VISIBLE REFLECTANCE SPECTROSCOPY
TO OBSERVE THE EFFECT OF COPPER
EMBEDDED SOCKS ON ERYTHEMA IN FEET

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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled:

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presented by LISA JEAN HUHMAN ,

a candidate for the degree of Master of Science,

and hereby certify that, in their opinion, it is worthy of acceptance.

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VISIBLE REFLECTANCE SPECTROSCOPY TO OBSERVE THE EFFECT
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Dr. John A. Viator, Thesis Supervisor

ABSTRACT

Objective assessment of skin characteristics is necessary to aid in the clinical detection of erythema and debilitating skin conditions. The ability to treat different skin pathologies based on individual patient skin conditions will lead to fewer amputations and complications experienced by amputees, diabetics and the elderly. Copper has been used in medical applications for over a hundred years. A process has recently been developed that incorporates copper into textile materials. As an antimicrobial fabric, copper impregnated socks have a broad spectrum of potential including reduction or elimination of erythema. Though previous studies have concluded that copper ion socks reduce or eliminate erythema, those studies were based on clinical observation only and not empirical data. Expanding on preliminary studies, we were interested in scientifically measuring the change in erythema utilizing VRS methods. This study focused on the detection of erythematic changes associated with copper socks using a visible reflectance spectrometer in two randomized, double blind studies. Using the spectral data collected over the duration of the pilot study using a visible reflectance spectrometer, optical diffusion theory and knowledge of normal and pathological skin components, we were able to determine the amount of erythema in skin by determining an erythema index.

Chapter 1

Skin and Copper

1.1 Human Skin

Skin is the largest organ of the human body accounting for about 7% of total body weight. The skin, which varies in thickness from 1.5 to 4 mm, or more in different regions, protects us from the environment, helps regulate body temperature and provides receptors for sensations such as touch, pain, and pressure. Human skin has great variations in structural and optical properties from one person to another and from site to site on the same person. However, skin is relatively homogenous in terms of histological structures including the component structures of the epidermis and dermis (Figure 1.1).

1.1.1 Skin Pathologies

Contact dermatitis, edema, erythema, blisters, sores, and other related foot pathologies frequently persist with current sock materials. Skin trauma due to frictional forces affects people differently. For instance, athletes develop skin irritations on their feet because of constant rubbing of a material, normally a sock, against their skin. When skin comes into contact with moisture, frictional forces increase, especially at the hot spots, exacerbating blister formation [1]. Frictional forces are at a minimum when there is no moisture. As moisture is
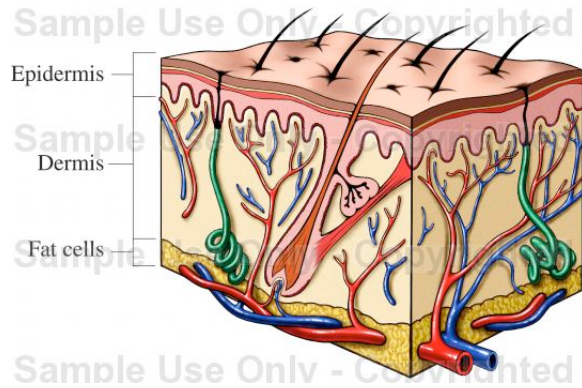
Figure 1.1: Schematic of the epidermis and dermis of the skin. Art courtesy of Nucleus Medical Art.

added, these forces increase. These mechanical irritations can cause cell necrosis, which results in separation of layers of the skin. A gap forms between the epidermis and dermis, similar to Figure 1.3, which is then filled with fluid producing a blister [2]. Contact dermatitis is intercellular edema, or swelling, of the epidermis which may result in intraepidermal vesicle and bullae formation in acute cases and papules, scaling, lichenification in chronic cases [3]. Figure 1.4 illustrates the effects of edema in the case of diabetics who have much more sensitive skin.

With aging, the epidermis and blood vessels of the dermis thin as connective tissue loses strength and elasticity. With a reduction in the stability of the skin, it is not as resilient against forces of friction and pressure. The more fragile the blood vessels become, the more bleeding under the skin will occur with minimal force to the skin. As individuals age they become more susceptible to injury and infection of the skin. Skin will lose its ability to sense touch, pressure, vibration, and temperature. Rubbing or pulling can easily cause skin tears or cracks. Wound healing may actually occur 4 times slower than that of a young person which contributes to the exacerbation of infection with minor injury. Diabetes
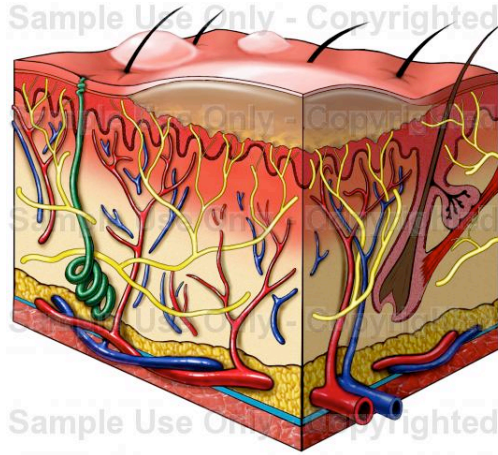
Figure 1.2: Schematic of blisters and sores forming in the epidermis due to rubbing or friction. Art courtesy of Nucleus Medical Art.

can complicate the stability of the skin even further by lowering immunity and sensory of nerves in the wounded area resulting in undetected ulcers. It has been estimated that more than 90% of all older people have some type of skin disorder [4]. These disorders can be caused by many diseases including diabetes, liver disease or heart disease, in conjunction with natural breakdown of the cellular layers of the skin. As a person ages it becomes increasingly more important to prevent skin pathologies and growth of infection.

Diabetes can induce many changes in the integumentary, circulatory and nervous systems that predispose people to pressure ulcers and hinder the healing process. Figure 1.4 illustrates the proliferation of a skin ulcer and the depth of skin it can affect. Skin of a diabetic is more prone to skin trauma due to damage of the nerves and blood vessels causing a loss of feeling at these areas termed *peripheral neuropathy*. These skin traumas go unnoticed due to the inability to feel pain, making irritations worsen over time and can become infected due to bacterial or fungal growth. Many of the complications of diabetes, including retinopathy and neuropathy, are due to impaired microcirculation. There can

Figure 1.3: Schematic of a diabetic foot with serious edema. Art courtesy of Nucleus Medical Art.

be thickening of the capillary basement membrane and localized microvascular occlusion preventing growth of new blood vessels. Therefore, diabetics typically do not have sufficient blood flow to the legs or feet preventing immunity factors from reaching the site of infection.

Amputees who utilize prosthetic limbs for daily activities encounter repetitive loading and accumulation of moisture between the prosthetic sock and limb much like athletes wearing tennis shoes, thus the elicitation of skin trauma. When an amputee experiences these problems, they may be at risk for infection if blisters or open sores are present. The skin inflammation varies from mild irritation and erythema to open sores, depending on the type of irritant, the body part affected, and the sensitivity of the individual. Pressure ulcers represent a major secondary complication for amputees with diabetes. Ulcers are particularly hard to heal in this population and are not easily detected using visual monitoring [5]. Between 1990 and 1994, the number of diabetes related hospital discharges after lower extremity amputation averaged 56,000 per year in the US [6]. Currently, it is clinically accepted to visually monitor tissue response to pressure for appearance

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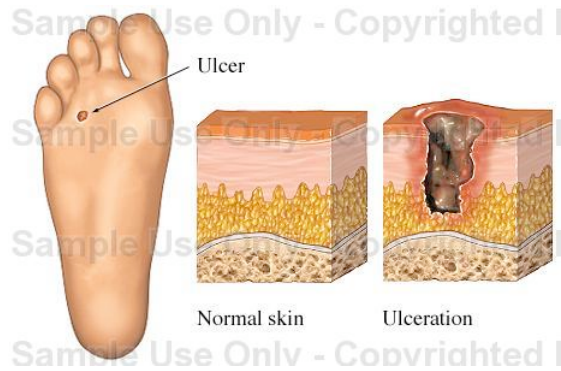
Figure 1.4: Schematic of ulcer formation on the foot due to complications of diabetes. Ulcers of this nature can also form on the residual limb of an amputee when using poor sock materials. Art courtesy of Nucleus Medical Art.

of erythema. No data is available to guide clinicians in identifying the damage to tissue in response to pressure experienced on the residual limb. As a result, severe tissue damage can go undetected as visual assessments can be difficult. Even an amputees own melanin can mask the visual appearance of erythema leading to misdiagnosis.

1.1.2 Erythema

Erythema is classified as redness of the skin caused by increased blood flow to the capillaries. There are many symptoms of erythema ranging from itchiness of skin to red, hard, painful lesions. [7]. While mild cases may not require treatment, bed rest and medication may be necessary for more severe cases. Erythema is a result of different causes such as heat, drugs, ultraviolet rays, ionizing radiation, over-exposure to sunlight, allergic reactions, pressure or repetitive loading on

skin. This disorder is believed to involve damage to the blood vessels of the skin with subsequent damage to skin tissues. Erythema can be a descriptive parameter in both clinical and scientific evaluations as clinical observations are often inaccurate [8]. Erythema left unidentified and untreated can lead to serious medical conditions as discussed above. Objective analysis of skin color and the ability to track redness changes can help clinicians treat patients more effectively.

1.2 Socks

Health and comfort of one's foot or residual limb is dependent on several criteria. Socks should protect the skin against destructive forces of pressure and friction while also preventing perspiration and odor while allowing for ventilation. Additionally, socks should prevent formation and inhabitation of fungus and bacteria. Socks are used as a barrier between the skin and a shoe (or skin and prosthetic device for amputees) maintaining a smooth, wrinkle-free environment. Preferrably, the interface between the sock and skin should remain dry, clean, well ventilated and free of any kind of skin trauma or pathological conditions.

Materials used as a protective layer between the skin and prosthetic limb are ineffective in preventing irritation and withstanding long term use [9]. It is estimated that 70 to 85 percent of prosthetic office visits involve sock wear or modification issues [10]. Even a minor skin eruption may, through neglect or mistreatment, become an extensive disorder that can seriously threaten the amputee's mental, social, and economic rehabilitation [11]. Currently there are no regulations about what is used for prosthetic sock materials and a lack of true testing of skin reaction or characteristics of socks by independent groups [12]. For amputees, the primary advantage of wearing a sock is that it is removable allowing for cleansing and elimination of oils, odors, and contaminants produced by the residual limb. Without a minimum level of comfort and safety between

the prosthetic and residual limb, even the most advanced prosthetic device can be rendered useless and can lead to a variety of pathological conditions.

1.3 Copper

1.3.1 Copper Background

Copper has been known for centuries to be an essential metal for human health. Copper is an antibacterial, antifungal and antiviral metal found in the human body that helps promote, maintain, and repair connective skin tissues while also acting during proliferation and remodeling phases of wound healing [13]. It is estimated that the adult body actually contains between 1.4 and 2.1 mg of copper per kg of body weight [14]. As a nutrient, copper is vital to healthy looking skin and in a non-soluble form, it can be incorporated into fabrics [13].

1.3.2 Medical applications of copper

Cupron^{TM}Inc. has created a cost effective platform technology that utilizes the qualities of copper and binds copper to textile fibers allowing for the production of woven, knitted and non-woven fabrics containing copper-impregnated fibers creating a sock with antimicrobial protection against microorganisms such as bacteria and fungi that attack the fibers. This process has been developed to incorporate copper into textile materials as well as latex, and other polymer products [15]. As an antimicrobial fabric, copper impregnated materials have a broad spectrum of activities including destruction of gram positive and negative bacteria as well as viruses [13, 14, 16]. This technology is being considered for significant medical applications to eliminate nosocominal infections in hospitals and alleviation from allergens and asthma related pathogens [16]. Of interest to this particular study, copper has been investigated for use in sock materials to prevent skin pathologies.

Copper impregnated in a sock lasts for the lifetime of the material and can withstand detergent and hot water exposure [13]. Copper does not deplete over time with regular wear or laundering, thus contributing to their long-term appeal for various foot pathological conditions. Additionally, in animal and human studies, no test subjects displayed or reported any negative effects or skin irritation when exposed to the copper material [15]. It has also been documented that there is a very low risk of adverse skin reactions associated with copper and will likely not elicit any reaction [17].

Currently, the most common treatment for skin conditions such as erythema, edema, burning/itching, and other related foot pathologies are topical or oral steroids and anti-fungal agents. Some of these remedies can take several months to control microbial organisms responsible for these conditions. Copper socks have been shown to greatly reduce and prevent pathological conditions in feet without compromising the sock to skin interface through protection against infection, perspiration management and odor control. A preliminary study performed by a podiatrist involving 56 patients, explored *in vivo* activity of copper infused fabrics for treatment of *tinea pedis* (or athletes foot) [13]. The results showed that subjects who wore Cupron yarn HealthStrides^{TM}Socks had significant improvement or resolution of erythema after only nine days (Figure 1.5).
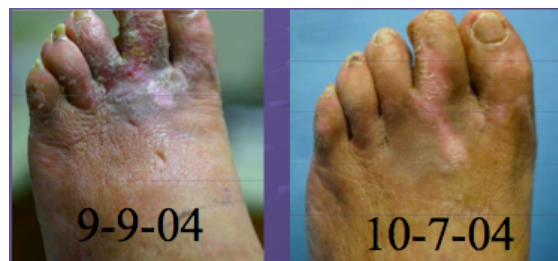
Figure 1.5: Example results of a diabetic patient who wore the copper ion socks for one one month during Dr. Zatcloff's study.

Focus needs to be directed to clinically observing changes in the sub-cutaneous layers of skin using a high-resolution spectrometer. Two pilot studies were designed and executed with a focus on the detection of erythematic changes associated with copper socks using a visible reflectance spectrometer. The pilot studies followed the requirements of a double-blind, randomized study. Though previous studies have concluded that copper ion socks reduce or eliminate erythema, those studies were based on clinical observation only and not empirical data. The first study consisted of 20 healthy, non-diabetic elderly volunteers and the second study focused on 10 healthy, non-diabetic volunteers between the ages of 18 and 35. Volunteers were assigned either control (non-copper) socks or copper socks to wear everyday for the full duration of the study. VRS measurements were collected in regular intervals where spectra were observed, recorded and analyzed to find the erythematic effects of the copper socks. An optical diffusion theory was used to find the erythema index and compare copper and control subjects.

Chapter 2

Detection of Erythema

Since many prosthetic wearers, diabetics, and elderly individuals are plagued with erythematic symptoms, it is fortunate that this redness of the skin can be easily measured using spectroscopy. Visible reflectance spectroscopy (VRS) of human skin involves measurement of diffuse reflectance in the 400-800 nm wavelength region. The measured spectrum can then be used to determine changes in erythema based on a layered skin model and optical diffusion theory. VRS is widely used as a diagnostic tool for skin characterization because it is a quick, non-invasive and relatively inexpensive method. Erythematic changes over a specified segment of time can be observed, recorded, and analyzed utilizing this technique.

2.1 Visible Reflectance Spectroscopy

VRS is a commonly used, noninvasive measurement for investigating skin color and has been used in dozens of documented studies to measure several different parameters including erythema, hemoglobin, and skin lesions [8, 18–21]. In particular, extensive research in reflectance spectroscopy has been performed within the visible [18, 22] and in the infrared [23] spectrums. VRS has been used to probe skin for determination of optical properties, including blood content

and oxygenation. In Viator et al, 2004, a comparison was performed between photoacoustically determined melanin content in Fitzpatrick skin phototypes I-VI with melanin content derived using VRS. A photon diffusion theory was then used to match parameters including epidermal thickness, background scattering, oxygenated and deoxygentated hemoglobin content, epidermal melanin, hair melanin, and water content. This diffusion theory model agreed with the physiologically expected blood content and the values derived photo-acoustically.
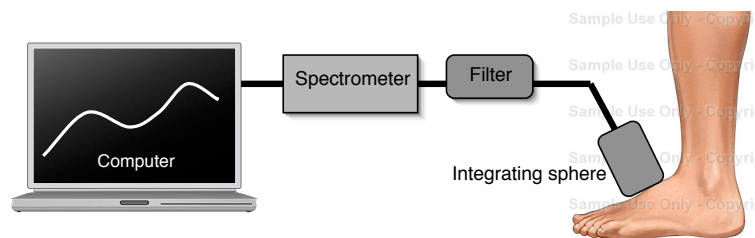
Figure 2.1: Schematic of the VRS setup. The integrating sphere port was 10 mm in diameter. The integrating sphere is placed next to the skin during measurement. Schematic of foot courtesy of Nucleus Medical Art.

Using a high-resolution spectrometer can allow users to clinically observe changes in the sub-cutaneous layers of skin. Ocean Optics HR2000 spectrometer performs up to 1000 full spectral scans per second with an optical resolution of 0.035 nm. The Ocean Optics HR2000 consists of a spectrometer, white light source, and integrating sphere connected to a computer. The spectrometer (HR2000, Ocean Optics, Dunedin, FL) can be optimized for detection of 190–820 nm light, although erythema analysis is best confined to 500–820 nm, with a wavelength accuracy of 2 nm. The light source is a tungsten halogen lamp within the integrating sphere (RSA-HP-84, Labsphere, North Sutton, NH) and is coupled to the spectrometer controlled by a computer. There is no risk or

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harm caused by the light beam and it takes only a few seconds to get an accurate reading. The skin surface of human subjects can be positioned at the 10 mm diameter port of the integrating sphere and sampled with a desired integration time. This device uses the characteristic absorption of light by chromophores of the skin to measure the amount of chromophores present [19]. Absorption of light in the skin is calculated by comparing the measured reflectance with a 99% diffuse reflectance standard (WS-1, Ocean Optics, Dunedin, FL). The spectrometer produces a graph of reflected light from the skin pigments and structures in SpectraSuite software (Ocean Optics, Dunedin, FL).
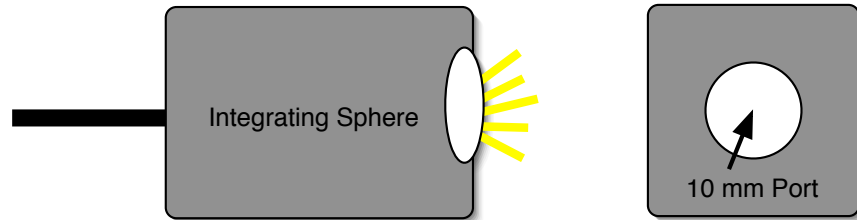
Figure 2.2: Schematic of the integrating sphere. The light source is a tungsten halogen lamp that is projected through the 10 mm port of the integrating sphere.

2.2 Diffusion Theory

As previously mentioned, photon diffusion theory to match parameters including epidermal thickness, background scattering, oxygenated and deoxygentated hemoglobin content, and epidermal melanin can be used to determine an experimental erythema index [24, 25].

2.2.1 Background

Diffusion theory, by definition, is the modeling of photon transport in a medium dominated by scattering where the photon moves down a concentration gradient. This theory is best suited for medium with scattering, rather than absorption, so each photon can undergo multiple scattering events before its transport is ended with absorption. Photons in this model have a relatively long residence time allowing for random movement in the medium. In the medium, the instantaneous fluence rate, is proportional to the concentration of optical energy, and the speed of light, c, as seen in equation 2.1.

$$F(r,t) = cC(r,t) \tag{2.1}$$

Time resolved spatial distribution of photon concentration, C(r,t), in a light scattering medium is based on the relationship between the fluence rate and concentration of optical energy. Diffusion occurs due to a concentration gradient. This concentration gradient is expressed as the change in concentration due to a change in position, $\delta C/\delta x$. Fick's first law is defined for steady-state diffusion when the concentration within the diffusion volume does not change with respect to time. Fick's first law of diffusion defines the flux, J, as proportional to the diffusivity, χ, and the negative gradient of concentration, $\delta C/\delta x$. The negative sign in equation 2.2, indicates that movement is going down the gradient in the direction of increasing x. For light, equation 2.3 displays diffusivity, χ is proportional to the diffusion length D and the speed of light, c, where D equals $1/(3\mu_s(1\text{-g}))$.

$$J = -\chi \frac{\partial c}{\partial x} \tag{2.2}$$

$$\chi = cD \tag{2.3}$$

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Fick's first law defines optical diffusion in terms of the flux proportional to the diffusion constant D and negative fluence gradient, (Equation 2.4). Diffusion constant, D, is proportional to the velocity of the diffusing particles, which depends on the position or length of χ.

$$J = -D\frac{\partial F}{\partial x} \tag{2.4}$$

Fick's first law can be used to define the optical transport theory. One must consider a model where light scattered at position r passes through a small aperture with area A contributing to the flux, J_+. In this model it is assumed there is a homegenous isotropical scattering medium with scattering coefficient μ_s, small aperture with area A at the origin (r=0) and fluence rate F(0) at the origin. Also, assumed that the fluence rate F(r) at a position r near the aperture is approximated by equation 2.5 where higher order terms are neglected.

$$F(r) \approx F(0) + r\frac{\partial F}{\partial r} + \dots \tag{2.5}$$

The net flux can be calculated through the small aperture with area A due to scattering from all the surrounding volume where the scattered flux at r follows equation 2.6.

$$\mu_s F(r) = \mu_s(F(0) + r(dF(r)/dr) \tag{2.6}$$

The fraction of scattered flux from r that survives a pathlength r without being scattered or absorbed is exp(-μ_tr), where $\mu_t = \mu_s + \mu_a$. The fraction of surviving scattered flux from r that passes through A is Acosθ/(4πr2). When you integrate the flux from r over all the possible θ and r for the hemisphere above the aperture you will have:

$$J_+ \approx \int_0^\infty \int_0^{\frac{\pi}{2}} \mu_{s_o} F(r)\frac{A\cos\theta}{4\pi r^2}\exp(-\mu_t r)2\pi r\sin\theta r d\theta dr \tag{2.7}$$

With another integration for the scattering from the hemisphere below the aperture yields a positive flux passing through A in the other direction opposite to J_+. The connections between Fick's first law and the optical transport theory is based on the linearization of F(r) for F(0) and the approximation of D by the value $1/(3\mu_s)$. The final expression for next flux J is in the same form as Fick's first law where the diffusion constant, D has the values shown in Equation 2.8. For photon transport this equation describes isotropic scattering.

$$J = -D\frac{\partial F(r)}{\partial r}\bigg|_{at\,r=0} \; where\, D = \frac{1}{3\mu_s} \tag{2.8}$$

Fick's second law is used in non-steady or continually changing state diffusion where the concentration within the diffusion volume changes with respect to time. The second law states the rate of accumulation of concentration within a volume is proportional, dC/dt, is proportional to the diffusivity and the second derivative of the concentration. Equation 2.9 is positive when the concentration gradient is generating a greater flux moving into the front end of the planar volume.

$$\frac{\partial C}{\partial t} = \chi\frac{\partial^2 F}{\partial x^2} \tag{2.9}$$

Fick's second law allows us to create a differential equation for optical diffusion as seen in equation 2.10 using substitutions from Fick's first law.

$$\frac{\partial F}{\partial t} = cD\frac{\partial^2 F}{\partial x^2} \tag{2.10}$$

As with all theories there are limitations to the diffusion theory and its applications. There are two significant areas where curvature can affect the accuracy of the diffusion theory. First, when a gradient is very steep near a point source or collection of point sources there can be changes in the curvature of the gradients caused by the exponential terms exp(-r/(4cDt)) and the 1/r. When there is distance from point sources the gradients become more gradual, making the

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diffusion theory more accurate. Second, if there is strong absorption the theory can be affected. Strong absorption prevents photons from having extended scattering causing the approximation $\mu_t = \mu_s$ to be inaccurate. The diffusion constant D, is commonly approximated as:

$$D \approx \frac{1}{3} \frac{1}{\mu_s' + \mu_a} \tag{2.11}$$

But Monte Carlo simulations have shown that the approximation is closer to equation 2.12 where if $3\mu_s$ is much greater than μ_a, the effects of absorption can be neglected.

$$D \approx \frac{1}{3} \frac{1}{\mu_s' + \frac{\mu_a}{3}} = \frac{1}{3\mu_s' + \mu_a} \tag{2.12}$$

Using Fick's second law we can define a time-resolved diffusion theory. The solution of Fick's second law of diffusion for a point source of energy, U_o, placed a time zero (t=0) and at origin of zero (r=0) is shown in equation 2.13. This expression describes spherically symmetric diffusion from a point impulse source on a homogeneous medium with no boundaries. In this equation, r is the distance from the source to the point of observation, t is the time of observation and χ is the diffusivity.

$$C(r,t) = U_o \frac{\exp \frac{-r^2}{4\chi t}}{(4\pi\chi t)^{\frac{3}{2}}} \tag{2.13}$$

For optical diffusion, if you let F=cC, χ=cD, and the point source to be an impulse energy of U_o you will have fluence rate, F(r,t) equal to equation 2.14.

$$F(r,t) = cU_o \frac{\exp \frac{-r^2}{4cDt}}{(4\pi cDt)^{\frac{3}{2}}} \tag{2.14}$$

For steady-state diffusion, fluence rate F_{ss} is described in response to an isotropic point source of continuous power, P_o. The steady-state fluence rate is derived from equation 2.15 and is shown in equation 2.16. T(r,t) and T_{ss}(r) are

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the transport factors that distinguish the source, the transport and the fluence rate. The transport rate for steady-state is obtained from integrating T(r,t) from equation 2.15 over time to yield the total accumulated amount of photon transport to each position r. The factor exp(-$\mu_a$ct) accounts for the absorption of photons causing photon concentration to approach zero as time goes to infinity.

$$F(r,t) = U_o T(r,t) \, where \, T(r,t) = \frac{c \, \exp(\frac{-r^2}{4cDt})}{(4\pi cDt)^{\frac{3}{2}}} \qquad (2.15)$$

$$F_{ss}(r) = P_o \, T_{ss}(r) \; where \; T_{ss}(r) = \frac{\exp(-r\sqrt{\mu_a/D})}{4\pi Dr} \qquad (2.16)$$

T_{ss}(r) is derived from equation 2.17 with substitions that remove diffusion length, D, with optical penetration depth, δ. The optical penetration depth is the incremental distance from the source.

$$T_{ss}(r) = \int_0^\infty T(r,t) \exp^{(-\mu_a ct)} \; dt = \frac{\exp^{(-r\sqrt{\mu_a/D})}}{4\pi Dr} = \frac{\exp^{(-r/\delta)}}{4\pi\mu_a\delta^2 r} \qquad (2.17)$$

2.2.2 Diffusion Theory for Skin

The diffusion theory was used to calculate reflectance spectra based on a simple two-layer model of skin consisting of an epidermis and dermis layer based on Svaasand *et al*, 1995. Each layer was treated as optically homogenous which allows for an analytical solution of the diffusion equations for light and the calculation of reflectance [20]. First, light passes through a 100 micron epidermis (or melanin layer) followed by a plexus of blood vessels in the semi-infinite dermis (or hemoglobin layer) to the light finally being reflected off collagen in the lower dermis [19]. The light returned carries spectral characteristics from the chromophores present in the skin. There are three human tissue chromophores that most influence the measurement of erythema: hemoglobin, oxyhemoglobin and melanin [5]. Absorption in the epidermis is primarily due to melanin and a

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small fraction of blood in the vascular dermal papillae extending into the most superficial 100 micron of human skin. Dermal absorption is primarily due to oxygenated and deoxygenated hemoglobin. Both layers have wavelength dependent scattering, following λ^{-1} where λ is wavelength, and a small background component of absorption. Svaasand *et al* proportionally averages the optical properties of the epidermis and melanin in the first layer and those of dermal blood for all the underlying layers to create the composite optical properties of human skin. Fitting with the diffusion theory can produce information about the amounts of hemoglobin present in the skin and its level of oxygenation. A simple estimate of the redness of skin can be found from the ratio between the diffuse reflectances at 550 nm and 650 nm [26]. The parameters that are varied are shown in Table 1 as well as the range in which they were varied.

2.2.3 Erythema Index

Erythema index, which as originally discussed by Dawson et al, 1980, is defined as the total volume fraction of blood, oxygenated and deoxygenated [27]. The blood absorption coefficient at 540 nm for oxygenated blood and 760 nm for deoxygenated blood can be used to compare to the coefficients of whole blood to derive the volume fractions. These wavelengths are chosen as they represent local maxima for each blood type and are readily identified from the spectrum. The total volume fraction is the sum of the oxygenated and deoxygenated blood. This fraction is dimensionless and is used to compare erythema measurements between different patients and for individual patient measurements taken at different times during the study.

We used VRS to determine an experimental erythema index. Measurement of the dorsal surface of the foot is best as the epidermis will be 50–100 micron thick and may be more amenable to VRS measurements. Whereas the plantar surface can be prone to calluses and blisters resulting a unpredictable and thicker

epidermis. In the case of the two pilot studies conducted for this research a blood absorption coefficient at 540 nm for oxygenated blood and 560 nm for deoxygenated blood was used as these were the local maxima generated in the spectral field used between 500 - 600 nm for each VRS measurement.

Chapter 3

Pilot Study 1

3.1 Introduction

The purpose of this pilot study was to determine if copper induces a change in erythema in feet. Though previous studies have concluded that copper ion socks reduce or eliminate erythema, those studies were based on clinical observation only and not empirical data. Expanding on preliminary studies, we were interested in scientifically measuring the change in erythema utilizing VRS methods. Our study consisted of 20 non-diabetic volunteers from two elderly living communities. Volunteers were assigned either control (non-copper) socks or copper socks to wear everyday for 12 weeks of the study. VRS measurements were collected every other week where we observed, recorded and analyzed the erythematic effects of the copper socks.

3.2 Materials and Methods

3.2.1 Copper Sole^{TM} Socks and Study Protocol

This pilot study followed the requirements of a double-blind, randomized study. The socks used in this study were Copper Sole^{TM} Socks provided by Renfro Corporation (Mount Airy, NC) containing Cupron^{TM} copper bonded polyester

yarn and control socks that did not contain copper impregnated fibers. There were a total of 128 pairs of socks. The sock pairs were divided into four groups and labeled with a designated letter that was identifiable only by the supplier of the socks, Renfro Corporation. Each study participant was randomly assigned 6 pairs of socks of the same letter.

Twenty active, non-diabetic, healthy volunteers from two area retirement living communities participated in this study. The volunteers were required to wear only their assigned socks for at least 8 hours each day for 12 weeks. Participants were allowed to launder the socks as they would normally without restriction on detergents or water temperature.

On day one, each volunteer had the dorsal side of their left and right foot measured with a visible reflectance spectrometer. Immediately following measurement, the socks were provided for wear. The same measurement protocol was followed every two weeks from the start of the study through week 12, totaling 7 sets of data collection for each volunteer. Each individual was seen on the same day of the week and at the same time of day throughout the study.

There were strict inclusion and exclusion criteria for the volunteers in this study. To be included participants needed to be in good health as determined by medical care or major procedures performed in the 6 months prior to the study. Additionally, the volunteers were required to have the ability to understand and carry out instructions for the study. Exclusion criteria included diabetic individuals, participants with pathological conditions on their feet such as blisters, sores, edema, fissures, cracking, leakage or vesicular eruptions as these conditions can alter the measurement of the VRS. Participants were also excluded if they had used topical or oral steroids or antifungal agents regularly in the 6 months prior to the study. Concurrent use of topical or oral steroids was strictly prohibited for the duration of the study, as was concurrent use of topical or oral anti-fungal agents. Use of these products could alter the behavior of the skin and underly-

ing tissues and influence the measurements. Finally, participants were prohibited from participating in any drug or drug device studies at the same time as this study.

This pilot study was approved by the University of Missouri Health Sciences Institutional Review Board and all volunteers signed a detailed consent to participate in this pilot study.

3.2.2 Experimental Setup for Visible Reflectance Spectrometer

The apparatus for performing VRS measurements consisted of a spectrometer, white light source, integrating sphere, and an Apple laptop computer (Figure 3.1). The spectrometer (HR2000, Ocean Optics, Dunedin, FL) is optimized for detection of 190–820 nm light, although our analysis was confined to 500–600 nm with a wavelength accuracy of 2 nm. The light source was a tungsten halogen lamp within the integrating sphere (RSA-HP-84, Labsphere, North Sutton, NH). The integrating sphere was coupled to the spectrometer and controlled by an Apple laptop computer (Powerbook G4, Apple Computers, Cupertino, CA). A 99% diffuse reflectance standard (WS-1, Ocean Optics, Dunedin, FL) was used as a reference for the spectrometer. The skin surface of human subjects was positioned at the 10 mm diameter port of the integrating sphere. SpectraSuite software (Ocean Optics, Dunedin, FL) was used as an interface to the spectrometer and samples were collected with an integration time of 10 msec and scans to average was set to 128. The spectrometer then displayed a wavelength reading of reflected light from the skin pigments and structures. Each wavelength display was saved and analyzed using the diffusion theory. In order for the spectrometer setup to reach a temperature equilibrium, the halogen light source was turned on 30 minutes prior to measurements. Care was taken to place the integrating
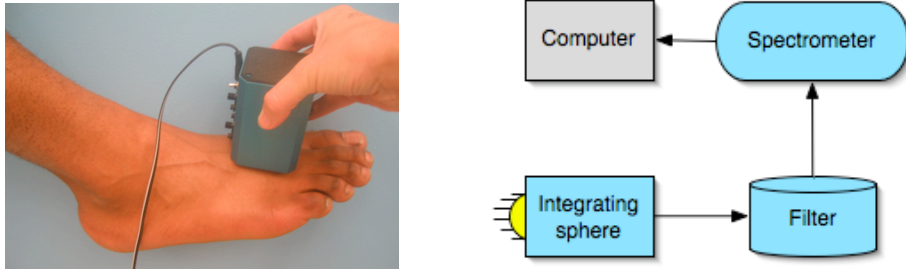
Figure 3.1: LEFT: Integrating sphere placed next to the skin on the dorsal side of the foot. RIGHT: Schematic of visual reflectance spectrometer device setup. The integrating sphere has a light source and a sensor that sends a reading to the filter. The signal is then sent to the spectrometer and a wavelength is displayed in the computer program.

sphere on the skin gently, applying minimal pressure to avoid possible occlusion of the skin structures.

VRS measurements resulted in spectra from 500-820 nm as percent diffuse reflectance. The spectra was fitted to a diffusion model based on Svaasand et al, 1995, which explains the entire development of the diffusion model and treats the subject in detail in an appendix of that paper [26]. The two layer model developed consisted of a thin 100 micron epidermis over a semi-infinite dermis. Absorption in the epidermis was primarily due to melanin and a small fraction of blood in the vascular dermal papillae extending into the most superficial 100 micron of human skin. Dermal absorption was primarily due to oxygenated and deoxygenated hemoglobin. Hematocrit and relative blood oxygenation were variable, normally set at 0.41 and 70%, respectively. Both layers have wavelength dependent scattering, following λ^{-1} where λ is wavelength, and a small background component of absorption. Absorption and scattering were expressed as analytic functions incorporated into a computational model using Matlab® (R2006b, MathWorks, Inc., Natick, MA). Included in the model was absorption

coefficient of epidermal melanin (mm^{-1}), scattering coefficient of the epidermis and dermis (mm^{-1}), erythema index (also known as total blood fraction), blood vessel radii (mm), oxygen saturation, hematocrit and depth of epidermal thickness (mm). Fitting was accomplished primarily by varying melanin and blood concentrations in the model, though relative blood oxygenation and background absorbance levels were also varied to improve the fit.

Hematocrit	HbO$_2$	melanin	vessel radii	epi depth	blood (epi)
0.40–0.50	0.5–1.0	0.2–1.3	0.004–0.015	0.05–0.13	0.01–0.1

blood (derm)	σ_{epi}	σ_{derm}
0.01–1.0	30–80	25–50

Table 3.1: The parameters varied during the fit of the VRS data. Deoxygenated hemoglobin was 1-HbO$_2$. Vessel radii, melanin and epidermal and dermal scattering (σ_{epi} and σ_{derm}, respectively) are given in mm^{-1}. Other values are in volume fraction.

3.2.3 Erythema Index

Erythema index was defined as the total volume fraction of blood, oxygenated and deoxygenated, determined by VRS. The blood absorption coefficient at 540 nm for oxygenated blood and at 560 nm for deoxygenated blood were used to compare the coefficients of whole blood in order to derive volume fractions. The wavelengths chosen represent the local maxima for each blood type and are readily identified from the VRS spectrum. The total volume fraction was the sum of the oxygenated and deoxygenated blood. This fraction is dimensionless and was used to compare erythema measurements between each volunteer and for individual volunteer measurements taken at the 7 collection dates in the study.

3.2.4 Statistical Analysis

A linear regression line was determined for each volunteer for all dates of measurements plotting erythema index versus time. From this graph, a linear regression line was found to obtain the slope of each data set. Using the slopes obtained from each regression line, statistical results were collected using $Prism^{TM}$ software (Graphpad, Inc., San Diego, CA). A one-way ANOVA analysis was used to compare 4 groups of slopes - left foot control, right foot control, left foot copper, right foot copper. In addition, we compared all the copper measurements against the controls in an unpaired T-test. P values, f, and R squared were determined.

3.3 Results

At the end of the 3 month study, there were only 14 volunteers who participated in the entire duration of the study. After identification of the sock groups, 4 volunteers represented the copper group while 10 represented the control group.

3.3.1 Diffusion Theory

Using the diffusion theory a fit was made between the diffusion theory model curve and the actual curve from VRS measurements. Figures 3.2 shows four examples of the type of curve matching that was performed to determine the erythema index. Figure 3.2 shows the Matlab diffusion theory fit of the control volunteers 21-132, 122-13, 22-126 and 118-11. The wavelength was examined from 500 nm to 600 nm and is plotted with the reflectance. The graph for volunteers 21-132 and 122-13 were expected to be good fits due to the lack of visual erythema prior to testing. The graph for volunteers 22-126 and 118-11 were expected to have bad fits due to the visual appearance of the skin prior to testing. However, the VRS measurement was easy to fit with the diffusion theory

model for these two individuals. Graphs like this were collected and analyzed for each volunteer after each measurement.
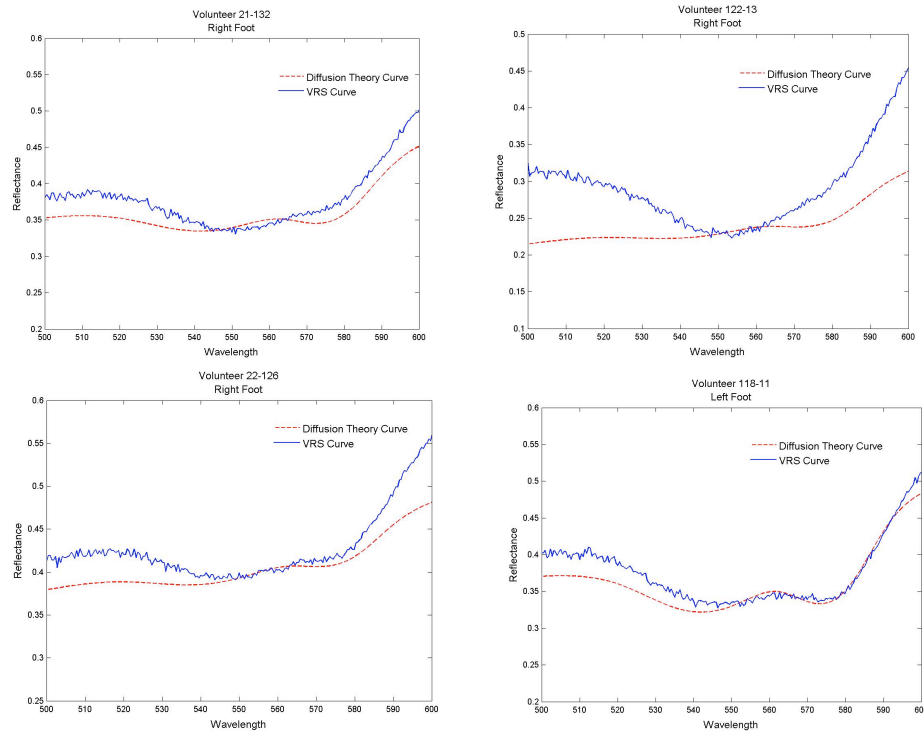

Figure 3.2: Matlab diffusion theory fit curves of 4 volunteers. The top two graphs show the fits for two volunteers that visually had no erythema on their feet but the curves were difficult to fit. The bottom two graphs show the fits for two volunteers that visually had redness in their feet but the fits were easy to make.

3.3.2 VRS Spectra

The VRS spectra for each person was collected for each measurement as mentioned in section 3.3.1. Figure 3.3 and 3.4 shows the left and right foot spectra collected over time from 2 of the study volunteers. Volunteer 2146 in Figure 3.3 was assigned the copper socks, while volunteer 21-232 in Figure 3.4 was assigned the non-copper socks.
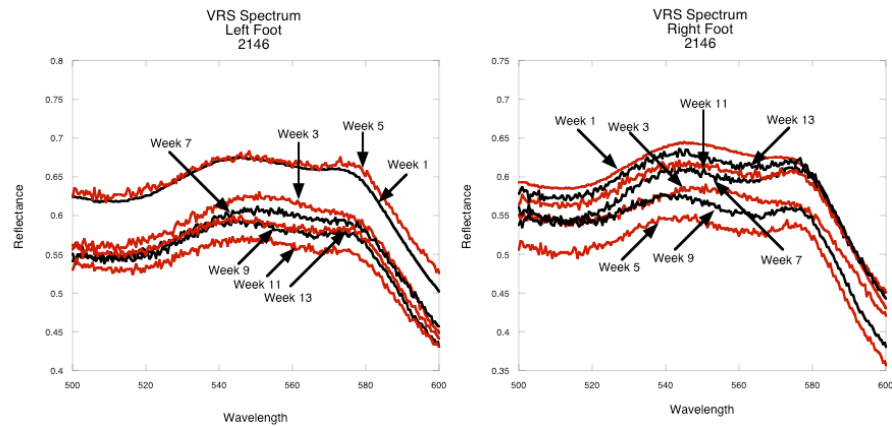
Figure 3.3: VRS Spectra over the duration of the study. Measurements were collected every 2 weeks. Volunteer 2146 was assigned copper socks to wear during the study.

3.3.3 Statistical Analysis

A linear regression line was determined for each volunteer for all dates of measurements. Figure 3.5 shows the linear regression line for 2 study volunteers that wore the copper socks and Figure 3.6 shows the linear regression line for 2 study volunteers who wore the control socks. The graphs are in terms of erythema index from the first week of the study to the last.

Using the slopes obtained from each regression line statistical results were collected. One-way ANOVA resulted in a p value of 0.4959, F of 0.8194 and R squared of 0.09291. The unpaired T-test revealed a p value of 0.0.9029 and R squared value of 0.0005828. Neither test showed significant differences between copper and control.

3.4 Discussion

This pilot study was executed as a double blind, randomized test. It was discovered at the conclusion of the study, when the identities of the sock groups were revealed to us by the supplier, that only 30% of the study group had the control and the other 70% had copper. The study was designed to have a 50%

27
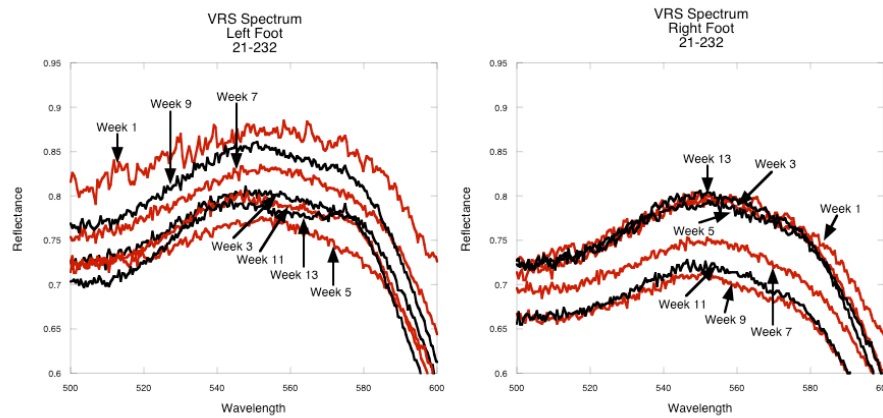
Figure 3.4: VRS Spectra over the duration of the study. Measurements were collected every 2 weeks. Volunteer 21-232 was assigned the control socks to wear during the study.

representation of both the control and copper. However, due to the double blind factor of this experiment we were not able to correct this imbalance before the study began. Additionally, due to compliance issues from 6 of the study volunteers, their data was removed from the study thus affecting the ratio of copper to control participants.

The spectrometer was used on the dorsal surface of the foot due to the ease of modeling the thinner epidermis on the top of the foot with the diffusion theory. The plantar surface of the foot can vary in thickness from heel to toe due to different levels of friction, stress and pressure experienced by the epidermis. The heel is also prone to thick calluses while the plantar side of the toes are prone to blister formation. Additionally, there can be a much greater difference in thickness from person to person on the plantar surface, especially in elderly individuals, and can be very difficult to predict in the diffusion theory model. The dorsal surface of the foot is not a load bearing or a stressed area of the foot. The epidermis here more closely follows the parameters of the diffusion theory model.

28
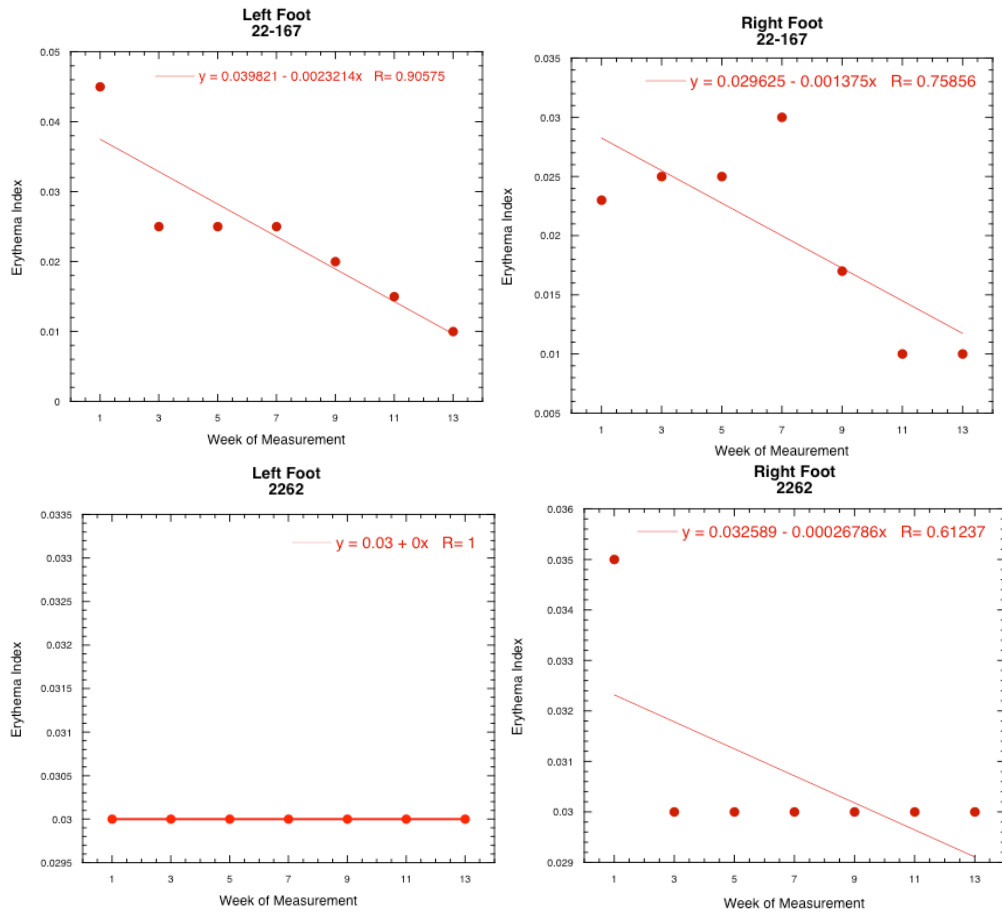
Figure 3.5: This is a sample linear regression plot of 2 study volunteers who wore the copper socks. The first row shows the linear regression results on the left and right foot of volunteer 22-167. The second row shows the linear regression results for study volunteer 2262.
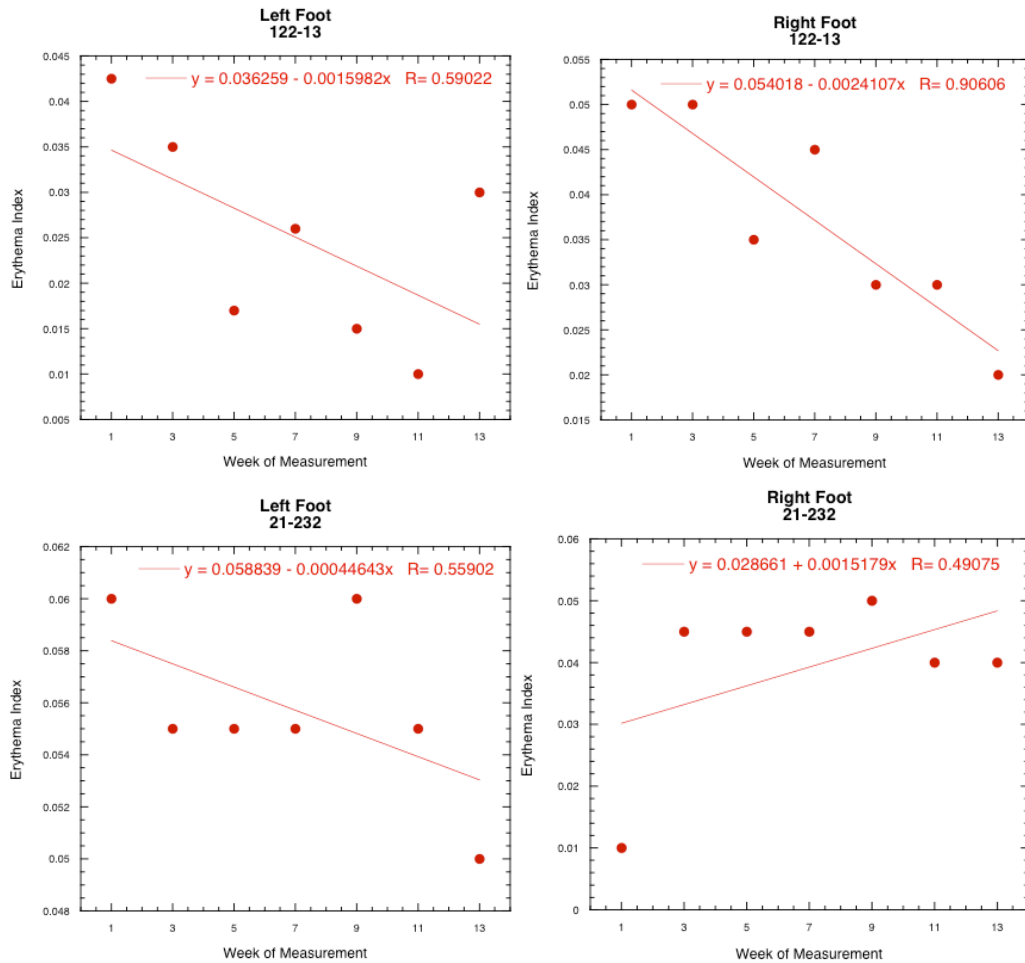
Figure 3.6: This is a sample linear regression plot of 2 study volunteers who wore the control socks. The first row shows the linear regression results on the left and right foot of volunteer 122-13. The second row shows the linear regression results for study volunteer 21-232.

The methods we used did not show any significant difference between the control group and the copper group. Both a one-way ANOVA and t-test showed that there was no significant difference between the two groups. One-way ANOVA P value was greater than 0.05 as was the P value from the t-test resulting in no significant difference between the copper and control using the t-test. There are a few factors that could have affected the significance of the results.

First, it was very difficult to control the compliance of the study volunteers. Six individuals were removed from the study for not wearing their socks regularly or for not attending every scheduled measurement time. The length of the study coupled with only seeing the volunteers every two weeks were primary factors affecting compliance. Due to the age of the volunteers, it was naturally difficult for them to complete and follow all study protocols. Most volunteers had a difficult time attending scheduled visits every two weeks and wearing only their study socks for the 12 week duration. It was also difficult for the study participants to get out of their homes and come to the main building at their living community for measurement due to the winter weather during the study duration. The volunteers would either walk or drive from their homes to the building where measurements were collected and some days the weather made that a difficult challenge.

Participants had appointments at the same time in the same room but they were coming directly from outside. The cold temperatures likely influenced the parameters of the skin in their feet due to blood flow restriction to the extremities in cold weather. The volunteers should have been allowed to reach temperature equilibrium in the building of measurement before data was collected to ensure accurate readings.

The volunteers of this study were selected due to their overall health and lack of skin pathologies on their feet. Among all study volunteers, no adverse effects from the socks were reported. None of the feet showed any signs of allergic

reaction or irritation of any kind. Volunteers could not tell the difference between wearing these socks and wearing any other type sock. Additionally, none of the volunteers were able to identify if they were wearing the copper or the control socks. With healthy skin there would be a limit to the effect that the copper socks could have on erythema. Statistical testing showed there was no significant change detected by the spectrometer. Without the presence of a skin pathology for the copper to effect, there would likely be no change in the erythema index from the start of the study to the end. There would also be no difference between the volunteers that wore the copper and those that wore the control sock. Copper works to alter and effect bacteria that cause skin pathologies. Thus, in the case of healthy feet, neither sock type would have a positive or negative influence on the skin. The linear regression with copper and control individuals support the lack of statistical significance and are demonstrated in the figures above. Both graphs demonstrate that there was no significant difference in the effect of wearing either sock group for the duration of the study as they both show the same linear trend and slopes.

It has been stated that the test volunteers were healthy people with no skin pathologies. However, the health of their circulatory system was not taken into account. If any of the volunteers were cigarette smokers, the nicotine would have been acting as a vasoconstrictor and there would have been a comprised flow of blood into the feet. The opposite is true for volunteers who may have been taking vasodilators. With the widening of blood vessels there could have been another level of compromise occuring within the body affecting the measurements.

Additionally, the way we fit the data and the computer model we used each contribute to why we were unable to detect a significant change in erythema. Fitting of data to the diffusion theory was done by manually adjusting the optical parameters of the skin. Manually fitting the curves makes it very difficult to correctly identify the parameters acting in each person's foot. There were feet

that visually appeared to have erythema prior to testing. However, this visual assessment was not seen after using the diffusion theory. Some graphs were easily fit using the diffusion theory model while others were very challenging.

Furthermore, the diffusion model used did not consider the age of the skin we were evaluating. Elderly skin may have different parameters to follow then the skin of a healthy young person. The skin of an elderly person can have different absorption coefficients of epidermal melanin, scattering coefficients of the epidermis and dermis, blood concentration, blood vessel radius, oxygen saturation, hematocrit and depth of epidermal thickness. The skin is far more sensitive to pressure, friction and abrasion as production of skin cells is not as efficient in elderly skin.

3.5   Conclusion

This pilot study focused on the detection of erythematic changes associated with copper socks using a visible reflectance spectrometer. The methods we used did not show any significant difference between the control group and the copper group. Despite statistical results, this study does not mean that there is not a change due to the copper socks, it simply means that we were unable to detect a change using our study protocol, VRS and diffusion theory methods. Length of the study and environmental conditions made it very challenging for volunteers to comply with the study rules. Compliance issues resulted in the removal of 15% of the data. In order to further investigate the effects of copper socks on erythema, we propose another study using volunteers of a younger age and increased physical activity and health. Study length and frequency of measurement should also be considered to influence compliance and detection of erythema. Further development of the diffusion theory model to provide optical solutions using improved methods to fit the VRS data would also impact the results of a future study.

Chapter 4

Pilot Study 2

4.1   Introduction

The purpose of this pilot study was to determine if copper induces a change in erythema in young, healthy feet. Due to the results of the elderly foot study, this study was designed to test the same theories of copper on a younger set of subjects. Though the previous study concluded that copper ion socks showed no change in erythema, there were many compliance issues and experimental designs that may have influenced those results. This study consisted of 10 non-diabetic volunteers from the University of Missouri - Columbia with ages ranging from 18-35. Volunteers were assigned pairs of socks where the right sock was the control (non-copper) and left was the copper sock. Volunteers were instructed to wear their assigned socks everyday for the full duration of the study. VRS measurements were collected every third day for 2 weeks where we observed, recorded and analyzed the erythematic effects of the copper socks.

4.2 Materials and Methods

4.2.1 Copper Sole^{TM} Socks and Study Protocol

This pilot study followed the requirements of a double-blind, randomized study. The socks used were Copper Sole^{TM} Socks provided by Renfro Corporation (Mount Airy, NC) containing Cupron^{TM} copper bonded polyester yarn and control socks that did not contain copper impregnated fibers. There were a total of 60 pairs of socks with each individual sock labeled with either an "L" for left foot or an "R" for right foot. It was not known to study participants or researchers which sock group contained the copper fibers. Assignment was designated by the supplier of the socks, Renfro Corporation. Each study participant was supplied with 6 pairs of socks where each pair consisted of one "L" and one "R" sock. This resulted in each volunteer being assigned one copper sock ("L") to wear on their left foot and one control sock ("R") to wear on their right foot for the duration of the study.

Ten active, non-diabetic, healthy students from the University of Missouri participated in this study. The volunteers were required to wear only the assigned study socks for at least 8 hours each day for 2 weeks. Participants were allowed to launder the socks as they would normally without restriction on detergents or water temperature.

On day one, each volunteer had their left and right foot measured with a visible reflectance spectrometer. Immediately following measurement, the socks were provided for wear. The same measurement protocol was followed every third day from the start of the study totaling 6 sets of data collection for each volunteer. Each individual was seen at the same time of day throughout the study in the same room.

There were strict inclusion and exclusion criteria for the volunteers in this study. To be included participants needed to be in good health as determined by

medical care or major procedures performed in the 6 months prior to the study. Additionally, the volunteers were required to have the ability to understand and carry out instructions for the study. Exclusion criteria included diabetic individuals, participants with pathological conditions on their feet such as blisters, sores, edema, fissures, cracking, leakage or vesicular eruptions as these conditions can alter the measurement of the VRS. Participants were also excluded if they had used topical or oral steroids or antifungal agents regularly in the 6 months prior to the study. Concurrent use of topical or oral steroids was strictly prohibited for the duration of the study, as was concurrent use of topical or oral anti-fungal agents. Use of these products could alter the behavior of the skin and underlying tissues and influence the measurements. Finally, participants were prohibited from participating in any drug or drug device studies at the same time as this study.

This pilot study was approved by the University of Missouri Health Sciences Institutional Review Board and all volunteers signed a detailed consent to participate in this pilot study.

4.2.2 Experimental Setup for Visible Reflectance Spectrometer

The apparatus for performing VRS measurements consisted of a spectrometer, white light source, integrating sphere, and a Apple laptop computer (Figure 3.1). The spectrometer (HR2000, Ocean Optics, Dunedin, FL) is optimized for detection of 190–820 nm light, although our analysis was confined to 500–600 nm with a wavelength accuracy of 2 nm. The light source was a tungsten halogen lamp within the integrating sphere (RSA-HP-84, Labsphere, North Sutton, NH). The integrating sphere was coupled to the spectrometer and controlled by a Apple laptop computer (Powerbook G4, Apple Computers, Cupertino, CA). A 99% diffuse reflectance standard (WS-1, Ocean Optics, Dunedin, FL) was used

as a reference for the spectrometer. The skin surface of human subjects was positioned at the 10 mm diameter port of the integrating sphere. SpectraSuite software (Ocean Optics, Dunedin, FL) was used as an interface to the spectrometer and samples were collected with an integration time of 10 ms and scans to average was set to 128. The spectrometer then displayed a wavelength reading of reflected light from the skin pigments and structures. Each wavelength display was saved and analyzed using the diffusion theory. In order for the spectrometer setup to reach a temperature equilibrium, the halogen light source was turned on 30 minutes prior to measurements. Care was taken to place the integrating sphere on the skin gently, applying minimal pressure to avoid possible occlusion of the skin structures.

4.2.3 Diffusion Theory

VRS measurements resulted in spectra from 500-820 nm as percent diffuse reflectance. The spectra was fitted to a diffusion model based on Svaasand et al, 1995, which explains the entire development of the diffusion model and treats the subject in detail in an appendix of that paper [26]. The two layer model developed consisted of a thin 100 micron epidermis over a semi-infinite dermis. Absorption in the epidermis was primarily due to melanin and a small fraction of blood in the vascular dermal papillae extending into the most superficial 100 micron of human skin. Dermal absorption was primarily due to oxygenated and deoxygenated hemoglobin. Hematocrit and relative blood oxygenation were variable, normally set at 0.41 and 70%, respectively. Both layers have wavelength dependent scattering, following λ^{-1} where λ is wavelength, and a small background component of absorption. Absorption and scattering were expressed as analytic functions incorporated into a computational model using Matlab$^{®}$ (R2006b, MathWorks, Inc., Natick, MA). Included in the model was absorption coefficient of epidermal melanin (mm^{-1}), scattering coefficient of the epidermis

and dermis (mm^{-1}), erythema index (also known as total blood fraction), blood vessel radii (mm), oxygen saturation, hematocrit and depth of epidermal thickness (mm). Fitting was accomplished primarily by varying melanin and blood concentrations in the model, though relative blood oxygenation and background absorbance levels were also varied to improve the fit.

4.2.4 Erythema Index

Erythema index was defined as the total volume fraction of blood, oxygenated and deoxygenated, determined by VRS. The blood absorption coefficient at 540 nm for oxygenated blood and at 560 nm for deoxygenated blood were used to compare the coefficients of whole blood in order to derive volume fractions. The wavelengths chosen represent the local maxima for each blood type and are readily identified from the VRS spectrum. The total volume fraction was the sum of the oxygenated and deoxygenated blood. This fraction is dimensionless and was used to compare erythema measurements between each volunteer and for individual volunteer measurements taken at the 7 collection dates in the study.

4.2.5 Statistical Analysis

A linear regression line was determined for each volunteer for all dates of measurements plotting erythema index versus time. From this graph, a linear regression line was found to obtain the slope of each data set. Using the slopes obtained from each regression line, statistical results were collected using Prism^{TM} software (Graphpad, Inc., San Diego, CA). A comparison of all the copper measurements against the controls (left sock versus right sock) was done with a paired T-test. P value and R squared were determined from this analysis.

4.3 Results

4.3.1 Diffusion Theory

Using the diffusion theory a fit was made between the diffusion theory model curve and the actual curve from VRS measurements. Figure 4.1 shows an example of the type of curve matching that was performed to determine the erythema index. This figure shows the Matlab diffusion theory fit of study volunteers 12-519, 12-1019, 22-312, and 12-128. The wavelength was examined from 500 nm to 600 nm and is plotted with the reflectance. Graphs like this were collected and analyzed for each volunteer after each measurement.
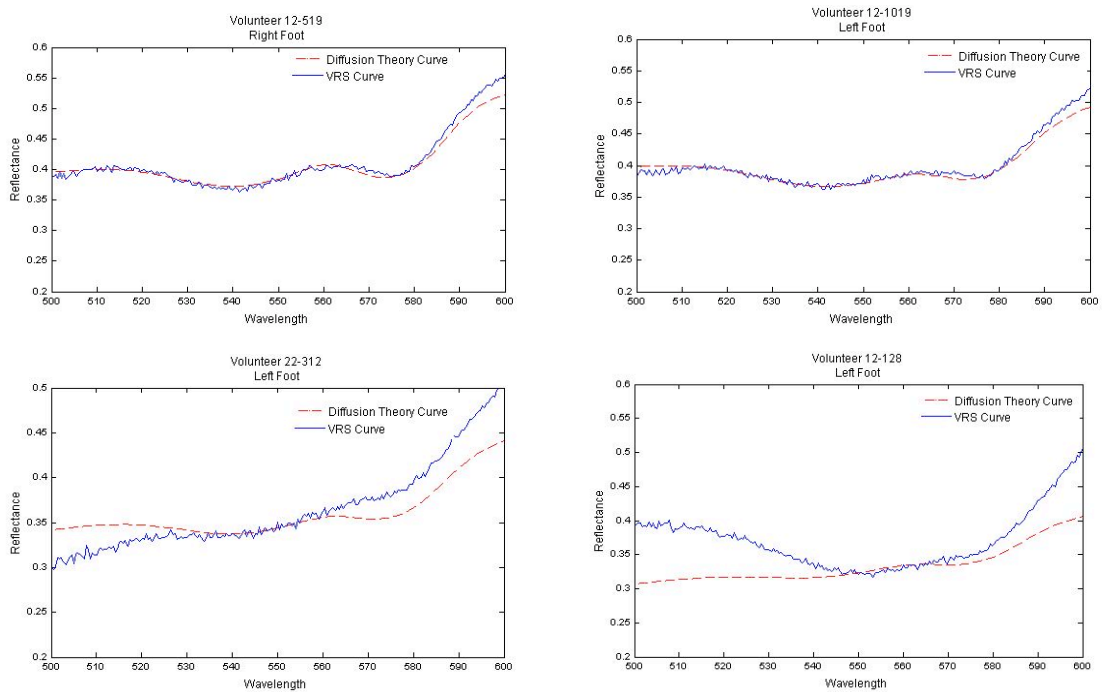
Figure 4.1: Left: Matlab diffusion theory fit of study volunteers to demonstrate the fit between the diffusion theory and the measured VRS curve.

4.3.2   VRS Spectra

The VRS spectra for each person was collected for each measurement. Figure 4.2 shows a sample of spectra collected over time for the left and right foot of study volunteers 12-128 and Figure 4.3 shows volunteer 21-923. Study participants wore copper socks on their left foot and the control on their right.
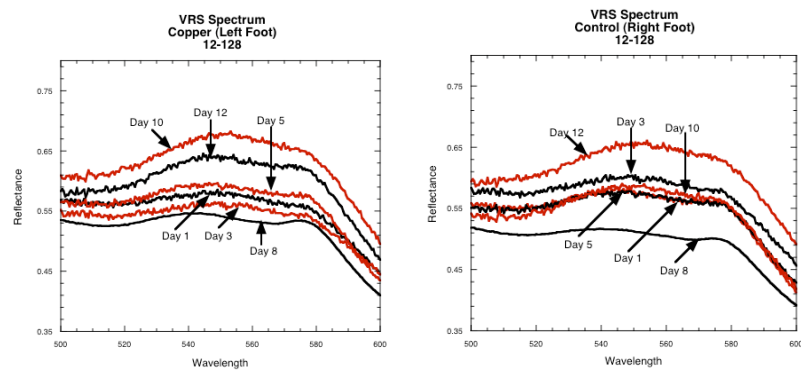
Figure 4.2: VRS Spectra over the duration of the study. Measurements were collected every other day for 2 weeks. Volunteer 12-128 wore copper socks on the left foot and control socks on their right foot.
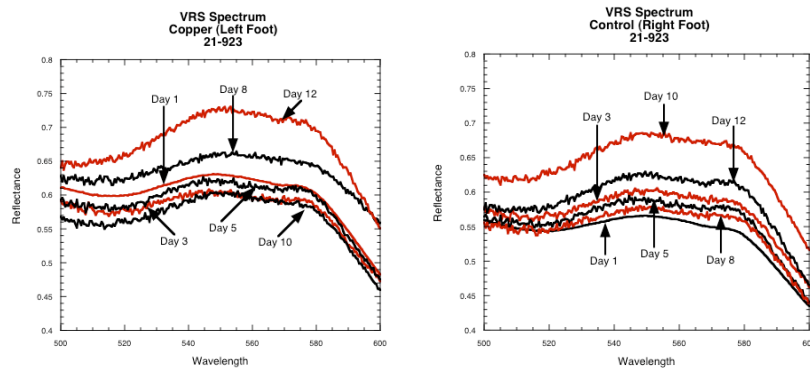
Figure 4.3: VRS Spectra over the duration of the study. Measurements were collected every other day for 2 weeks. Volunteer 21-923 wore copper socks on the left foot and control on the right.

4.3.3 Statistical Analysis

A linear regression line was determined for each volunteer for all dates of measurements. Figures 4.4 illustrates the difference in erythema index between the left copper and right control sock for study volunteer 11-1820 and 21-182. The graphs are in terms of erythema index from the first date of the study to the final date. Using the slopes obtained from each regression line statistical results were collected. A p value of 0.8659 and R-squared equaled 0.003346.
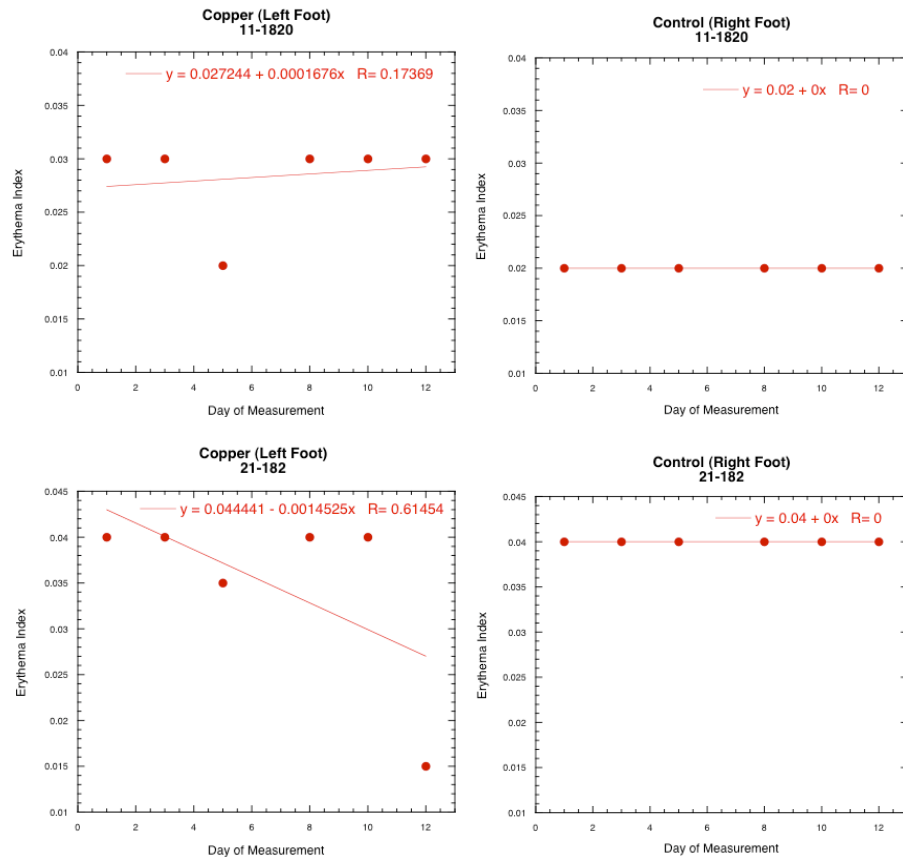
Figure 4.4: This is a sample linear regression plot of the copper (left) foot and control (right) foot of study volunteers 11-1820 and 21-182. The equation shown is the linear regression.

4.4   Discussion

This pilot study was executed as a double blind, randomized test. Upon completion of the study is was revealed by Renfro Corporation that the left sock contained the copper fibers while the right was the control. The spectrometer was used on the dorsal surface of the foot due to the ease of modeling the thinner epidermis on the top of the foot with the diffusion theory. The plantar surface of the foot can vary in thickness from heel to toe due to different levels of friction, stress and pressure experienced by the epidermis. The heel is also prone to thick calluses while the plantar side of the toes are prone to blister formation. Additionally, there can be a much greater difference in thickness from person to person and can be very difficult to predict in the diffusion theory model. The dorsal surface of the foot is not a load bearing or stressed area of the foot. The epidermis here has not been compromised with skin pathologies and follows the parameters of the diffusion theory model.

Each volunteer was under complete compliance, wearing the socks daily for the two week study period. Each person was present for their regularly scheduled testing as well. No adverse effects from the socks were reported from any volunteers. Additionally, none of the feet showed any signs of allergic reaction or irritation of any kind. Volunteers could not tell the difference between the left and right sock thus there was no identification of which sock held the copper. Despite this, the testing methods used did not show any significant difference between the control group and the copper group on erythema in the feet. A paired t-test showed that there was no significant difference between the left and right foot. While the P value from the t-test resulted in no significant difference between the copper and control either. There was very little variation, if any, in the erythema index between each volunteer's left and right foot for the duration of the study. The erythema index between the left and right foot of each person showed no significant variation.

The age and health of the study volunteers could be a primary contributer to the negative results of this study. Each volunteer was between the age of 18 and 35 and was healthy, meaning they had no signs of skin pathologies of any kind. Perhaps, since there were no pathologies to correct with the copper, any effect of the copper was so minimal that is was not detected by the spectrometer. Figure 4.1 above shows all the VRS spectra for the left and right feet of two study volunteers from day 1 to day 12. There is no pattern or consistent change of erythema from left to right foot spectra, despite the left foot being exposed to the copper and the right as the control. Based on these results, had an individual had a skin pathology, it is likely that there would be a detectable difference or pattern of erythema with the spectrometer. The skin in a young, healthy person is much more resilient to exterior forces and bacteria. In contrast, the skin of an elderly diabetic is highly compromised and susceptible to friction, abrasion, and bacteria. It is reasonable to suggest that if the study volunteers actually had a skin pathology, this study may have shown different results.

The way we fit the data and the computer model we used could be another contributer to why we were unable to detect a change. Fitting of data to the diffusion theory was done by manually adjusting the optical parameters of the skin. Manually fitting the curves makes it very difficult to correctly identify the parameters acting in each person's foot. There were no feet that visually displayed forms of erythema similar to the elderly study. however there may still have been some level of erythema acting in each foot.. This indicates the complexity of manually fitting with the diffusion theory. It is difficult to predict which chromophores are active in the skin causing the VRS spectra and therefore the diffusion theory may not be accurately depicting erythema.

Furthermore, the diffusion model used does not consider the age of the skin we were evaluating. We used the same testing methods and parameters as were used in the elderly foot study. It is difficult to predict the parameters of skin

such as absorption coefficients of epidermal melanin, scattering coefficients of the epidermis and dermis, blood concentration, blood vessel radius, oxygen saturation, hematocrit and depth of epidermal thickness accurately using this model. In the future, a modified or more advance diffusion theory model should be used to more accurately predict the physiology of the skin being observed. Age and the factors associated with aging skin should be taken into account to ensure accurate readings.

4.5   Conclusion

This pilot study focused on the detection of erythematic changes associated with copper socks using a visible reflectance spectrometer. The methods we used did not show any significant difference between the control group and the copper group. This does not mean that there is not a change due to the socks, it simply means that we were unable to detect a change using our study protocol, VRS and diffusion theory methods. To further research the effect of copper fabrics we propose a new study that focuses on a group of people with one skin pathological condition. Individuals inflicted with this condition could be provided copper and non-copper socks as a treatment rather than drug treatment. For the duration of the study the VRS could be used to detect changes due to the copper. The next study should also have more measurement collection to track erythema in the feet and an improved diffusion theory model. Continual develop of the diffusion theory model to provide optical solutions using improved methods to fit the VRS data will greatly influence the sensitivity of these studies.

Chapter 5

Summary and Conclusions

5.1 Summary

This thesis introduced the need for sock materials that can reduce skin patho-
logical conditions in order to prevent serious harm or injury as well as presented
a means to test the current sock technology that is available today. Currently,
there are copper ion socks on the market that claim to heal the skin and feet of
the people who wear them. However, there is no current technology to test their
effectiveness. Chapter 1 described the skin pathologies that are most common
for diabetics, amputees, athletes and the elderly. This chapter discussed the
structure of the skin and how these pathologies lead to serious injury and harm
including a dedicated discussion of eryhtema, or redness of the skin. Addition-
ally, this chapter discussed the medical history of copper and how copper is now
being investigated as an additive to sock materials to prevent skin pathologies
from occurring. Chapter 2 described the methods that can be used to detect
erythema in feet. Background information of visible reflectance spectroscopy
and the diffusion theory are discussed as well as their application in detecting
erythema in feet. VRS coupled with the diffusion theory generates an erythema
index that can be compared between test subjects to determine the amount of
change over a period of time.

Chapters 3 and 4 describe two pilot studies designed and executed with a focus on the detection of erythematic changes associated with copper socks using a visible reflectance spectrometer. The pilot studies followed the requirements of a double-blind, randomized study. Though previous studies have concluded that copper ion socks reduce or eliminate erythema, those studies were based on clinical observation only and not empirical data. The first study consisted of 20 healthy, non-diabetic elderly volunteers and the second study focused on 10 healthy, non-diabetic volunteers between the ages of 18 and 35. Elderly volunteers were assigned either pairs of control (non-copper) socks or copper socks to wear everyday for the full duration of the study while the volunteers in the second study each wore one copper sock on their left foot and a control sock on their right. Each volunteer had their feet measured with the VRS at regular intervals for the duration of the study. VRS measurements were then observed, recorded and analyzed to find the erythematic effects of the copper socks. The optical diffusion theory was then used to develop the erythema index for each person and used to compare between copper and control measurements. Statistical analysis performed revealed there was not change in erythema as a result of wearing the socks in either study.

5.2   Conclusions

Skin pathologies associated with the skin of a diabetic or amputee can be very dangerous and harmful to the overall health of a person inflicted with them. Copper is becoming more popular for use in textiles as a means to prevent skin pathologies from occurring. However, the is a current lack of research to support the claimed effects of copper ion sock technology. Focus needs to be directed to clinically observing changes in the sub-cutaneous layers of skin using a high-resolution spectrometer. VRS is a widely used diagnositc tool due to its quick, non-invasive, and relatively inexpensive nature. Visible reflectance spectroscopy

coupled with an enhanced diffusion theory model show great potential to detect the clinical effect of copper on the skin under the right testing parameters.

We have shown that the diffusion theory and VRS together can be used to detect erythema changes in feet. However, the diffusion theory is difficult and cumbersome to use to determine the erythema index. A non manual fitting of the data curves would be much effective in detecting the skin parameters acting a the time of measurement including absorption coefficients, hematocrit, deoxygenated and oxygenated blood levels. The pilot studies discussed were ineffective in resulting in a clear distinction in erythema index between copper sock and control sock wearers. We believe that the lack of significant difference between copper and the control was not due to the methods used but rather to the lack of skin pathologies present in the skin of the healthy volunteers we tested.

Copper ion technology promises to become vastly used technology across personal, military, and hospital use in the coming years. It is my hope that more work will be done to test the effectiveness of this technology to ensure that diabetics and amputees will no longer be plagued with painful, and often times, life or limb threatening skin pathologies. As more is learned about how to improve the diffusion theory and VRS techniques, I believe more clinical studies can be successfully performed on diabetics and amputees with serious skin conditions. The development of copper ion technology could be the cure for many people, but it is very important to first have research that can support the healing claims currently being made by the manufacturers of the product.

Appendix A

Matlab Diffusion Theory Code

The following matlab code was used to fit the VRS data with a curve using the diffusion theory. The model has several parameters that were adjusted to find the optimal curve fit. The fit was done manually by adjusting the chromophore values within their range.

```
%clear all;
clf
load -ascii /Users/lisahuhman/Desktop/RightTop_070416;
load -ascii /Users/lisahuhman/Desktop/wavelength;

% ---------------------------------------------------------------
% Variables to change for curve fitting
Mu_a_M_694=.200;  %absorption coeff. of epidermal melanin mm^(-1), range: 0.2~1.3

Mu_s_E=45;% scattering coeff. of epidermis mm^(-1), range: 30-80

Mu_s_D=40; % scattering coeff. of dermis mm^(-1), range: 25~50

B(2) =0.015; % blood concentration in 2nd layer; range: 0.01~0.1
B(3) =0.00; % blood concentration in 3rd layer; range: 0.01~0.1

R(2) =0.015;  % blood vessel radii in each layer
% Note: only change R(2), range: 0.004~0.015 mm

p_Oxy=0.9; %oxygen saturation range: 0.5~1.0
H= 0.40;   % Hematocrit; range: 0.4~0.5
d1= 0.05;    % depth of the epidermal thickness; range: 0.05~0.13 mm

% end of changing variables
% ---------------------------------------------------------------

B(1)=0.002;
R(1)=0.004;
R(3)=0.025;
L0=100; % eliminated anyway, value do not affect output
Rs1=0.04; % can be fixed
R1 = 0.5577 ;%  First hemispherical moment of Fresnel Reflection Factor;
R2 = 0.42; % Second hemispherical moment of Fresnel Refl. Factor;
Pinc=2*pi*L0/(1-Rs1);

d2=0.230;
z1=d1; z2=z1+d2;
z=[z1 z2  ];
%C=[1 1 1];         % correction factor
M=[1 0 0]; % Melenin in epidermis only
g_B=0.995; % eqn.(3) in Douven paper

lambda_jo=[268 315 350 395 414 430 435.7 530 542.5 575 907];
ao=[5.88 2.88 2.59 8.43 23.49 5.38 1.67 0.78 2.72 3.21 0.07];
bo=[23.41 70.71 31.82 14.07 12.30 17.68 99.0 35.36 16.97 11.31 159.1];

lambda_j=[265 315 325.4 360 380 389.4 410 430 555 755 900.5];
a=[5.46 0.64 0.99 3.43 2.63 1.56 11.59 30.64 2.45 0.04 0.04];
b=[25.74 56.57 247.49 51.48 35.36 10.61 11.74 11.31 35.00 15.00 84.85];


startLambda=450;
endLambda=800;


    index=1; %index discretizes the wavelengths

    % calculate A from Maple @ ns=1.44 ???
    A= (1+R2)/(1-R1);

    for lambda=startLambda:2:endLambda
```

```matlab
% Calculate Mu_a_HbO2:
Mu_a_HbO(index)=0;
for j=1:11
    Mu_a_HbO(index)=Mu_a_HbO(index)+25*H*ao(j)*exp(-((lambda-lambda_jo(j))/bo(j))^2);
end


% Calculate Mu_a_Hb:
Mu_a_Hb(index)=0;
for j=1:11
    Mu_a_Hb(index)=Mu_a_Hb(index)+25*H*a(j)*exp(-((lambda-lambda_j(j))/b(j))^2);
end

% Calculate Mu_s_Blood:
Mu_s_B(index)=440.72*H*(1-H)*(1.4-H)*(685/lambda);

% Calculate Mu_a_Blood:
Mu_a_B(index)=p_Oxy*Mu_a_HbO(index)+(1-p_Oxy)*Mu_a_Hb(index);

% Calculate Mu_a_Melanin:
Mu_a_M(index)=Mu_a_M_694*(694/lambda)^4;

% Calculate Correction factor
Mu_a_tr(index)=Mu_a_B(index)+(1-g_B)*Mu_s_B(index);
for i=1:3
    C(i)=(1-exp(-2*R(i)*Mu_a_tr(index)))/2/R(i)/Mu_a_tr(index);
end

% Calculate Mu_a(i)
Mu_a_T=7.84*10^7*lambda^(-3.255);
for i=1:3
    Mu_a(i)=B(i)*C(i)*Mu_a_B(index)+(1-B(i))*Mu_a_T+M(i)*Mu_a_M(index);
end

% Calculate Mu_s_T(i)
for i=1:3
    if i==1 Mu_s_T(i)=Mu_s_E*(577/lambda);
    elseif i==2|3 Mu_s_T(i)=Mu_s_D*(577/lambda);
    end
end

% Calculate Mu_s(i) & Mu_t(i)
for i=1:3
    Mu_s(i)=B(i)*C(i)*Mu_s_B(index)+(1-B(i))*Mu_s_T(i);
    Mu_t(i)=Mu_a(i)+Mu_s(i);
end


% Calculate g_T(i) & g(i)

for i=1:3
    g_T(i)=0.62+29*10^(-5)*lambda;
    g(i)=(B(i)*C(i)*Mu_s_B(index)*g_B+(1-B(i))*Mu_s_T(i)*g_T(i))/Mu_s(i);
end

% Calculate Mu_tr(i)
for i=1:3
    Mu_tr(i)=Mu_a(i)+Mu_s(i)*(1-g(i));
end

% calculate h(i), k(i)
for i=1:3
```

```
        h(i)=2/(3*Mu_tr(i));
        k(i)=sqrt(3*Mu_a(i)*Mu_tr(i));
    end

    % below, results for the diffuse Keijzer Model (equations a)
    C1(1) = -8*exp(k(1)*z1)^2*pi*L0*(k(1)*Mu_tr(2)*exp(k(2)*z2)^2*k(2)*Mu_tr(3)+k(1)*Mu_tr(2)^2*exp(k(2)*z2)^2*k(
    C2(1) = -8*pi*L0/(-k(1)*Mu_tr(2)*k(2)*Mu_tr(3)*exp(2*k(1)*z1+2*k(2)*z1)-Mu_tr(1)*k(3)*Mu_tr(2)*k(2)*exp(2*k(1)*
    gamma_d(index)=((C1(1)+C2(1))/(2*A*Pinc) ) - (((1-R2)/(1+R2)) * (1-Rs1));


    index=index+1; % next lambda
  end % end variable loop


figure(1);
plot([startLambda:2:endLambda],gamma_d,'r-');
hold on
plot(wavelength, 1-RightTop_070416)
axis([500 600 0 1])

tgamma_d=gamma_d';
save mspectra tgamma_d -ascii
```

Appendix B

Blood Concentration Tables

Figure B.1: Blood Concentrations from each volunteer on each measurement day from the first pilot study. The blood concentrations were used to find the erythema index of each volunteer.

Pilot Study #1 Blood Concentrations		Volunteer 22-1012	Volunteer 118-11	Volunteer 22-233	Volunteer 21-132	Volunteer 2262	Volunteer 22-126	Volunteer 122-13	Volunteer 22-1119	Volunteer 22-167	Volunteer 21-232
Left Foot	Date										
	12/4/06	0.035	0.045	0.01	0.035	0.03	0.045	0.0425	0.035	0.045	0.06
	12/18/06	0.035	0.045	0.01	0.035	0.03	0.03	0.035	0.027	0.025	0.055
	1/1/07	0.035	0.045	0.015	0.035	0.03	0.02	0.017	0.02	0.025	0.055
	1/15/07	0.035	0.05	0.015	0.04	0.03	0.015	0.026	0.013	0.025	0.055
	1/29/07	0.035	0.02	0.015	0.035	0.03	0.01	0.015	0.02	0.02	0.06
	2/12/07	0.035	0.05	0.01	0.04	0.03	0.01	0.01		0.015	0.055
	2/26/07	0.035	0.035	0.01	0.035	0.03	0.01	0.03	0.01	0.01	0.05
Right Foot	Date										
	12/4/06	0.02	0.035	0.04	0.04	0.035	0.03	0.05	0.02	0.023	0.01
	12/18/06	0.025	0.035	0.015	0.03	0.03	0.025	0.05	0.014	0.025	0.045
	1/1/07	0.025	0.033	0.01	0.03	0.03	0.01	0.035	0.025	0.025	0.045
	1/15/07	0.02	0.045	0.01	0.01	0.03	0.01	0.045	0.01	0.03	0.045
	1/29/07	0.025	0.035	0.01		0.03	0.01	0.03	0.015	0.017	0.05
	2/12/07	0.025	0.045	0.01	0.2	0.03	0.01	0.03		0.01	0.04
	2/26/07	0.025	0.03	0.01	0.035	0.03	0.01	0.02	0.01	0.01	0.04

		Volunteer 22-105	Volunteer 22-162	Volunteer 225-13	Volunteer 2146	Volunteer 22-1916
Left Foot	Date					
	12/4/06	0.045	0.04		0.04	0.035
	12/18/06	0.035	0.03	0.04	0.04	0.024
	1/1/07	0.025	0.03	0.03	0.04	0.03
	1/15/07	0.02	0.025		0.02	
	1/29/07	0.01	0.02	0.02	0.02	0.015
	2/12/07	0.01	0.015		0.01	0.015
	2/26/07	0.01	0.015	0.01	0.01	0.01
Right Foot	Date					
	12/4/06	0.025	0.04		0.04	0.018
	12/18/06	0.02	0.02	0.015	0.015	0.013
	1/1/07	0.02	0.02	0.01	0.01	0.035
	1/15/07	0.02	0.01		0.01	
	1/29/07	0.01	0.01	0.01	0.01	0.015
	2/12/07	0.01	0.01	0.01	0.01	0.01
	2/26/07	0.01	0.012	0.01	0.01	0.01

Figure B.2: Blood Concentrations from each volunteer on each measurement day from the second, younger population, study. The blood concentrations were used to find the erythema index of each volunteer.

Pilot Study #2						
Blood Concentrations		Volunteer	Volunteer	Volunteer	Volunteer	Volunteer
Left Foot	Date	11-1820	12-128	12-519	21-103	21-182
	4/9/07	0.03	0.015	0.025	0.04	0.04
	4/11/07	0.03	0.01	0.03	0.04	0.04
	4/13/07	0.02	0.015	0.015	0.04	0.035
	4/16/07	0.03	0.02	0.01	0.04	0.04
	4/18/07	0.03	0.02	--	0.01	0.04
	4/20/07	0.03	0.02	--	0.02	0.015

Right Foot	Date					
	4/9/07	0.02	0.015	0.04	0.01	0.04
	4/11/07	0.02	0.025	0.03	0.025	0.04
	4/13/07	0.02	0.015	0.015	0.04	0.04
	4/16/07	0.02	0.02	0.015	0.04	0.04
	4/18/07	0.02	0.015	--	0.025	0.04
	4/20/07	0.02	0.025	--	0.02	0.04

		Volunteer	Volunteer	Volunteer	Volunteer	Volunteer
Left Foot	Date	21-1019	22-312	22-826	22-1919	21-923
	4/9/07	0.02	0.025	0.05	0.03	0.035
	4/11/07	0.02	0.025	0.04	0.04	0.035
	4/13/07	0.02	0.02	0.03	--	0.03
	4/16/07	0.02	0.025	0.03	0.03	0.03
	4/18/07	0.03	0.02	0.03	0.03	0.03
	4/20/07	0.02	0.02	0.03	0.03	0.03

Right Foot	Date					
	4/9/07	0.03	0.025	0.05	0.035	0.04
	4/11/07	0.03	0.02	0.04	0.03	0.03
	4/13/07	0.025	0.025	0.03	--	0.02
	4/16/07	0.02	0.025	0.03	0.03	0.015
	4/18/07	0.02	0.02	0.03	0.025	0.015
	4/20/07	0.02	0.02	0.03	0.025	0.015

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VITA

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