Progress in Organic Coatings 121 (2018) 130–141

Contents lists available at ScienceDirect

Progress in Organic Coatings

journal homepage: www.elsevier.com/locate/porgcoat

Antimicrobial ecological waterborne paint based on novel hybrid nanoparticles of zinc oxide partially coated with silver

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ARTICLE INFO

Keywords:
Zinc oxide-silver nanoparticles
Zinc pyrithione
Antimicrobial potential
Antibacterial activity
Antifungal activity

ABSTRACT

Antimicrobial activity of Zinc oxide partially coated with silver nanoparticles produced by Flame Spray Pyrolysis (FSP) as an additive to include in waterborne paints with promising applications in areas as schools, clinics or hospital environments and in food industry equipment and facilities has been demonstrated. Due to the current increase of multi-resistant microorganisms, the results obtained in this research would enable an enhanced sanitary conditions to avoid health problems of professionals and improve the Hazard Analysis Critical Control Point (HACCP) in the Food Industry like a way to control the biofilm formation on surfaces and beyond.

The nanomaterial was prepared by the use of Flame Spray Pyrolysis process obtaining nanoparticles of about 35 nm coated with ca. 5% (w/w) of silver (Ag). Antimicrobial trials showed that these ZnO-Ag nanoparticles has a potential effect against different bacteria (*Staphylococcus aureus*, *Pseudomona* spp, *Salmonella* spp, *Bacillus subtilis*, *Listeria monocytogenes*) and molds as *Aspergillus niger*. Furthermore, these nanoparticles were evaluated as an antimicrobial waterborne paints additive, and formulations with 0,15% (w/w) showed > 5 mm inhibition zone against the main bacteria and molds tested.

1. Introduction

Incorporation of antibacterial agents (biocides) in specific products can inhibit the growth of microorganisms on the surfaces of products when a possible microbial growing is able to occur. This technology is significant for the quality of life, not only in developed countries but also in developing countries [[17–19]]. These products can be used in different applications such as leather, stainless steel, plastics, ceramics, coating materials, etc and are of increasing importance in areas such as healthcare, home and personal hygiene, foods, active packaging, automotive and textiles. One of the main objectives of this technology is to control harmful microorganisms. However, these systems have to guarantee some characteristics, such as strong antibacterial efficacy, environmental safety, low toxicity, cost effectiveness and easy of fabrication [2]. Infections caused in hospitals in the last decades are related to resistant antibiotics bacteria such as strains of methicillin resistant *Staphylococcus aureus* (MRSA). Percentages of hospital infections as high as 70% have been attributed to MRSA strains that result in high mortality rates. This problem needs to be controlled using new techniques to avoid hospital infections caused by microorganisms such as MRSA [3,4]. On the other hand, some microorganisms such as *Listeria monocytogenes* are able to colonize environmental surfaces and produce a three-dimensional matrix of extracellular polymeric substances (EPS) called biofilm that can contaminate food products. Antimicrobial agents can lead to an inhibition of *L. monocytogenes* in the biofilm state due to a lower susceptibility [5,6]. Nanoparticle materials (NPs) have received increasing attention in recent years due to their different properties from their conventional counterparts [7]. Antimicrobial activity of different NPs including silver [8–11], copper [12,13], titanium dioxide [14–16] and zinc oxide [17–19] have been reported. The antimicrobial properties of silver (Ag) is based on the strong interaction between silver (Ag^{+} or NPs) with thiol groups present in the enzymes involved in the bacterial cell metabolism thus causing the cell death [20]. The antibacterial properties of Ag-NPs are influenced by morphology, size of NPs, and level of partial oxidation [21], and it has been demonstrated that materials with larger surface area show higher antibacterial activity [20–22]. The promising antibacterial activity against several microorganisms related to silver-based

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https://doi.org/10.1016/j.porgcoat.2018.04.018
Received 22 September 2017; Received in revised form 28 March 2018; Accepted 14 April 2018

materials has reemerged [23,24]. Inks, plastics or coatings are evaluated their bactericide activity including different silver materials (NPs or ions) [[29–31],25–28]. However, few antimicrobial additives based in silver, copper or zinc oxide NPs have been tested in coatings [29–31]. Zinc oxide nanoparticles (ZnO NPs) are unique in that they can be produced with high surface areas and with unusual crystal structures [32]. The microbiological properties of zinc have also been tested against different bacteria: *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens*, *Listeria monocytogenes*, *Salmonella senftenberg* and *Staphylococcus aureus* [17,19,33]. Recent interest lies in their NP forms. It is believed that the smaller the size of ZnO, the stronger antimicrobial activity it has [34]. Preliminary studies show that the antibacterial activity of ZnO NPs might be related to the formation of free radicals on the surface of the NPs, and the damage to the lipids in bacterial cell membrane by free radicals, which consequently lead to the leakage and breakdown of bacterial cell membrane [35,36].

As described before antimicrobial biocides are incorporated in small quantities in several products including paints to prevent microbial growth and to extend the functionality and esthetic appearance. The biocides should remain active as long as they are incorporated in the paint. Protection against microbial colonization should last at least a decade [37,38]. The placing of biocides or compounds with biocidal effects on the European market is regulated for the environment according to the EU Regulation 528/2012 (Biocidal Product Regulation or BPR). The main goal of the biocides for paints is in can and dry film protection (PT6 & PT7) [39]. Nevertheless, it is possible to develop new waterborne paints based in silver and zinc oxide NPs which show broad spectrum bactericide activity and it can be tested under the international standard ISO 22196:2011 was modelled after Japanese standard JIS Z 2801 [1]. The Ecologic paints on the European market is regulated for the environment according to the EU Commission Decision of 28th May 2014 establishing the ecological criteria for the award of the EU Ecolabel for indoor and outdoor paints and varnishes [40]. This regulation marks the criteria both technical, environmental and safety that have to accomplish a paint or varnish for getting the EU Ecolabel. For other markets we can find similar regulation as Green Seal for North America, Nordic Ecolabelling for the Nordic countries or Blue Angel for Germany.

All paints contain the same primary constituents; a matrix or binder, pigments and extenders (which confer color and build) and a solvent. The solvent is either organic in nature, for a solvent based paint, or water, for a water-based or latex emulsion paint. In addition to these common components, there are several other ingredients, which make up about 5% of the total system. These include emulsifiers (surfactants), defoamers, wetting agents, dispersants, biocides, co-solvents, plasticizers or coalescents, hydrophobic agents (silanes and polysiloxanes), extenders (colloids) and thickeners, based in organic materials like polymers (polyurethane or acrylic rheological additives) or cellulose, and modified inorganic minerals like bentonites, laponites, sepiolites or zeolites. Paints can also be classified in base of its kind of binder. It can be inorganic like on silicate paints produced with sodium or potassium silicate, or with colloidal silica (sol silicate paints). If the binder is organic, we can find natural latexes or synthetic polymers like alkyd, acrylic, poly (vinyl acetate), etc [41].

This work shows the bactericidal activity of a waterborne paint formulated with zinc oxide nanoparticles partially coated with silver and the effectiveness of the surfaces coated with this paint against different kinds of microorganisms such as bacteria and molds, responsible of the most important health problems in Sanitary Sector and Food Industry.

2. Materials and methods

2.1. Waterborne paint preparation

The following formulation was used to prepare a white waterborne paint: 40.0–45.0% distilled water, 0.30–0.50% rheological additive (Borchers Chemie GmbH, Germany), 0.40–0.60% thickener (SE Tylose GmbH, Germany), 0.30–0.45% defoamer (BYK GmbH, Germany), 0.10–0.30% dispersant (Elementis Specialties, UK), 0.10–0.30% wetting agent (Borchers Chemie GmbH, Germany), 15.00–20.00% titanium dioxide (Kronos GmbH, Germany), 4.00–6.00% micronized calcium carbonate (Reverté Minerals, Spain), 6.00–8.00% micronized talc (Minerales Roset, Spain), 6.00–8.00% Refined kaolin (Dorfner Minerals GmbH, Germany), 15.00–20.00% vinyl acetate/ethylene (VAE) emulsion (Celanese Emulsions GmbH, Germany). A high-speed disperser Netzsch Mastermix (Germany) of 1.1KW model was used to get an appropriate dispersion. The following biocide was used to prepare the control samples: Zinc pyrithione, CAS 13463-41-7 (Lonza GmbH, Germany), purity 98.8% and *n*-butyl-1,2-benzisothiazolin-3-one, CAS 4299-07-4 (Lonza GmbH, Germany), purity 99.6%.

2.2. Characterization of ecological paint

The specific weight, measured with a pycnometer of 100cc (UNE EN ISO 2811, ASTM 1475), was 1.38 g cm^{-3}. The pH, measured with a pH-meter (Crison 25, Spain) was 8.2 ± 0.2 (at 23 °C ± 0.5), and the viscosity, measured with a Viscometer model Neurtek (Spain) First RM (ASTM/EN ISO 2555 n°3 to 250 rpm, at 23 °C ± 0.5), was 1436 ± 100 mPa s.

The paint was tested and classified under the standard UNE EN 13300:2002 [42], necessary for accomplish with the EU Ecolabel technical criteria. The VOC and SVOC were measured under the standard ISO 11890-2:2013, by the external laboratory Tecnalia Research and Innovation (report num. 14_06667) [43].

The content of formaldehyde (UV/Visible Spectrophotometry), the covering and performance (UNE EN ISO 6504-3:2007), and the wet scrub resistance (UNE EN ISO 11998:2007) of the ecological paint were tested by the external laboratory Tecnalia Research and Innovation (report num. 13_03411) [44].

For the Tg measurements a differential scanning calorimeter (DSC) Mettler Toledo 12E with a DSC822 oven was used and a cooling unit with liquid nitrogen. For calibration, the fusion points of great zinc and indium purity were used. The calibration was checked with indium before each period of measurements. Nitrogen atmosphere was used and an empty aluminum capsule was used as a reference for the measurements. To measure the Tg of the dry films, small aliquots of about 10 mg were determined with a precision of 0.02 mg in an Mettler Toledo AG285 electrobalance. First, an empty aluminum capsule in the electrobalance was weighed. Next the piece of film in the capsule was put, while making sure that there was good thermal polymer-capsule base contact, the lid is put in place (which has had a hole in it), is closed and placed in the DSC chamber. Two temperature scans were carried out with scans of 20 °C/min and only the second scan was taken into account. The Tg was selected as the inflexion point of the thermogram, the difference of the onset temperature and the endset temperature was taken as the transition width (ΔT) and the quotient of energy exchange and sample mass as the heat transition capacity (ΔCp). Unless otherwise specified, the following standard protocol is used,

$$\text{r.t.} \rightarrow -50\,^{\circ}\text{C} \rightarrow 100\,^{\circ}\text{C}\ (3\ \text{min}) \rightarrow -50\,^{\circ}\text{C} \rightarrow 100\,^{\circ}\text{C} \rightarrow 25\,^{\circ}\text{C}$$

The morphology of the paint was observed by scanning electron microscopy (SEM) at 500, 50,000 and 200,000 magnifications. The following protocol was employed: metallization of surfaces with a coating of gold-palladium alloy using sputter-coater (Polaron Range SC7620 model) and getting pictures from surfaces at three different scales with a scanning electron microscope Hitachi S-3000N.

For the evaluation of antibacterial activity, tests were carried out on two types of surfaces: one side of a glass surface (50 × 50 mm) and another one on polypropylene thin film and then, the dry film of paint was removed.