^*, a^*, and b^*, respectively. An increase in

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