

In-situ evaluation of barrier-cream performance on human skin using laser-induced breakdown spectroscopy

QING SUN, MICHAEL TRAN, BENJAMIN SMITH AND JAMES D. WINEFORDNER

Department of Chemistry, University of Florida, Gainesville, FL 32611–7200, USA

Laser-induced breakdown spectroscopy (LIBS) was used to evaluate the effect of barrier creams (skin protective creams) on human skin. A Nd: YAG laser at 1064 nm was used with a pulse energy of 100 mJ. A method was developed to measure the effectiveness of barrier creams against zinc ion absorption from aqueous zinc chloride solution and oil paste zinc oxide, which represent model hydrophilic and lipophilic metal compounds, respectively. Zinc was chosen since it posed no risk to human skin. 3 representative commercial barrier creams advertised as being effective against lipophilic and hydrophilic substances were evaluated by measuring zinc absorbed through the stratum corneum. 4 consecutive skin surface biopsies (SSB) were taken from biceps of the forearms of 6 volunteers at time periods of 0.5 h and 3 h after application of the protective cream. Results were compared with control skin where no barrier cream was used. The zinc atomic emission line at 213.9 nm was selected. Gate delay and gate width time was optimized to obtain the best signal-to-noise ratio (SNR) and precision. This method provided a facile and rapid screening of the effectiveness of skin barrier creams against zinc ion penetration. The barrier creams were shown to provide appreciable protection against the penetration of both ZnCl_2 and ZnO into the skin.

Key words: laser-induced breakdown spectroscopy (LIBS); skin protection; barrier creams; human skin; skin care products; cosmetics; prevention; zinc chloride; zinc oxide. © Munksgaard, 2000.

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Healthy skin consists of dead, hardened skin cells, which are surrounded by water, various lipids, oils, acids, and salts. This hydrolipic layer is also known as the acid shield because of its low pH; it forms a natural protective outer skin, which is flexible, keeps out micro-organisms and dangerous substances, reduces physical damage, and retains moisture. When water comes into contact with skin, the components of this layer disintegrate; this process is accelerated when the water is warm and contains detergents and when there is prolonged exposure. Such washed-out skin distends and loses its natural biological and physical protective function.

Skin barrier creams theoretically reduce the penetration into, and absorption through, the skin of hazardous materials in the workplace and home environments. In practice, protective gloves remain the most important means of protection from corrosive agents (acids, alkalis). Some barrier creams are recommended to be used under gloves to pro-

vide valuable secondary protection against acid burns, in case the gloves develop pinholes or fail altogether. In fact, the effectiveness of barrier creams is debated. Some reports have doubted the usefulness of barrier creams (1–5).

Zhai & Maibach (6) used a simple method to measure the effectiveness of skin barrier creams. In their study, methylene blue in water and oil red O in ethanol, which represented models for hydrophilic and lipophilic compounds, were used. A colorimeter was used to determine the stain in the peeled skin. The method provided an easy, rapid measurement for evaluating barrier creams as protection from the dye. However, no information was provided as to how the barrier cream prevented the penetration of cations, such as nickel, lead, iron, copper, and several others used in the chemical and cosmetic industries. It is well known that some metals, like Ni and Pb, result in skin problems (7).

Laser-induced breakdown spectroscopy (LIBS) is, for the 1st time, used to evaluate barrier creams

as a means of protection against the absorption of metal ions. LIBS has the advantages of being rapid and simple, with little or no sample preparation being required compared to other analytical methods, and providing simultaneous analysis of multiple elements (8, 9). In this study, zinc is chosen as our model element because it is considered safe to skin.

Materials and Methods

Experimental

Spectrometer

The experimental set-up is shown in Fig. 1 and consisted of a laser, a motorized x , y , z translational stage carrying the sample, a spectrometer, an intensified charged coupled device (ICCD) (Princeton Instruments, USA) detector, detector gating and control electronics, and a computer for control and data acquisition. A Nd:YAG laser (Quintel Brilliant, France) with a maximum pulse energy of 360 mJ at the fundamental wavelength of 1064 nm, a 5 ns pulse duration and a maximum 10 Hz repetition frequency was used. A laser energy of 100 mJ was used to form the plasma and a laser repetition rate of about 1 Hz was used in this study. The laser was focused onto the sample surface and aligned through a pierced mirror tilted at a 45° angle with respect to the laser beam. Emission was collected from the plasma top using the same pierced mirror and a simple optical system including 2 short focal-length lenses directing the emission to a 0.5-m grating monochromator (SpectraPro-500I, Acton Research Corporation, USA); the grating of 3600 lines/mm was used in this study.

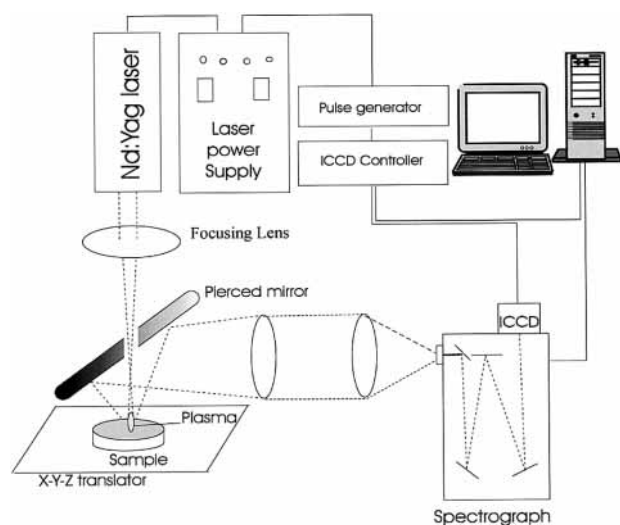


Fig. 1. Schematic setup of laser-induced breakdown spectrometer used to measure zinc signals.

A positioning system consisting of a helium-neon laser, lenses and a position-sensing detector was used to monitor the position of the sample surface with respect to the laser beam focus. The sample motion was accomplished with a motorized x , y , z stage (Slo-Syn motor, MO61-LS08, Superior Electronic, U.S.A). For all measurements, the sample was moved horizontally between shots to allow laser sampling from a fresh location to improve the reproducibility of mass ablation.

By adjusting the delay and gate times, the signal-background ratio, signal-background noise ratio and relative standard deviation were optimized. A delay time of 1.0 μ s and an integration time of 4.0 μ s were selected. Each sample measurement consisted of 3 replicates of 20 laser shots; each laser shot was on a fresh spot with no overlap between shots. The signal intensity was calculated by subtracting the total intensity of background from the total intensity of Zn from the 20 single laser shots.

Subjects

Six healthy volunteers (1 female and 5 male) participated in the study, after providing written consent. This project was approved by the Institutional Review Board (IRB) of the University of Florida.

Reagents and sample preparation

Microscope slides (precleaned) and zinc chloride were purchased from Fisher Scientific. Quick Gel (super glue) and *Equate* (Clay-Park Labs, Inc., Bronx, NY 10457) diaper relief ointment were purchased in a grocery store.

Barrier creams: *Brand A* is a water-insoluble skin protection ointment. Recommended use to protect skin from water and other aqueous substances, such as detergents, chemicals, plant juices, insecticides, and water-based paints. *Brand B* is a skin protection ointment. It has a wide range of uses based on forming a fine, net-like web inside the skin to guard against harmful, damaging and irritating substances, and is recommended for protection of skin from alkaline and acid solutions, oils and greases, paints, inks and lubricants and minerals, metals and chemicals. *Brand C* is a protective skin barrier against chemicals and is recommended for protection of skin from harsh chemicals in the industrial workplace, such as solvents, oils, acids (not HF) and household cleaners, etc.

ZnCl₂ solution: ZnCl₂ was dissolved in de-ionized water and several drops of HCl were added

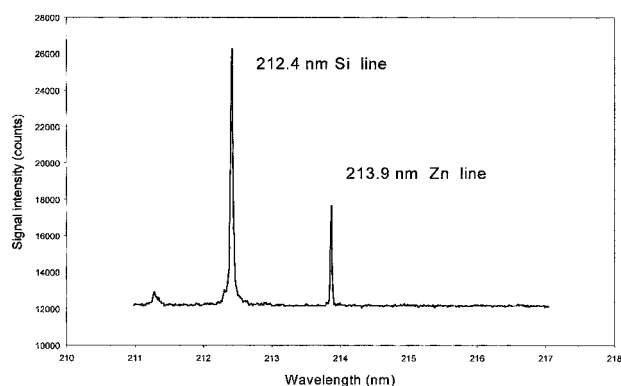


Fig. 2. Spectrum of a single laser shot on a skin sample of glass slide, which was exposed to a ZnCl_2 solution.

(pH~4) to prevent $\text{Zn}(\text{OH})_2$ from precipitating. The Zn concentration was diluted to give a solution of 6740 ppm of Zn (w/w).

Preparing skin sample on glass slide: Skin of the biceps area of both forearms was shaved and washed with soap before testing. Prior to the skin biopsy, different skin barrier creams were applied on different people following the instruction label on the barrier cream. An aqueous ZnCl_2 or an oil paste ZnO (diaper relief ointment) was saturated in the center of a bandage and the bandage then applied to the biceps skin area. For the control skin, no barrier cream was used before applying the aqueous ZnCl_2 solution or the ZnO ointment. The application times on the skin were 30 min and 3 h for each volunteer. The skin was washed again before the skin was peeled off. The skin peeling process was similar to the skin surface biopsy technique previously described (10). A thin layer of cyanoacrylate glue (QUICK GEL) was coated on a glass slide. The glass slide was then gently pressed onto the skin of the biceps area for about 30 s and then slowly removed. To be sure that the next 3 samples of peeled skin came from the same location, the skin was covered by Scotch tape so that only the area of interest was exposed. The skin area was about $1.7 \times 1.8 \text{ cm}^2$. By using this method, skin could be peeled layer by layer up to 6 success-

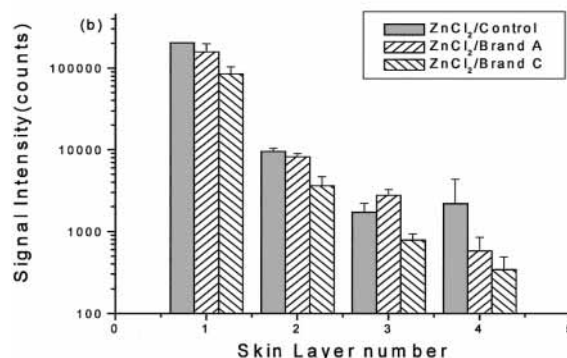
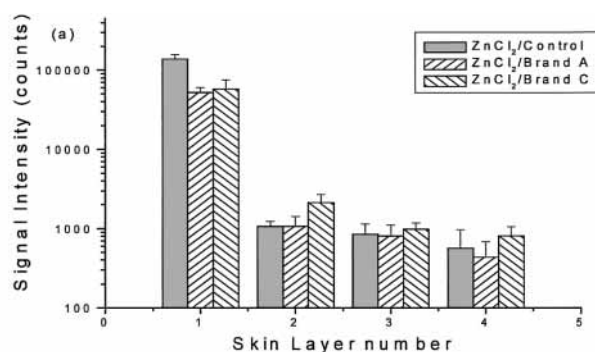


Fig. 3. Signal intensity of Zn for ZnCl_2 absorbed into control skin and skin treated with barrier cream for different skin layers: (a) after 30-min exposure, (b) after 3-h exposure. The y-axis is in log scale. See Table 1 for information concerning the volunteers.

ive layers with no pain or bleeding of the volunteers. In this study, a total of 4 successive strips were peeled off.

Results

Fig. 2 shows a typical spectrum of Zn in the 1st layer of a peeled skin sample. Fig. 3 shows the effectiveness of barrier creams compared to the unprotected skin samples (control), after applying aqueous ZnCl_2 solution for several exposure times.

Table 1. Zinc signals for different skin layers^a by applying different barrier creams at different time periods compared to control skin layers. 0.1 M ZnCl_2 solution was used

Skin layer	Control ZnCl_2		Brand A/ ZnCl_2		Brand C/ ZnCl_2	
	30 min	3 h	30 min	3 h	30 min	3 h
1	138,555	203,216	52,347	159,544	57,520	85,503
2	1073	9602	1066	8196	2158	3654
3	856	1718	811	2786	989	790
4	564	2195	434	580	810	343
total	141,048	216,731	54,658	171,106	61,477	90,290

^aThe results for the control, brand A and brand C was for 3 different volunteers.

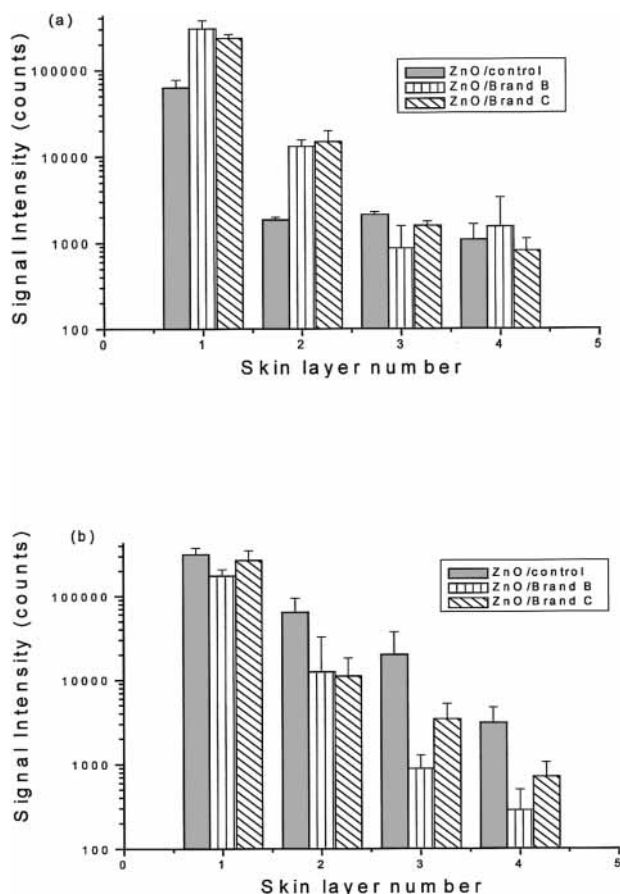


Fig. 4. Signal intensity of Zn for ZnO absorbed into control skin and skin treated with barrier creams for different skin layers: (a) after 30-min exposure, (b) after 3-h exposure. The y-axis is in log scale. See Table 2 for information concerning the volunteers.

Table 1 gives the signal intensities with $\pm 1\sigma$ (standard deviations) obtained from Fig. 3. Fig. 4 shows the effectiveness of barrier creams compared to the unprotected skin samples (control) for oil-based ZnO (diaper relief ointment) for several exposure times. Table 2 gives the signal intensities with $\pm 1\sigma$ (standard deviations) obtained from Fig. 4.

Discussion

From the emission spectrum in Fig. 2, an intense silicon peak at 212.4 nm indicated that the laser beam penetrated into the glass slide and therefore the entire depth of the skin was ablated. Because each skin layer had approximately the same thickness, the signal measured at each skin layer could be compared. In this study, 4 successive skin layers were removed and measured, and 3 or 4 measurements were performed on each strip. The signal was determined by background subtraction from the total signal intensity. The signals of control samples with application of aqueous ZnCl_2 were significantly higher than the signals where barrier creams had been applied for both 30-min and 3-h periods of time, especially in the 1st skin layer (Fig. 3 and Table 1). Since the signal intensities varied greatly in different skin layers, the y-axis on Fig. 3 has a log scale to observe the wide range of signals in each skin layer. The signal intensities in Fig. 3 are listed in Table 1 for convenience. All of the signal changes for the 3-h exposure and only those for the 1st skin layer during the 30-min exposure were statistically significant (see error bar in Fig. 3). Brand C cream achieved good skin protection, since it greatly reduced the amount of zinc absorbed into the skin in the 30-min and 3-h time periods compared to the control samples. The signal intensity under the protection of brand C cream was about 44% and 42%, respectively, of the control skin layers for the 30-min and 3-h exposure times. For the 3rd and 4th skin layers, the signals under the protection of brand C cream did not significantly change from 30 min to 3 h, which indicated that brand C cream reduced the penetration of zinc ions into the skin and essentially restricted penetration to the upper skin layer. Brand A was very effective in the first 30 min but almost lost its effectiveness in 3 h. The total signal intensity under the protection of brand A cream was about 39% and 79%, respectively, of the control skin layers for the 30-min and 3-h exposure times. Since the aqueous ZnCl_2 solution was in contact with the skin continuously (the bandage was saturated by ZnCl_2

Table 2. Zinc signals for different skin layers^a by applying different barrier cream for 2 time periods with comparison to the control skin layers; 40% ZnO ointment was used

Skin layer	Control ZnO		Brand B/ZnO		Brand C/ZnO	
	30 min	3 h	30 min	3 h	30 min	3 h
1	62,749	315,718	300,985	174,457	234,852	267,041
2	1855	64,465	13,121	12,424	14,911	11,073
3	2117	19,934	865	890	1584	3448
4	1085	3102	1528	285	817	709
total	67,806	403,219	316,499	188,056	252,164	282,271

^a The results for the control, brand B and brand C was for 3 different volunteers.

solution and attached to the arm), this might have been the reason that brand A barrier cream protected the skin from zinc absorption for a time period of less than 3 h.

The signals of each control skin layer with application of oil-based ZnO were significantly increased for the 3-h period in comparison to the 30-min period (see Fig. 4 and Table 2). Therefore, the longer the skin is in contact with ZnO, the greater the amount of zinc absorbed into the skin. Also, zinc signals of each control skin layer were greater than those protected by barrier creams for the 3-h exposure time (for convenience, the signal intensities are listed in Table 2). All of the signal changes for the 3-h exposure and only those for the 1st and 2nd skin layers during the 30-min exposure were statistically significant (see error bar in Fig. 4). The total signal intensity for the 4 skin layers under the protection of brand B and brand C creams were about 47% and 70%, respectively, of the control skin samples for the 3-h exposure time. However, for the 30-min application, the signals with the barrier cream were much higher than the signals from the control skin in both the 1st and 2nd skin layers. We know that the barrier cream forms a fine net-like web inside the skin and cannot be washed away during the effective time. Therefore, a residue of oil-based ZnO ointment may have been left on the skin surface, together with the barrier cream, especially for the short exposure time. Therefore, the signals from the 1st and 2nd skin layers were higher than in the control samples for the 30-min time period (because, in the control sample, no barrier cream was applied and the ZnO ointment on the skin surface was cleaned away by washing with soap). For the 3-h exposure time, the protection of the barrier cream was less effective; therefore, the ZnO ointment on the skin surface was relatively easy to wash away. Based on the signal intensity from the 2nd to the 4th skin layer, the penetration of zinc ion with these 2 creams was about 16% and 17%, respectively, of the control skin samples for the 3-h exposure time. Therefore, most of the Zn was still in the 1st skin layer and these 2 barrier creams had good protection for oil-based ZnO for the 3-h exposure time.

Conclusions

This study provided an in-situ method to evaluate the effectiveness of barrier creams for protecting

skin from zinc ions. As an analytical instrument, LIBS provided an easy and fast method for each layer of peeled skin. We believe that this method will provide an effective tool in screening barrier cream candidates and in the formulation of barrier cream vehicles. The ultimate proof of effectiveness of barrier creams will reside in controlled clinical assays and field experience in humans. The measurement approach (LIBS analysis of peeled skin) should also be applicable to other metal ions applied to the skin.

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Address:

James D. Winefordner
Department of Chemistry
University of Florida
Gainesville, FL 32611-7200
USA