International Journal of Pharmaceutics 479 (2015) 88–95

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Zinc oxide as a new antimicrobial preservative of topical products: Interactions with common formulation ingredients

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ARTICLE INFO

Article history:
Received 28 October 2014
Accepted 13 December 2014
Available online 16 December 2014

Keywords:
Zinc oxide
Excipients
Interactions
Preservative
Topical formulations
Challenge tests

ABSTRACT

Zinc oxide (ZnO) appears as a promising preservative for pharmaceutical or cosmetic formulations. The other ingredients of the formulations may have specific interactions with ZnO that alter its antimicrobial properties. The influence of common formulation excipients on the antimicrobial efficacy of ZnO has been investigated in simple model systems and in typical topical products containing a complex formulation. A wide variety of formulation excipients have been investigated for their interactions with ZnO: antioxidants, chelating agents, electrolytes, titanium dioxide pigment. The antimicrobial activity of ZnO against *Escherichia coli* was partially inhibited by NaCl and $MgSO_4$ salts. A synergistic influence of uncoated titanium dioxide has been observed. The interference effects of antioxidants and chelating agents were quite specific. The interactions of these substances with ZnO particles and with the soluble species released by ZnO were discussed so as to reach scientific guidelines for the choice of the ingredients. The preservative efficacy of ZnO was assessed by challenge testing in three different formulations: an oil-in-water emulsion; a water-in-oil emulsion and a dry powder. The addition of ZnO in complex formulations significantly improved the microbiological quality of the products, in spite of the presence of other ingredients that modulate the antimicrobial activity.

1. Introduction

Zinc oxide (ZnO) is widely used in topical formulations because of several specific properties. It is currently used on a large scale as pigment and sunscreen for its optical properties (Gasparro et al., 1998; Hewitt and Woodruff, 2000; Nesseem, 2011; Serpone et al., 2007), but also as a soothing and protective coating against skin irritation and abrasion. As a pharmaceutical ingredient, it is used to treat diaper rash and minor burns (Arad et al., 1999). An increasing attention is paid to ZnO for the control of bacterial contaminations of aqueous suspensions (Franklin et al., 2007; Jones et al., 2008), by also solid materials such as ceramics (Hewitt et al., 2001; Hirota et al., 2010; Yamamoto, 2001) and food packaging (Perez Espitia et al., 2012). The antimicrobial properties of ZnO are quite attractive for its use as an antimicrobial preservative of pharmaceutical or cosmetic formulations (Pasquet et al., 2014a), although it has surprisingly been sparsely used as preservative in topical formulations. Favet et al. (2001) assessed the antimicrobial properties of ZnO used as an alternative to parabens in the ointment zinc gelatin described in the Swiss Pharmacopoeia.

The antimicrobial activity of ZnO depends on three main mechanisms. (i) A cascade sequence of photochemical reactions coming from the semiconductive properties of ZnO generates reactive oxygen species (ROS) that damage cell membranes of microorganisms (Sawai et al., 1998; Applerot et al., 2009). (ii) The partial dissolution of ZnO particles releases cytotoxic Zn^{2+} species in water (Doménech and Prieto, 1986; Atmaca et al., 1998; Yang and Xie, 2006; Franklin et al., 2007; Padmavathy and Vijayaraghavan, 2008; Pasquet et al., 2014b). (iii) The adsorption of ZnO particles onto the microbial cells destabilizes the microbial cell walls (Zhang et al., 2010).

To this end, Pasquet et al. (2014a) recently investigated the antimicrobial efficiency of various ZnO grades on the five microorganisms used for challenge tests described in the European and US Pharmacopoeia's. The antimicrobial efficacy was assessed on simple ZnO aqueous suspensions poured in broth medium at increasing concentrations. However, topical formulations are quite

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http://dx.doi.org/10.1016/j.ijpharm.2014.12.031
0378-5173/

complex and can contain a large number of ingredients, especially in the field of cosmetics. Some of the ingredients may show a specific interaction with ZnO and disturb its antimicrobial activity. As example, Zhang et al. (2007, 2010),) reported the protective effect of the bacterial membrane by poly(ethylene glycol) dispersing agents (PEG-400 or PEG-2000). Such effect disturbs the interaction by direct contact between the cell membrane and ZnO particles. Similarly, Applerot et al. (2009) reported that propylene glycol and poly(vinyl alcohol) dispersants had negative effects on the antibacterial properties of ZnO.

The aim of the present study was to identify such interactions between widely used ingredients of topical formulations and ZnO by evaluating their influence on the antimicrobial activity of ZnO. The first part was devoted to the investigation of the interactions between ZnO and such usual raw materials used in topical products such as antioxidant, chelating agent, inorganic powders and salts. The second part was an assessment of the antimicrobial efficacy of ZnO in three types of model topical formulations, two emulsions differing by their o/w and w/o types and a dry pressed powder.

2. Materials and methods

2.1. Materials

The ZnO powder from Rockwood Pigments (Beltsville, Maryland, US) was called ZnO-1 as in our previous paper (Pasquet et al., 2014a). This grade of pharmaceutical quality is characterized by a specific area of 39 $m^2 g^{-1}$ and a porosity of 0.18 $cm^3 g^{-1}$ (combined volume of all pores between 1.7 and 300 nm size); the crystallite size is about 15 nm and the apparent density of the dry powder is 0.38 g mL^{-1}. Transmission electron microscopy (TEM) observations were done at the centre 'Centre Technologique des Microstructures' facility (University of Lyon) on a Philips CM120 microscope. A dilute aqueous suspension (0.1%) was spread on formvar/carbon grids and dried before TEM observation at 80 kV acceleration. The TEM picture presented in Fig. 1 shows the platelet shape of the elementary particles. A full account of the characterization has been reported in our previous paper (Pasquet et al., 2014a).

Several ingredients frequently used in topical formulations were investigated for an assessment of their interactions with ZnO:

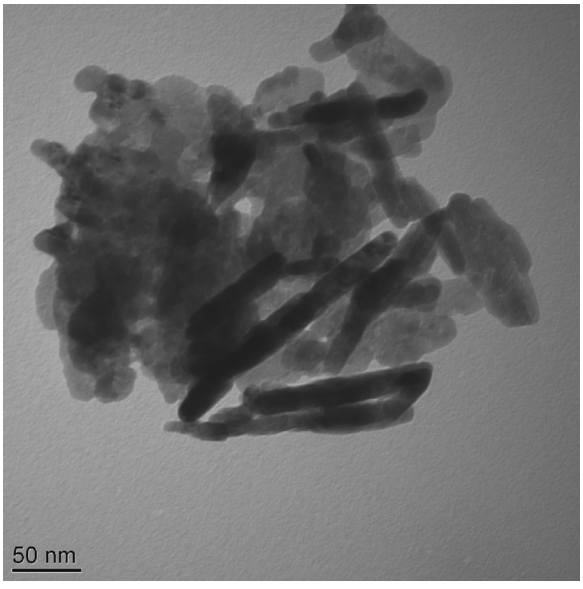

Fig. 1. TEM image of the ZnO-1 particles at ×140 magnification.

three antioxidants differing by their polarity: the hydrophilic ascorbic acid 2-glucoside (AA2G) and magnesium ascorbyl phosphate (MgAsc), and the hydrophobic butylhydroxytoluene (BHT); the chelatant of divalent cations ethylenediaminetetraacetic acid (EDTA), the NaCl and $MgSO_4$ salts widely used to stabilize w/o emulsions, and finally titanium dioxide (TiO_2) which is widely used in make-up and in sunscreens in combination with ZnO. The suppliers and the concentrations used in the present study are given in Table 1.

2.2. Microbiological assessment of the interactions with ZnO

ZnO-1 suspensions containing the raw materials were inoculated with *Escherichia* coli and examined by flow cytometry after 1 day incubation. For that purpose, ZnO-1 suspensions were prepared in the Mueller Hinton broth (AES Chemunex, France) at concentrations of 1% and 2% (w/w). Then, the raw material was introduced in the suspension at its usual concentration (Table 1). A calibrated inoculum of *E. coli* suspension was added to each sample in order to obtain a final concentration of 1.5×10^6 CFU mL^{-1}, mixed by vortex homogenization and incubated overnight at 32.5 °C under gentle agitation (20 oscillations per minute). After 24 h of contact, an aliquot was collected and analyzed for the concentration of viable bacteria by flow cytometry (BactiFlow ALS, AES Chemunex – BioMérieux, France). Such fluorescence-based method allows counts of viable bacteria previously marked with a viability fluorescent marker. The non-fluorescent substrate was split by enzymes to release fluorescent dyes inside the cytoplasm of the viable cells, allowing a specific labeling of the non-damaged viable cells. Control samples containing the bacteria and ingredient without ZnO were prepared as negative controls that ensure that the raw materials did not exhibit an antimicrobial activity.

The results of samples containing the potential interacting ingredient were compared to those collected for a suspension containing only ZnO-1 and bacteria: a significant lower population meant that the tested raw material enhanced the antimicrobial mechanism of ZnO particles whereas a significant higher population revealed an inhibition of the antimicrobial activity.

2.3. Formulations

Three types of formulations differing by their physicochemical properties were prepared: an oil-in-water emulsion, a water-in-oil emulsion with pigments and a compact powder. These formulations were developed free of any usual preservative or additive such as fragrance or active substance. Moreover, these products were free of the tested raw materials mentioned in the previous section, except sodium chloride which was incorporated to stabilize the water-in-oil emulsion. Controls of the physicochemical parameters of the finished products were realized as well as the stability at 20 °C, 4 °C, 40 °C, 50 °C, 4–40 °C cycles and under UV light.

2.3.1. Preparation of emulsions

The ingredients of the aqueous phase of each product were mixed in a beaker (Tables 2 and 3). In the presence of ZnO, the aqueous phase was stirred with a rotor–stator mixer (Silverson, US) at 9800 rpm until no more agglomerate of ZnO was detected (usually for 10 min). The water phase was then heated up to 80–85 °C in a water bath. The ingredients of the oil phase were mixed in another beaker and heating up to 80–85 °C concomitantly to the aqueous phase. For the w/o emulsion that contained pigments (Table 3), a pre-mixed paste containing fatty acid esters and pigments was prepared by passing it three times in a three roll mill to break up any agglomerate and introducing it in the oil phase under rotor–stator agitation. When both phases reached 80 °C, the