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CHARACTERIZATION OF SNAKE SKIN BY THERMOANALYTICAL TECHNIQUES

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Abstract

Snake skin is a viable and readily available material as a model for human skin. Pharmaceutical applications use shed snake skin to study the effects of sunscreens on exposure to UV radiation (e.g. benzophenone on Boa integument). In order to understand the effects of radiation or drug transport through this model skin, one must determine its basic physical properties. This preliminary study evaluated two types of snake skin, namely Cuban Boa a 'dark' skin (*Epicrates angulifer*) and Green tree python a 'light' skin (*Morelia viridis*). Previous studies by other investigators have used pig, rabbit and snake skin as a human skin substitute. The structure of both snake skins was comparable based on IR spectroscopy and were functionally amino acids and moisture. Photomicrography by light and scanning electron microscopy revealed strong anatomic similarities. Morphologically there were two structures visible, namely a cellular and hinge-fibrous area. The thermal techniques indicated a phase transition at 35–75°C, which is associated with lipid melting. There was an 8 and 12% mass loss for the light skin and dark skin, respectively, which is interpreted, in part, as moisture loss at <100°C. The physical and analytical properties establish a base line that will be used in the future to differentiate various sunscreen types, such as benzophenone and octyl salicylate. Study was also done to determine the effect of an application of a commercially available sunscreen using SEM.

Keywords: DSC, *Epicrates angulifer*, *Morelia viridis*, SEM, snake skin, thermal analysis, TMA, UVA, UVB, UVC

Introduction

Anatomically, snake skin is different from human skin. Human skin is fairly homogeneous in contrast to the heterogeneous reptilian integument. It has been widely used in a large number of studies to imitate the human skin [1, 2]. Studies relating to the effect of penetration enhancers used snake skin. The main advantages involving the use of snake skin is that it presents a negligible biohazard and is readily available. Snakes periodically shed the outer layers of their skin, which is incapable of growth. This prevents the need for the sacrifice of live snakes.

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The skin provides a physiological and mechanical barrier between the internal milieu and the external environment. This primary function is attributable to the properties of the generalized integument rather than that of its appendages. The ability of the reptilian integument to act as a barrier to water or solute movement is clearly a physiological problem. It cannot be understood unless its mechanical attributes are borne in mind. Skin often is conceptualized in biophysical terms as a homogeneous membrane, but the epidermis (especially that of reptiles) is, in fact, a heterogeneous system. Thus, any study on the skin must be analyzed in relation to its morphological features and physical properties [3].

Snake epidermis has a hinge and scale construction with no appendages. The shedding of the old epidermal layer of snake skin occurs either in single sheets or as smaller portions. The shed epidermis of snake skin consists of four layers: the oberhautchen, the β-layer (protein), the mesos layer (lipid) and the α-layer (protein). The oberhautchen consists of β-cells that plays a major role in the shedding process. The β, mesos and α-layers contribute to the barrier functions [2]. The mesos layer is suggested to be similar to the human stratum corneum [1]. The hinges do not possess clear oberhautchen and mesos layers. These are all essentially keratoic (protein). The reptilian epidermis differs from that of mammals in having two different types of keratinaceous protein, α (hair-like) and β (feather-like) [3].

In order to determine the physical properties of the typical skins thermal mechanical analysis (TMA), dielectric thermal analysis (DETA), differential scanning calorimetry (DSC) and thermogravimetric analysis (TG) were performed from room temperature to ≤250°C. Light and scanning electron microscopy were used to determine the integumentary morphology. The snake skins studied had maximum dielectric values (permittivity and conductivity) from 60 to 120°C. DSC scans revealed a broad diffuse maximum at 60°C and a shoulder at 70°C on the first run and a reversible peak at 70°C on the second and third DSC runs. TMA revealed a compaction at 40 to 60°C and degradation and shrinkage at 180 to 200°C. Published DSC curves of human, rabbit and snake skins suggest the physical changes at <90°C are due to the dissolution of lipids in the mesos layer [1]. The skins studied were amorphous by X-ray diffraction analysis with short range ordering peaks at 2.4 and 5.0 Å. The baseline information gathered here can now be used to study the effects of UV light on and drug diffusion in snake skins based on the properties defined by the thermal analytical techniques and microscopic evaluation. The snake skin was further exposed to UV light and the effects were noted using SEM.

Experimental

Small quantities of snake skin were examined by DSC in sealed 40 μL aluminum pans. The pan was placed on the sample head of a Mettler Toledo DSC 20 Instrument (with STAR^e software) and was scanned from room temperature to 120°C. The heating rate was kept at 10°C min^{-1} in an atmosphere of nitrogen with a flow rate of 50 mL min^{-1}. The DSC run was also done using a TA Instrument Model 910. The

DSC curves were reproducible over the temperature range of 25 to 120°C. The sample was reheated to study the reversibility of the thermal transitions.

A TA Instrument Model 2940 thermal mechanical analyzer was used to study the modulus of snake skin. A 2–3 mm square sample of the snake skin was evaluated from 23 to 200°C at a heating rate of 5°C min^{-1} in an atmosphere of nitrogen.

The unhydrated snake integument was examined for dielectric properties in a TA Model DETA 2970 instrument with gold-coated ceramic single surface sensor. A flat sample, 10×10 mm, was placed on a 100-micron interdigitated electrode and flattened with a No. 1 Whatman filter paper. The heating rate was 5ºC min^{-1} in nitrogen with a flow rate of 50 mL min^{-1} over a temperature range of 23 to 120°C.

A 2–3 mg sample of both dark and light snake skin was studied using Thermo Haeke thermogravimetric analyzer. The experimental conditions were: 10°C min^{-1}, sample size 2–3 mg, temperature range 23 to 400°C in nitrogen at 200 mL min^{-1}.

A Phillips-Expert wide angle X-ray diffraction analyzer was used to study the XRD properties of the snake skin samples. The snake skin samples were evaluated from 10 to 60° 2θ with copper radiation.

The snake skin structure was evaluated by a Fourier transform 60 SX Nicolet analytical transmission IR from 4000 to 400 cm^{-1}.

A scanning electron microscope (JSM-6100) was used to determine the surface morphology of the snake skin. The sample was sputter-coated with a thin layer of gold and examined at a magnification of 50 and 1 000× using a JSM-6100 scanning microscope. A Bausch–Lamb optical microscopy was also used to evaluate the skin at 5 ca. 5 and 10×.

A 2×8 cm sample of the dark snake skin *Epicrates angulifer* was placed in a Petri dish and exposed to UV light from an UV bulb for 45, 90, 135, 180, 240 mts. Small square samples 5×5 mm, were taken at the end of the specified time intervals and SEM performed.

Results and discussion

Physical properties

The DSC demonstrates a broad melting endothermic peak at 60°C and a shoulder at 70°C in the first run as seen in Fig. 1. The enthalpy of the transition (with shoulder) was very small 1.24 mJ. The shoulder endotherm was reversible upon reheating. The peaks are very broad and range from 30–100°C and hence it is a diffuse rather than a definitive calorimetric process. Earlier literature [1] has reasoned this to be due to lipid melting in the mesos layer.

Figures 2 and 3 present the thermal mechanical analysis of light and dark snake skin, respectively, at a heating rate of 5°C min^{-1}. The TMA for light snake skin showed a softening at 50–56°C having a maximum at 51°C at the scale and softening at 63–77°C at the hinges with final decomposition at 206–225°C having a maximum at 216°C with shrinkage of skin. The TMA for dark snake skin showed a softening at 36–64°C having a maximum at 48°C and final decomposition at 189–217°C. The

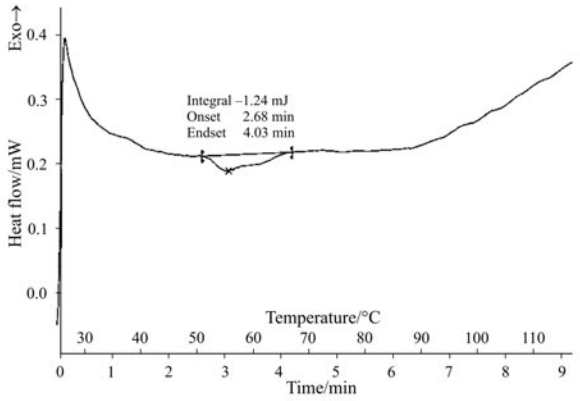

Fig. 1 DSC curve of dark snake skin (*Acrantophds dunerill*)

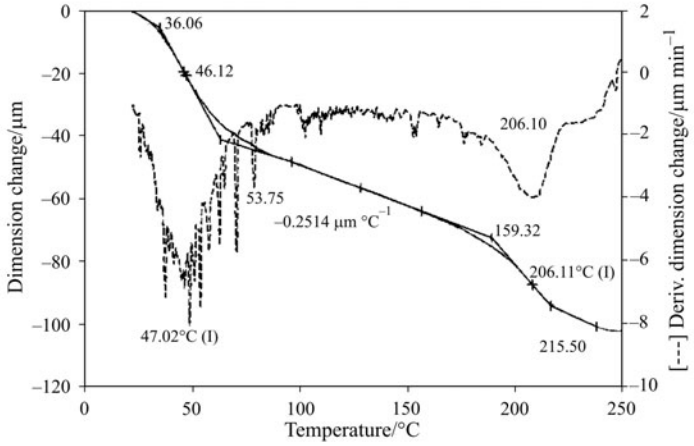

Fig. 2 TMA curve of dark snake skin (*Acrantophds dunerill*)

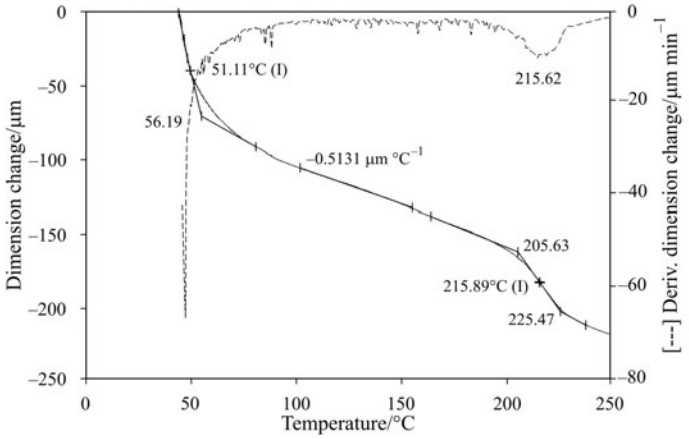

Fig. 3 TMA curve of light snake skin (*Morelia viridus*)

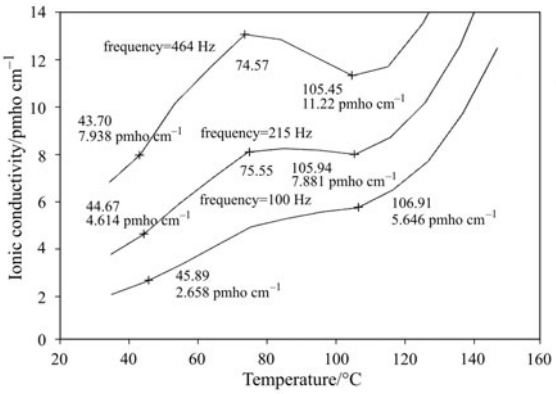

Fig. 4 Dielectric thermal analysis of dark snake skin (*Acrantophds dunerill*)

two snake skins showed a resemblance to each other with respect to their thermal changes by TMA.

The DEA, as seen in Fig. 4, revealed permittivity and conductivity variations from 44 to 105°C with a maximum at 74–75°C at a frequency of 464 Hz and 45–106°C with maximum at 75–76°C at 215 Hz.

A TG for the dark (Fig. 5) and light (Fig. 6) snake skin showed a mass loss of 12 and 8%, respectively, at 150°C. The small percentage mass loss suggests the removal of small molecules from the skin. This can possibly be a loss of moisture from the skin at higher temperatures.

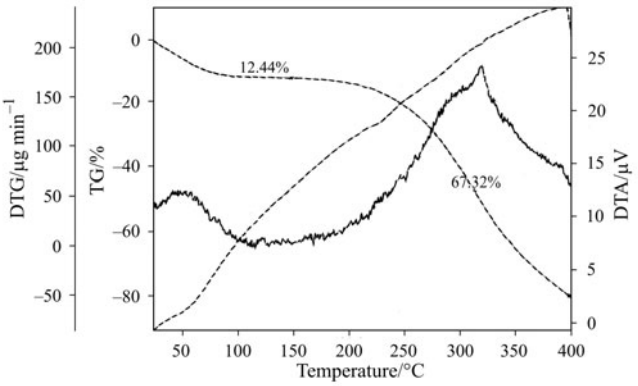

Fig. 5 TG curve of dark snake skin (*Acrantophds dunerill*)

SEM was used to study the detailed morphology of the snake skin. Figure 7a is the 50× magnification of the region showing the scale and hinge area of the dark snake skin. Figures 7b and c are the 1000× magnification of the scale region and 7d is the 1000× magnification of the scale region. There is distinct variation in the morphology of the two major areas of the snake skin. Figure 7d gives a fibrous appearance and might be attributed to the structure of the proteins making up the hinge region.

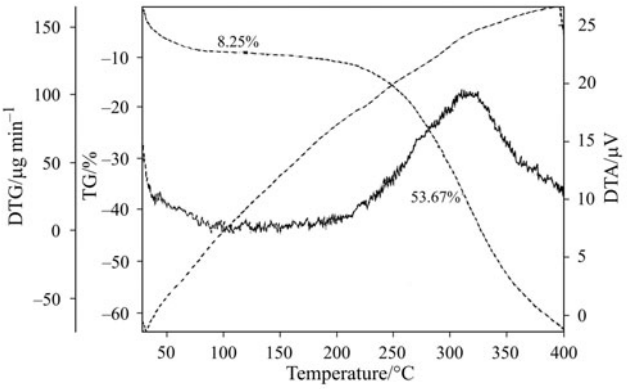

Fig. 6 TG curve of light snake skin (*Morelia viridus*)

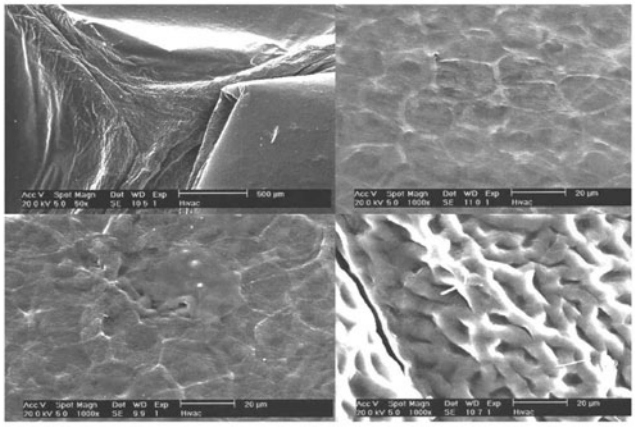

Fig. 7 SEM of dark snake skin at magnification of 1000×. a – region showing scale and hinge region at a magnification of 50×, b – and c – scale region at a magnification of 1000×, d – hinge region at a magnification of 1000×

Structure of snake skins

The IR curves for both the light (Fig. 8a) and dark (Fig. 8b) snake skin were very similar. The IR curve had a broad peak in the range of 3400–2500 cm^{-1} suggesting the presence of a carboxylic group and moisture. There are two overlapping peak areas one in the range of 3100–3000 cm^{-1} and another, four peaks in the range of 3000–2800 cm^{-1}. The first peak area is due to the primary amino group and the second due to methyl and methylene C–H stretch. The weak band at 2200–2000 cm^{-1}, and strong bands in 1250–1100 cm^{-1} confirmed the presence of the amino group. The observed IR structure for the snake skin is predominantly due to amino acids (proteins) and moisture.

The XRD for both skins (figure not provided) indicates that they are amorphous structures with non-crystalline scales at 2.4 and 5.0.

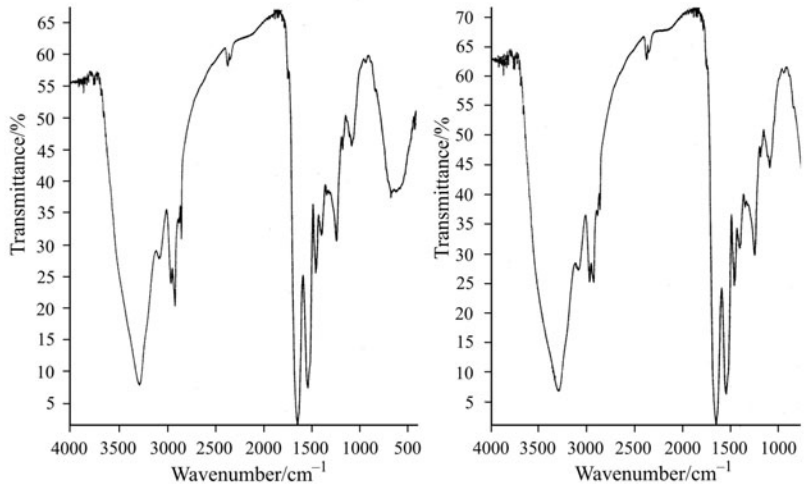

Fig. 8 IR spectroscopy of a – dark (*Acrantophds dunerill*) and b – light (*Morelia viridus*) snake skins

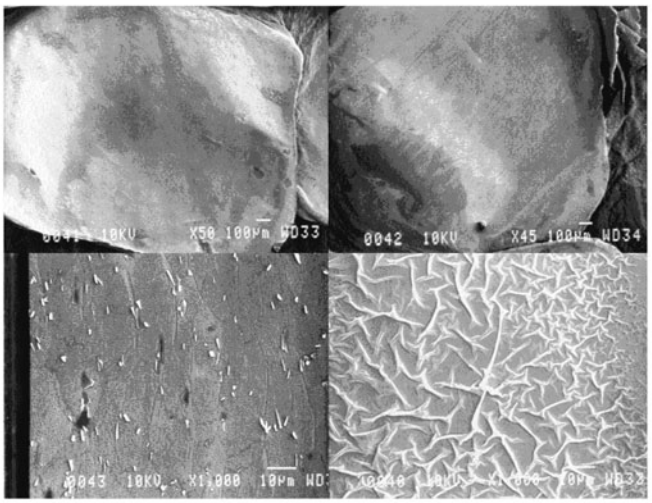

Fig. 9 SEM of UV irradiated for 240 mts of dark snake skin (*Acrantophds dunerill*) a – with sunscreen at 50×, b – without sunscreen at 45×, c – normal snake skin at 1000×, d – irradiated snake skin at 1000×

Snake skin samples exposed to UV light

The SEM of samples (Fig. 9) show patches of blisters over the snake skin. It was seen that the blisters are not uniformly distributed over the whole skin surface. It could be because of the wrinkled surface of the skin resulting in the non-uniform radiation of UV light. It was also noted that the blisters on the snake skin without the application

of sunscreen was darker as compared to the one with sunscreen. This could possibly be the effect of sunscreen and further studies using the thermal analytical techniques can be done in this direction.

Conclusions

Thermal analysis coupled with other techniques seems to be useful tool for the preliminary study of snake skin similar to the other material of biological origin [7–9]. It can be concluded that the DSC, TMA, DEA and TGA studies performed on snake skin showed a remarkable similarity in the thermo-physical properties of the two skins. The XRD and IR showed morphological resemblance between the two skins. The study can be further used as a baseline for the evaluation of sunscreens applied to these skins as a means of evaluating their effectiveness.

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