Applied Clay Science 53 (2011) 66–72

Contents lists available at ScienceDirect

Applied Clay Science

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

Research paper

Substituting non-natural agents in UV-protection cream by a mixture of clay with *Ganoderma pfeifferi* extract

Thao Hoang-Minh [a,*], Thi Lai Le [b], Jörn Kasbohm [c], Reto Gieré [d]

[a] Hanoi University of Science, Vietnam National University, Hanoi, Viet Nam
[b] Institute of Geological Sciences, Vietnamese Academy of Science and Technology, Viet Nam
[c] GeoENcon Ltd. & Institut für Geographie und Geologie, Ernst-Moritz-Arndt-Universität, Greifswald, Germany
[d] Institut für Geowissenschaften, Albert-Ludwigs-Universität, Freiburg, Germany

ARTICLE INFO

Article history:
Received 17 December 2010
Received in revised form 27 April 2011
Accepted 29 April 2011
Available online 19 May 2011

Keywords:
Ultraviolet radiation
Clay
Fungal extract
Layer charge

ABSTRACT

To investigate the potential of substituting non-natural agents in sunscreen, mixtures of some clays with *Ganoderma pfeifferi* extract were examined in regard to their protection abilities against ultraviolet (UV) radiation in the wavelength range 250–400 nm. Some mixtures showed remarkably high UV-protection potential in comparison with corresponding clay cream devoid of the fungal extract. Depending on interlayer charge of the dominant clay mineral contained in the clays, the interaction between the fungal extract and the clay mineral played a key role in the UV-protection ability of the mixtures. The optimal clay in the mixture with the *G. pfeifferi* extract for sunscreens was dominated by expandable clay minerals with low interlayer charge.

1. Introduction

UV radiation has well-known acute effects on human skin, comprising sunburn, and DNA-damage and, in the case of long-term exposure, carcinomas (Armstrong and Kricker, 2001; Baadsgaard, 1991; Diffey, 2002; Fuchs, 1998; Hu, 1990; Lim and Epstein, 1997; Longstreth et al., 1994; Peak and Peak, 1991; Ravanat et al., 2001; Young et al., 1998). UV-A radiation (320–400 nm) causes mainly sunburn as well as changes in the distribution of membrane fatty acids and impairment in the enzymatic defense system, which lead to skin aging (Bissett et al., 1989; Punnonen et al., 1991; Diffey, 2002), whereas the higher-energy UV-B radiation (280–320 nm) can cause DNA-damage and skin cancer (Setlow, 1974; Young et al., 1998).

Skin cancer has increased significantly in recent years because of changes in leisure behavior, which leads to increased UV exposure (Armstrong and Kricker, 2001). In 1988, for example, more than 1000 people died of skin cancer in Australia (Gies et al., 1998). It is also reported that about 66% of the Australian population were treated for skin cancer at some stage in their life (Unions, 2005). Therefore, sunscreens are essential if skin cancer incidence is to be reduced.

Sunscreens contain one or more UV filters of which there are two main groups: 1) organic molecules deliberately selected for their UV-absorption capacity (e.g., para- aminobenzoic acid, benzophenone), and 2) particles, both inorganic and organic, that absorb, reflect, or scatter UV radiation (e.g., titanium dioxide, zinc oxide, microfine polymeric molecules) (Gabard, 2009; Shaath, 1997B). Because of recent medical advances and enhanced expectations from consumers, there is a need for development of new and better sunscreen agents (see review by Shaath, 1997A). Furthermore, some non-natural UV filters may cause serious problems for the skin because of their photocatalytic effects, as are known for Padimate-A, Padimate-O, TiO_2, and ZnO (Clechet et al., 1979; Dunford et al., 1997; Hidaka et al., 1997; Knowland et al., 1993; Serpone et al., 2002; Sun et al., 2008; Tan et al., 1996).

Hoang-Minh et al. (2010) described the UV-protection of clays and clay minerals as substitutes for non-natural additives in sunscreens. Although the studied clay–creams were able to protect from UV radiation, most of the UV-transmission values of the samples were still higher than those of a commercial sunscreen with a sun-protection factor (SPF) of 20 (Ladival® allerg 20). Moreover, the clays with high UV-protection potential, such as the Thierfeld clay with its red color, are not well-suited for the production of sunscreen. Therefore, mixtures of clay with a material that has favorable properties as sunscreen could provide a solution because the mixture might combine the advantages of both components.

This study describes the UV-protection potential of some clays in mixtures with capsules composed of lipophilic extracts of the fungus *Ganoderma pfeifferi (G. pfeifferi)*, a saprophytic basidiomycete living on deciduous trees (mostly Fagus) of Europe. Extracts from *G. pfeifferi* are suitable for preparation of pharmaceutical and cosmetic products (Jülich et al., 2004; Niedermeyer et al., 2005). They also inhibit growth of microorganisms that are responsible for skin problems (Lindequist et al., 2005). Jülich et al. (2010) indicated that extracts of some

* Corresponding author at: 334 Nguyen Trai Road, Thanh Xuan District, Hanoi, Viet Nam. Tel.: +84 4 38585097; fax: +84 4 38583061.
E-mail address: hoangminhthao@vnu.edu.vn (T. Hoang-Minh).

0169-1317/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.clay.2011.04.024

Ganoderma species have a scavenging effect on free radicals so that they exhibit an anti-oxidative effect and cause a delay of the skin aging process. This finding is in contrast to the effects of the above-mentioned non-natural additives like TiO_2 which lead to formation of free radicals or photocatalysis.

Because of its mentioned beneficial characteristics, extracts from *G. pfeifferi* were mixed with some clays to investigate the potential of these mixtures as substitutes for non-natural agents in UV-protection creams.

2. Materials and methods

2.1. Materials

The studied clays included kaolin (German standard: Wolfka), bentonites (American Petroleum Institute standard: Al-rich Wyoming, Fe-rich Garfield), mixed-layer minerals-dominated clay (Fe-rich Friedland clay from Germany), mica-dominated clay (German standard: Fe-rich Thierfeld clay), and a purified clay (Thierfeld clay purified by the dithionite method: Thierfeld-dithionite). These samples were described in detail by Hoang-Minh et al. (2010).

Non-clay materials included a commercially available sun cream Ladival® allerg 20 (from STADA GmbH, Bad Vilbel, Germany, with an SPF of 20); titandioxid (Eu Rho ® Ph.Eur.4, from Euro OTC Pharma GmbH); cream matrix and cream admixtures (wool-wax-alcohol ointment [SR 90; composed of wool-wax-alcohol, sorbitanum trioleinicum GOT and mainly vaseline] from Bombastus Werke AG; emulsifier PLANTACARE® 2000 UP [aqueous solution of C8-C16 fatty alcohol polyglucoside], from Cognis; glycerol, from Merck); and fine powder of fruit bodies of *G. pfeifferi* (Institute of Pharmacy, Ernst-Moritz-Arndt-University of Greifswald, Germany; fruit bodies of *G. pfeifferi* were collected on *Fagus* in Ludwigsburg, Mecklenburg-Western Pomerania, Germany). The active substances extracted from *G. pfeifferi* included ganoderol B, applanoxidinic acid G or triterpenes (ganoderic acid A), and ganoderon B (triterpene 3,26-dihydroxylanosta-8,24-diene-7-one) (Jülich et al., 2004).

2.2. UV measurement

To perform the UV measurement, two kinds of cream samples were prepared: 1) clay-cream samples made from clay, glycerol, and wool-wax-alcohol ointment (for preparation details, see Hoang-Minh et al., 2010); and 2) clay-fungus-cream samples composed of clay, *G. preifferi* extract, and wool-wax-alcohol ointment.

The process to prepare a suspension of the fungal extract was similar to the examples described by Jülich et al. (2005, 2007). Firstly, the fine extract powder of *G. pfeifferi* and emulsifier PLANTACARE® 2000 UP were combined at 25 °C with a ratio of approximately 5 g and 45 mL and then heated to ~50 °C. PLANTACARE® 2000 UP is an aqueous solution of 51–55% of an active substance, namely the C8-C16 fatty alcohol polyglucoside (Cognis GmbH). Subsequently, a pre-suspension was manufactured using an agitator (rotor stator principle or ultrasonic) working at 45 rpm. The pre-suspension was then homogenized 4 times with the support of a high-pressure homogenizer, whereby the water contained in PLANTACARE® 2000 UP was also removed by evaporation. In the end, the active substance containing the fungal extract was precipitated in the form of stable microparticles and nanoparticles with a size range between 10 nm and 10 μm (Jülich et al., 2010).

For each sample, 1 mL of the extract was blended with 1 g of the wool-wax-alcohol ointment, followed by blending with 0.1 g of the clay. Subsequently, this mixture was stirred until the resulting clay–fungus creams were homogeneous, i.e. ready for UV-transmission measurement. Below, these mixtures are referred to as clay-fungus creams.

The UV measurements were carried out using an AnalytikJena AG SPECORD 50 photometer. The details of the experiments and data expression are identical to those reported for clay-cream samples by Hoang-Minh et al. (2010).

2.3. Lactate dehydrogenase test in vitro

The enzyme lactate dehydrogenase (LDH) is found in the cells of many body tissues. As a cell dies, its LDH is released, so that measurement of the released LDH can be used to assess the alteration level of the cells. In this study, an *in-vitro* test was performed at Biometec GmbH, Greifswald to investigate the safety of the *G. pfeifferi* extract in comparison with TiO_2 which is known for its harmful photocatalytic effect on human skin (Clechet et al., 1979; Dunford et al., 1997; Hidaka et al., 1997; Tan et al., 1996). The test used HaCaT cells which represent a spontaneously transformed human skin keratinocyte cell line. The HaCaT cells were incubated in a medium supplemented with 8 mass% fetal calf serum (FCS) at 37 °C, 95% humidity and 5 vol.% CO_2 in plastic culture dishes. The setup of the experiments was similar to the LDH test described by Boukamp et al. (1988). The cells were cultivated on 3 substrates: (1) a clean quartz plate as control sample, (2) a quartz plate with the *G. pfeifferi* extract, and (3) a quartz plate with a suspension of 0.5 mass% TiO_2. When the HaCaT cells had grown to reach confluence of 50% in the 60 mm^2 area dishes, the medium was replaced by 2 mL of phosphate-buffered saline solution. Subsequently, the cells were illuminated with UV light (medical UV-B, broad band, maximum 311 nm) using a dose of 20 mJ/cm^2.

2.4. X-ray diffraction (XRD)

XRD analyses were carried out on air-dried specimens to characterize the pure clay samples by a Freiberg Präzitronic diffractometer HZG 4A-2 equipped with a Seifert C3000 control unit (Co tube, $K\alpha_{1,2}$ radiation, 30 kV, 30 mA). The clay–cream and clay–fungus–cream specimens were prepared as smear slides for XRD analysis at the same conditions. Overlapping XRD reflections were deconvoluted using the WinFit program (Krumm, 1994).

2.5. Light microscopy

A light microscope (ZEISS-AXIOSKOP linked to a Carl Zeiss AxiCam HR system) was used to investigate selected cream samples. The samples were kept 0.003 mm thick between two glass slides. The images were converted into binary format by the Adobe Photoshop 7.0 software. Homogeneities of cream matrices were estimated by the standard deviation (STDV) of the darkness distribution from processed images.

3. Results

3.1. Safety of G. pfeifferi extract in comparison with photocatalytic TiO_2

During the UV-irradiation process, the LDH release was determined by measuring the optical density (OD) at 550 nm at selected time points. A higher toxicity was indicated by a higher OD value, which corresponds to a higher concentration of released LDH. The result of this test showed that the sample containing the *G. pfeifferi* extract released very low concentrations of LDH, reflecting lower numbers of dead cells in comparison with the sample containing the TiO_2 suspension, especially after six or more hours of UV irradiation (Fig. 1).

In conclusion, even at a very low concentration (0.5 mass%), the suspension of TiO_2 had a more deleterious effect on skin cells compared to the *G. pfeifferi* extracts. These experiments provided a justification for using the extracts from the fungus species *G. pfeifferi* in skin cream.