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Terahertz Scanning Reflectometry

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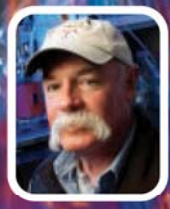

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TERAHERTZ SCANNING REFLECTOMETRY

Diffusion Kinetics & Permeation Concentration of Human Stratum Corneum Characterization by Terahertz Scanning Reflectometry

By: Anis Rahman, PhD; Scott Frenchek; Brian Kilfoyle; Leena Patterkine, PhD; Aunik Rahman; and Bozena Michniak-Kohn, PhD

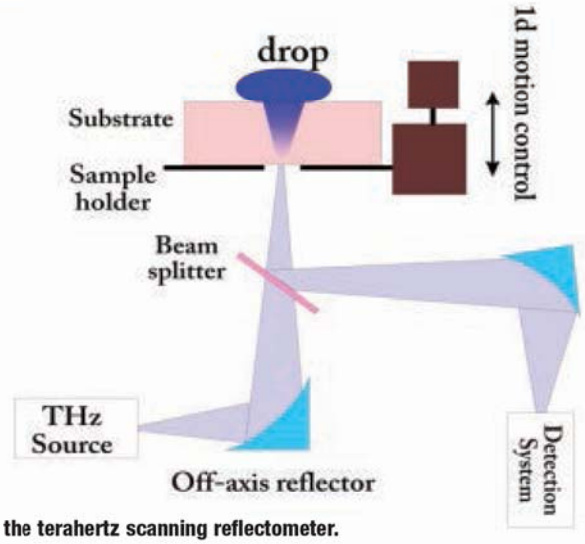
ABSTRACT

Terahertz reflectometry and spectrometry was used to investigate the permeation kinetics and concentration profile of active ingredients into the stratum corneum (SC). To our knowledge, this is the first effort of direct, non-invasive, and real-time measurement of kinetics and concentration gradient of analytes into the SC. Moreover, this is a general method that is applicable to any substrate and analyte combinations. It was found that the analyte concentration in the SC of 1% hydrocortisone solution in propylene glycol is significantly higher than 1% caffeine in deionized water. These findings are important for quantifying transdermal drug delivery formulation with these solvents and can be extended to other analytes and solvents. Terahertz spectra of untreated SC versus those treated with a 10-mM N-0915 solution were distinctively different. Additionally, the N-0915-treated specimen exhibits prominent absorption peaks in the 7.27 THz, 11.88 THz, and 18.42 THz region, while the spectrum of blank specimen exhibits a monotonous increase of absorbance with frequency. This indicates the importance of broadband terahertz spectroscopy of a range of 20 THz or more to be able to probe molecular events.

INTRODUCTION

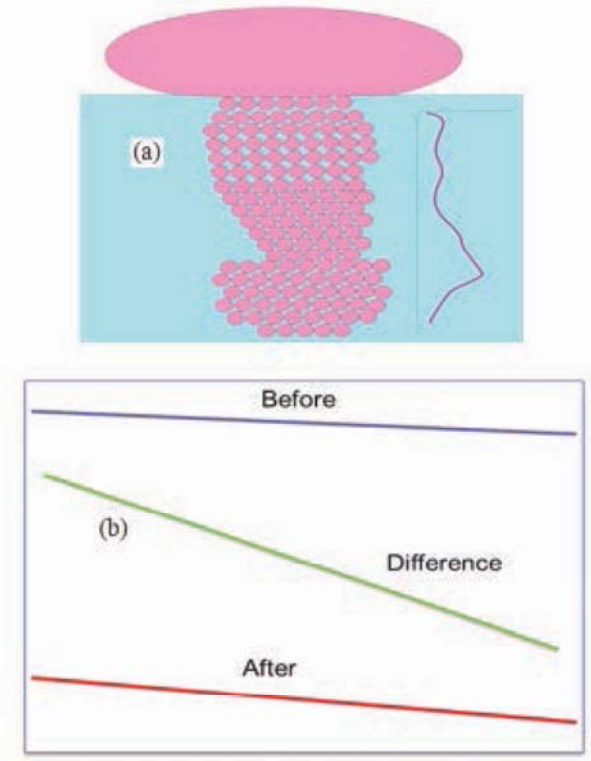
Terahertz spectrometry is an emerging novel technique that has great potential in diagnosis of certain disease conditions as well as in the analysis of actives in certain biological tissues. Broadband terahertz technology utilizes frequencies from ~100 GHz to over 30 THz that can be used to obtain tomographic information on the

FIGURE 1



Set-up of the terahertz scanning reflectometer.

FIGURE 2



(a) Cartoon of molecules permeating in to substrate, (b) computation of analyte concentration from scan of blank (before) and saturated substrate (after).

tissue surface and its interior, as well as interaction of the actives with tissue.[1-5]

The present study aims at investigating the field of transdermals/topicals and cosmetic formulations via terahertz spectroscopy and terahertz scanning reflectometry. Transdermals and topicals often involve use of compounds that enhance or retard the permeation of the active ingredients across the skin. The agents that enhance the permeation of the actives across the skin are termed permeation enhancers, and the agents that slow down the penetration of the active are known as retardants.[6,7] Permeation enhancers play a great role in increasing the bioavailability and efficacy of therapeutic agents by compromising the barrier properties of the skin and lead to enhancement in the delivery of the active across the skin. The retardants help in limiting the skin absorption of agents such as agrochemicals (pesticides), chemical warfare agents, mosquito repellants, sunscreens, and household chemicals that have the attributes of easily permeating through the barrier of the skin.

Many formulations used in transdermal and topical drug delivery use water and/or propylene glycol as solvents or penetration enhancers. For the present study, we examine permeation of two compounds in the SC: (1) hydrocortisone dissolved in propylene glycol (PG), and (2) caffeine dissolved in water.

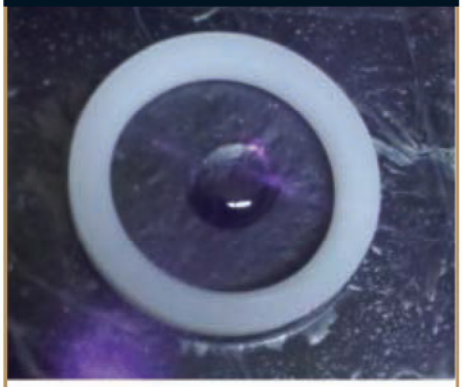
Propylene glycol (1,2-propanediol) is a diol with chemical formula $C_3H_8O_2$. It is a colorless, nearly odorless, clear, viscous liquid used as a solvent in many pharmaceuticals, moisturizers, hand sanitizers, and antibacterial lotions. Propylene glycol is used as a vehicle for penetration enhancers but is also considered a penetration enhancer in its own right. It permeates through the SC that alters the thermodynamic activity and partitioning of associated drug. Water is a common solvent; the water content of human SC is typically around 20% of the tissue dry weight but by soaking or occluding the skin, the SC water content can reach up to 400% of the tissue dry weight. Increased hydration can lead to increased permeation of associated drug as free water within the tissue alters the solubility of drug and therefore partitioning into the skin.

Additionally, terahertz spectroscopy was conducted on SC specimens treated with an active ingredient (N-0915). The spectra of blank SC and those saturated with N-0915 are also reported.

EXPERIMENTAL METHOD

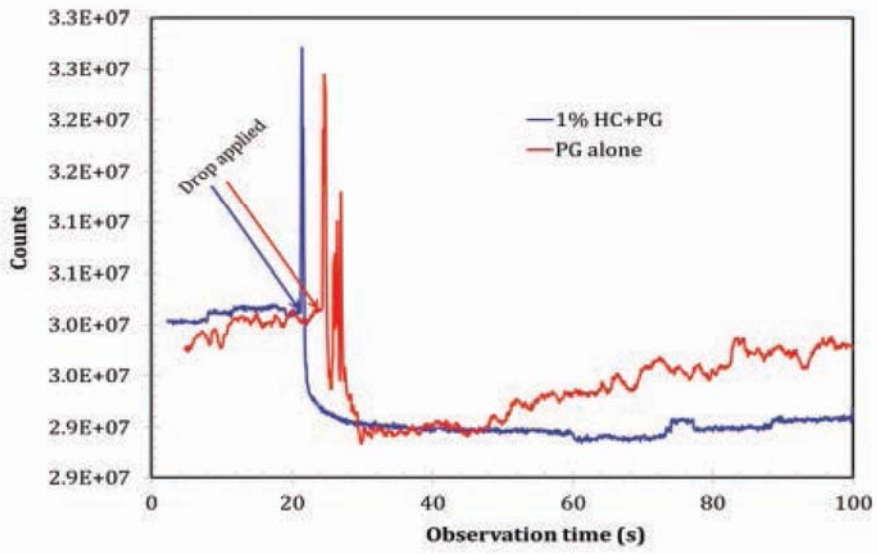
The measurements were carried out on a terahertz scanning reflectometer.[8] The experimental setup is shown in Figure 1. A CW terahertz source was

FIGURE 3



Human SC mounted on the sample holder on which a drop is applied.

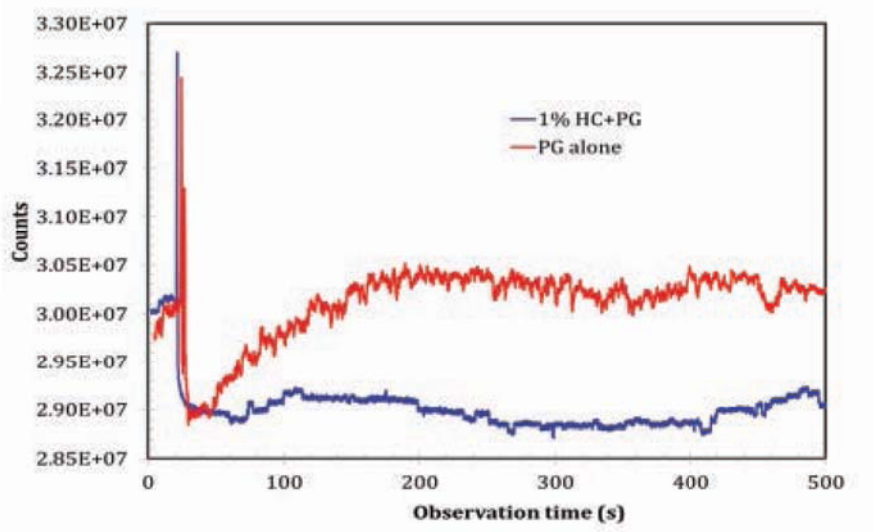
FIGURE 4



Kinetics of permeation of two solutions into SC (close-up view).

used that generates the terahertz radiation from an electro-optic dendrimer via a difference frequency method.[9] The terahertz beam was focused on to the specimen at a 90 degree angle via an off-axis parabolic reflector (normal incidence). The beam reflected by the substrate was directed to the detection system via a beam splitter. The specimen cell was composed of a platform controlled by a 1-d motion controller. The main purpose of this arrangement is as follows. The off-axis parabolic reflector was adjusted such that initially the terahertz beam remained focused on the substrate surface. At this position, the motion control can be engaged to move the focal point inside the substrate to interrogate the reflectance across the thickness; this gave the partial $\frac{\partial C}{\partial x}$ (Equation 1) when the blank substrate reflectance was subtracted from the reflectance of the same substrate treated with a desired ingredient (Figure 2).

FIGURE 5



Kinetics of permeation of two solutions into SC over longer time.

Equation 1.

$$\left|\frac{\partial C}{\partial x}\right|_{Analyte} = \left|\frac{\partial C}{\partial x}\right|_{Before} - \left|\frac{\partial C}{\partial x}\right|_{After}$$

However, when the beam remains focused at the surface, and the motion control is locked at that position, then the ingredient may be applied on the substrate to let it permeate across the thickness while the reflectance is measured in real time. In this case, the reflectance is directly proportional to the rate of permeation of the ingredient across the substrate partial $\frac{\partial C}{\partial t}$.

RESULTS & DISCUSSION

Analysis was carried out on two batches of dermatomed human skin samples supplied from the Human Skin Bank in New York City, NY. The SC was separated using the heat separation techniques described previously in the literature by Kligman and Christophers and others.[10-12] Two model compounds were selected for this study namely, hydrocortisone and caffeine. The former represented a lipophilic compound, and the latter a hydrophilic one. Solutions for

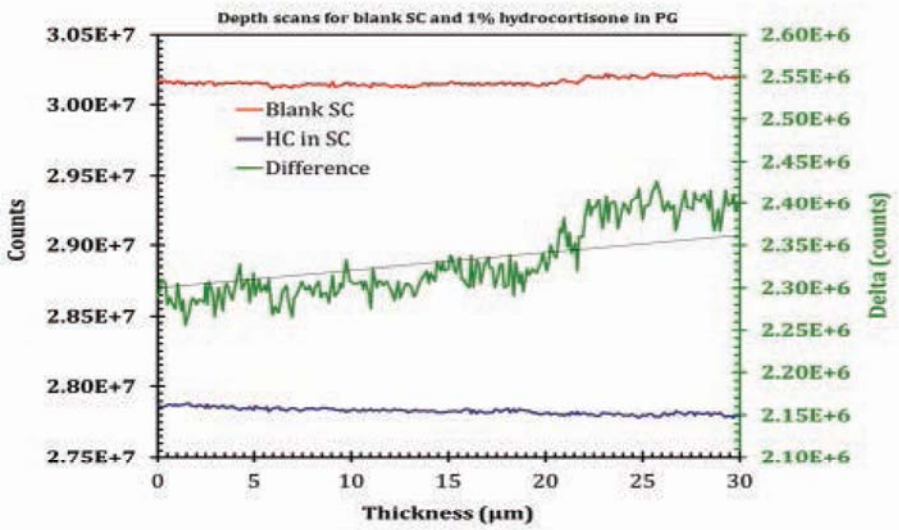
analysis (DI H_2O, propylene glycol [PG], 1% hydrocortisone in PG, and 1% caffeine in DI H_2O) were supplied by Rutgers University. Measurements were taken using a TeraScanR unit from Applied Research and Photonics, Inc. (Harrisburg, PA). An SC specimen mounted on the cell is shown in Figure 2. After all measurements were recorded, the results were imported to Microsoft Excel for visualization and analysis. Primary goals included: measuring the rate at which a given analyte diffused through the SC; and measuring the depth permeated by the analyte after stabilization (saturation).

Samples of SC were cut into squares large enough to cover a 5.31 cm^2 circle cut into a 5x5-cm^2 Plexiglass slide and fixed by the SC's inherent adhesiveness (Figure 3). All SC samples were oriented with the externa facing upward; they were fixed on the cell by a Teflon ring. The cell was then mounted in the TeraScanR reflectometer.

All SC samples that were to receive an analyte solution were vertically scanned to assess their reflectance at increasing depths; this was performed on all samples as a control before application of the analyte. Permeation kinetics, ie, the rate at which a solution penetrated the SC, were recorded after dropping 200 microliters of solution from an adjustable micro-pipette with the drop centered directly over the focal point.

Permeation was considered complete after the kinetics reached a steady state.

FIGURE 6

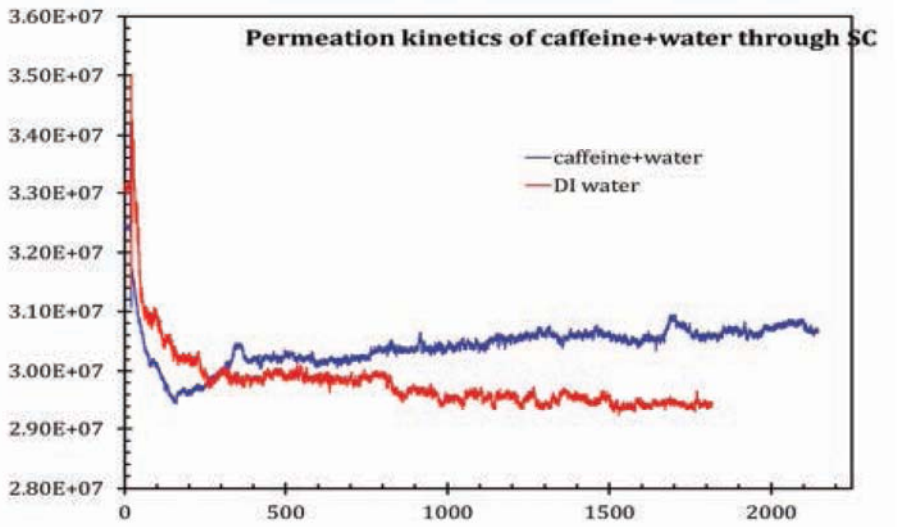


Depth scan of SC. Top (red) scan of blank SC, and bottom (blue) is the scan after the SC is saturated by 1% hydrocortisone solution in propylene glycol. The middle curve (green, right y-axis) is the difference of the top and the bottom curves, indicating the distribution of the hydrocortisone solution across the SC.

The solution was then pipetted off, and the remainder (on top) was carefully absorbed with a cotton swab. A second set of scans were performed to assess the concentration gradient of the analyte across the depth of the substrate. In all cases, at least three runs were taken, average of which is utilized for subsequent analysis.

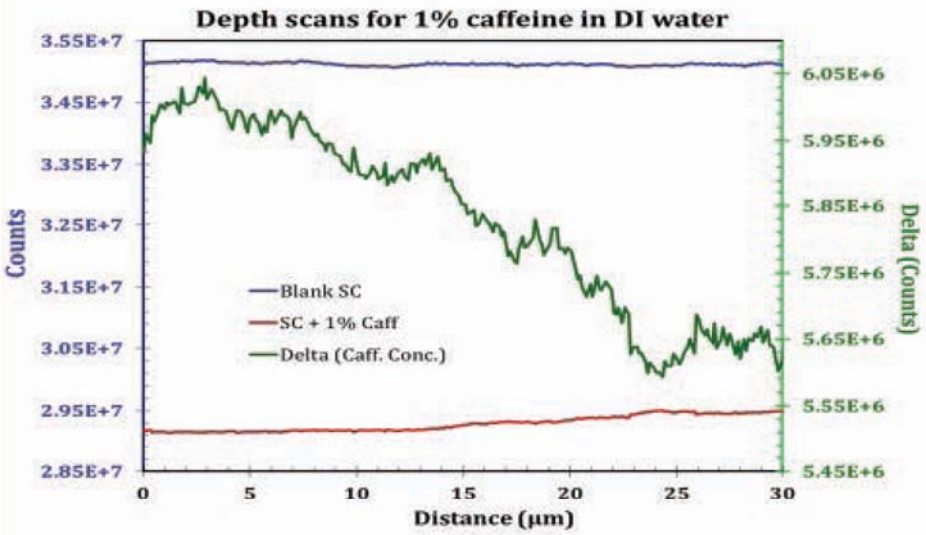
A pure sample of PG was tested as a blank for its permeation kinetics through the SC (Figure 4). This (kinetics) was later compared with that obtained for the hydrocortisone solution in PG. Upon the completion of kinetic measurement (when the kinetics reached saturation), its

FIGURE 7



Permeation kinetics of DI water and 1% caffeine in DI water in the SC.

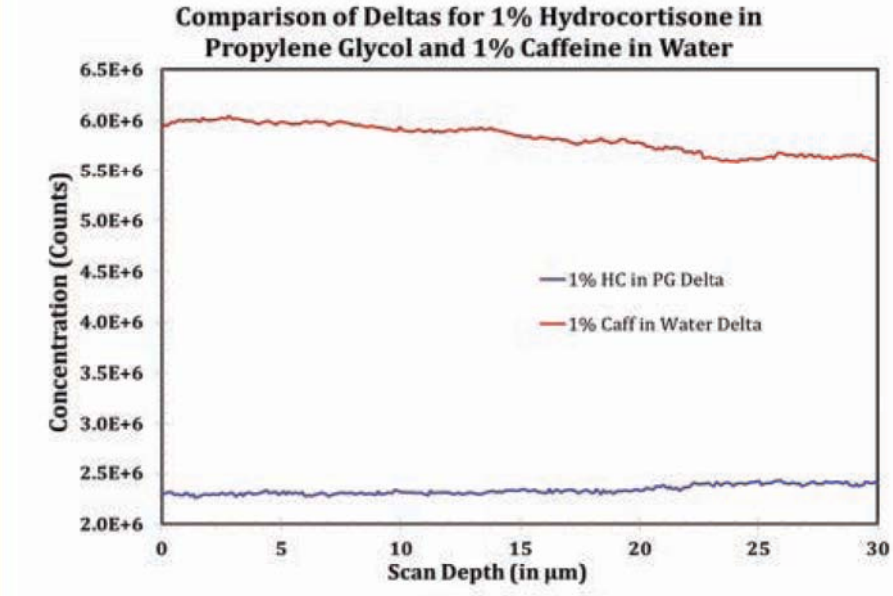
FIGURE 8



Concentration of caffeine solution (right y-axis) in SC.

depth scan was run, and the data stored in a file. Then a fresh specimen of SC was mounted. Three depth scans were performed on the blank SC. The average of these three runs is shown in Figure 6 (marked Blank SC). Kinetics measurement was then carried out with a solution of 1% hydrocortisone in PG (Figure 4). Figure 5 shows a close-up view of the data shown in Figure 4. After removal of analyte from the SC upper surface, three more depth scans were performed to assess the analyte's depth of permeation (Figure 6, marked HC in SC).

FIGURE 9



Comparison of the concentration profile of hydrocortisone and caffeine solution in the SC.

Measurements of kinetics and depth scan for blank SC, DI water, and 1% caffeine in DI water were carried out in sequence in a similar fashion as described earlier. Kinetics of DI water and 1% caffeine in DI water are shown in Figure 7, while Figure 8 exhibits the concentration profile of caffeine in the SC.

Figure 9 compares the concentration profile of both hydrocortisone and caffeine solution in the SC. It can be seen there are significantly more hydrocortisone in PG permeated through the SC than caffeine. This is expected and consistent with many observations from front-cell analysis experiments via HPLC.[13] As seen from Figure 9 (and also from Figure 6), the hydrocortisone profile shows that as we go deeper in the SC, the concentration of hydrocortisone is slightly increased, while the caffeine concentration profile (Figure 8) shows that less caffeine has penetrated deeper in the SC. This observation will be examined further by repeating the measurements and/or by utilizing other solvents.

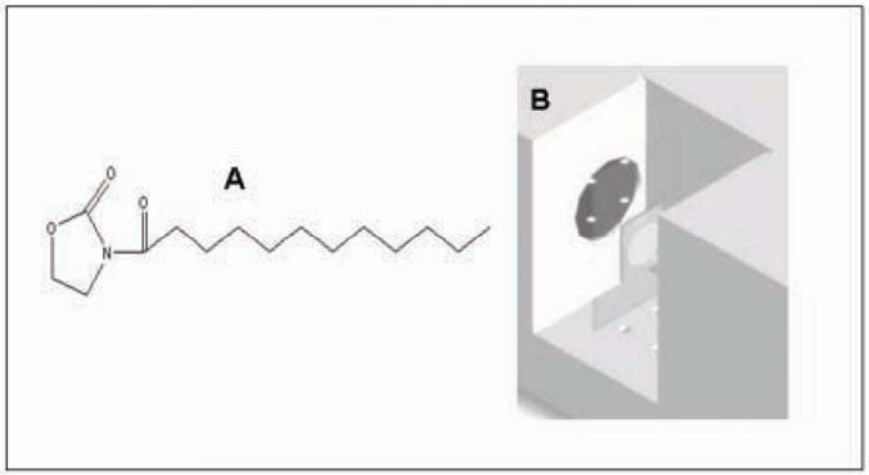
Figure 10 shows an experimental arrangement in which the SC was mounted in a terahertz spectrometer (TeraSpectra, Applied Research & Photonics, Harrisburg, PA). A blank specimen was measured first, and then another specimen was measured that was saturated with a 10-mM N-0915 solution. Here, the objective was to identify the

signals obtained in the spectrum to determine whether they attribute to the treatment with specific penetration modifier (N-0915) or to the components of the SC. Figure 11 shows the Fourier transform frequency-domain spectra of both blank- and N-0915-treated specimens. The spectra are distinctly different in that the SC treated with N-0915 showed prominent peaks in the 7.27 THz, 11.88 THz, and 18.42 THz regions whereas the control (untreated SC) showed a monotonous increase in absorbance as a function of frequency. While the significance of the peaks in the N-0915-treated specimen need to be explained, it is clear that if the spectra did not cover an extended window (up to 20 THz), then the peaks would not have been visible.

SUMMARY

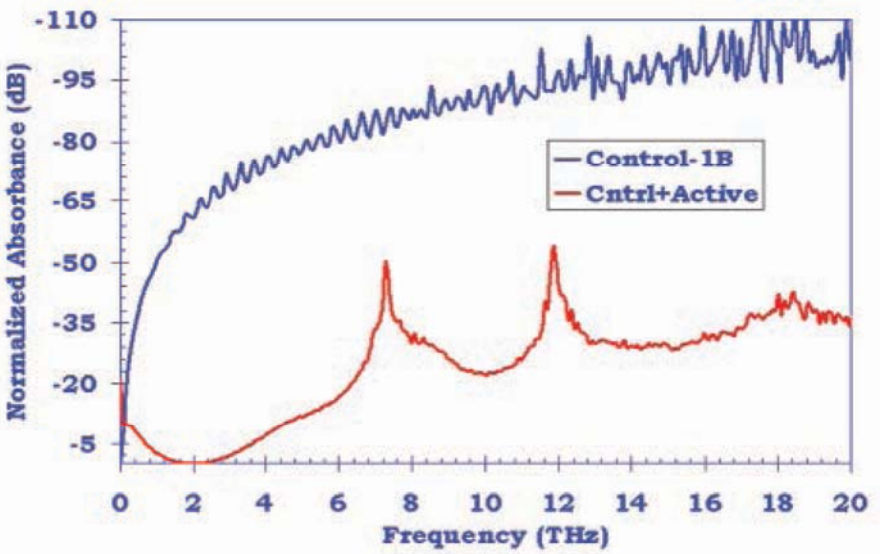
We conclude that terahertz scanning reflectometry is an effective tool for quantitative measurement of permeation kinetics and concentration profile of analytes in skin. To our knowledge, this is the only method for non-invasive quantitation of analytes in skin. These findings are important for quantifying transdermal drug delivery formulations with these solvents and can be extended to other tissues or substrates as well as to a variety of analytes. Unlike other methods, this is a simpler technique allowing direct quantification in a non-invasive fashion. Additionally, a wide broadband terahertz spectrometry allows spectroscopic inspection of differences between blank skin (substrate) and those treated with active ingredients. The N-0915-treated specimen exhibits prominent absorption peaks in the 7.27 THz, 11.88 THz, and 18.42 THz regions, while the spectrum of blank specimen exhibits a monotonous increase of absorbance with frequency. This indicates the importance of broadband terahertz spectroscopy over a wide range (20 THz or more) to be able to probe molecular events. ◆

FIGURE 10



(a) Molecular structure of the N-0915 and (b) the specimen (SC) mounted on the spectrometer.

FIGURE 11



Spectral signature of SC (control, blue line) and the SC treated with N-0915 (red). Terahertz can clearly detect the presence of an active as evidenced from the spectra.

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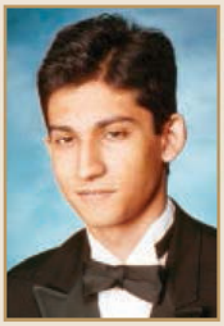
BIOGRAPHIES

Dr. Anis Rahman is the founder and CTO of Applied Research & Photonics (Harrisburg, PA). He is the originator of dendrimer-based photonics and terahertz science and technology. Here, electro-optic dendrimer is used to generate high-power CW terahertz radiation without requiring femto-second pulsed laser. Dendrimer is the "silicon for photonics," allowing fabrication of a number of important devices for sensing and communication.

Scott Frenchek is an Intern at Applied Research & Photonics. He is majoring in biotechnology at Harrisburg University of Science of Technology.

Brian Kilfoyle is a graduate student at Rutgers University, pursuing a doctoral degree in transdermal drug delivery mechanisms.

Dr. Leena Patterkine is an Associate Professor of Biotechnology at the Harrisburg University of Science of Technology.



Aunik Rahman is a Senior Engineer at Applied Research & Photonics. He is an inventor and a designer of the company's terahertz spectrometer, TeraSpectra.

Dr. Bozena B. Michniak-Kohn is a Professor in Pharmaceutics at the Ernest Mario School of Pharmacy, and Founder/Director of the Center for Dermal Research at Rutgers-The State University of New Jersey, Piscataway, NJ. She is an expert in the area of transdermal, topical, and buccal drug delivery and is a Fellow of the American Association of Pharmaceutical Scientists.