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Variables affecting the antibacterial properties of nano and pigmentary titania particles in suspension

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Abstract

The antibacterial activity of photoactivated nano and pigmentary titania particles in suspension was evaluated using *Escherichia coli* as the target organism. The antibacterial activity of nanotitanium particles was determined more by their intrinsic ability to generate radicals than to particle size. Indeed there was an inverse relationship between particle size and activity. The antibacterial activity of the particles was affected by multiple experimental parameters. The reliability and variability of the results were affected by the physiological status of the bacterial cells, the initial cell concentration, and the set up of the irradiation system and were also improved if the cell–particle mixture was stirred during irradiation. The development of appropriate in vitro testing methods is essential in the determination of antimicrobial effectiveness of these particles and this is examined here coupled with the use of the microwave spectroscopic method for determining the photoactivity of the pigments in terms of carrier generation.

Keywords: Photocatalytic; Antibacterial; Titanium dioxide; Pigments; Nanoparticles; *Escherichia coli*

1. Introduction

When light energy of wavelength below 400 nm is utilised by the photocatalytic action of titanium dioxide, the free radicals produced can decompose or destroy foreign materials. An antibacterial effect has been demonstrated, with several groups describing antimicrobial effects of suspensions of particles, or thin films [1–6]. Most of this work has been carried out using the anatase pigment P25 (Degussa Chemical Company, Germany).

The thermal and photocatalytic activity of a selection of nano versus micron-sized anatase and rutile titania pigments in polyethylene and alkyd based paint films has already been assessed in our laboratories [7–10]. Preliminary experiments demonstrated an antibacterial effect of the pigments by irradiation of a mixture of bacterial cell suspensions and pigment mixtures [9–11]. Further work was carried out to clarify the relative activities of the pigment series in terms of antibacterial effects, and to define experimental variables which may affect a demonstrable in vitro antibacterial activity. We believe that this is the first study using a range of nanoparticle TiO_2 pigments with different particle sizes for monitoring the inactivation/destruction of bacteria. Coupled within this study is the use of the photodielectric microwave method for analysis and comparing the titania activities for cross-referencing [12,13].

2. Experimental

2.1. Materials

A range of anatase and rutile pigments (supplied by Millenium Inorganic Chemicals, Grimsby, UK) were used in the

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study (Table 1). The most active photocatalysts are formulations based on the anatase crystal phase, and most work [15,16] has been carried out on pigment B (Degussa P25). In contrast to the remaining pigments, which were supplied as powders, pigment C is a colloidal dispersion of nanoparticles (colloidal suspension of TiO_2, 14–16%, stabilised with an amine, with a pH of 10–11.5). This suspension was neutralised with HCl to remove the amine, and to reduce the pH, and was then washed with water to remove the salt. It consisted of 1–10 nm primary particles, as determined by XRD, with 45–50 nm agglomerate size as indicated by transmission electron microscopy. This colloidal dispersion is the precursor product from which the rest of the pigments were produced, either by drying (pigment G), or calcining at increasing temperatures (pigments F, E, A). Pigments were characterised using microwave spectroscopy [7].

Stock suspensions were prepared by mixing 0.05 g TiO_2 in 5 ml deionised water. The dispersion was vortex mixed for 1 min, and was then diluted to 25 ml in order to produce a 0.2% stock suspension, from which required concentrations were prepared using deionised water.

2.2. Culture preparation

Escherichia coli NCTC 9001 was used as a model microorganism for all inactivation studies. In preparation for inactivation experiments, one colony from a 24 h culture on nutrient agar was inoculated into 10 ml nutrient broth and incubated at 37 °C for 24 h. Washed cell suspensions were prepared by centrifugation of cultures for 10 min at 3000 rpm. The supernatant was discarded and the pellet was re-suspended in deionised water, three times, with the final re-suspension in 10 ml deionised water, to prevent carryover of nutrient from the original culture medium [17], and hence any unintentional increase in cell numbers, or effect on cell physiology [19]. The optical density of the suspension at 460 nm was taken, and its value adjusted to approximately 0.91 by adding or removing (by centrifugation and re-suspension in a reduced volume) water as necessary. The concentration of the suspension was determined by diluting 1 ml of the sample 10-fold, plating 0.1 ml of each dilution onto nutrient agar in triplicate, and counting the resultant colonies. It is assumed that one cell (or one cluster of cells) will multiply on the medium to produce a visible colony.

The dilution at which the maximum number of countable colonies was obtained was used to calculate the number of viable cells per milliliter of original suspension. This was approximately 10^8 colony forming units (cfu) per ml. One milliliter of 10^6 cfu/ml was mixed with the required amount of TiO_2 stock suspension, and then diluted with deionised water to give a total volume of 25 ml with a final cell concentration of 10^5 cfu/ml.

2.3. Irradiation

After vortex mixing, the 25 ml cell–pigment mixture was poured into a 100 ml Petri dish on a magnetic cold plate and irradiated. Illumination was provided by a Hg–Zn–Cd (0.9 A) lamp (Philips) located 10 cm from the suspension. The suspension depth was approximately 5 mm. The mixture was mixed continuously using a sterilised Teflon coated magnetic stir bar placed in the Petri dish. Samples of 1 ml were removed at specific times during irradiation, and were diluted 10-fold, with 0.1 ml of each dilution being plated in triplicate onto nutrient agar for determination of survivors (expressed as cfu/ml).

It is important to maintain a septic technique when manipulating microorganisms, to prevent contamination. All materials were sterile. The irradiation experiments were performed at room temperature (25 °C), but during irradiation, the temperature within the suspension could rise to 32 °C.

2.4. Experimental variables

In order to devise the procedure outlined above, a number of experimental variables were monitored in the series of experiments:

(i) *Elimination of wavelengths with intrinsic antibacterial activity*: in later experiments, a Pyrex glass filter was placed over the Petri dish in order to eliminate wavelengths below and around 310 nm, since the Hg lamp emitted a high output below 300 nm, with intrinsic antimicrobial activity.

(ii) *Use of washed cell suspensions*: in later experiments, the cell suspension was not washed, but was mixed with titanium particles in the nutrient broth culture medium.

Table 1
Main properties of pigments used for antibacterial disinfection by TiO_2

Pigment	Crystalline modification	BET surface area (M^2/g)	Particle size	Preparation method	Dispersibility	Treatment
A	Micro-anatase	10.1	0.24 μm	Sulphate route	Medium	1% Alumina
B (Degussa P25)	Anatase-rutile	50	25–35 nm	Flame hydrolysis	Good	
C (colloid)	Nano-anatase	329.1	5–10 nm	Sulphate route	Good	
D	Micro-rutile	12.4	0.11 μm	Chloride route	Poor	3.4% Alumina
E	Nano-anatase	44.4	20–30 nm	Sulphate route	Poor	
F	Nano-anatase	77.9	15–25 nm	Sulphate route	Poor	
G	Nano-anatase	329.1	5–10 nm	Sulphate route	Poor	
H	Nano-anatase	52.1	70 nm	Sulphate route	Medium	Hydroxyapatite