## **Application Note (A6)**

Procedure for In-Vitro Sunscreen Testing

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## PROCEDURE FOR IN VITRO SUNSCREEN TESTING

A procedure for in vitro sunscreen testing is outlined below. Although there is some art involved with the procedure, it is essential that the source and measurement system be as stable as possible.

The source consists of an unfiltered, ozone-free, xenon arc and stable power supply. The spectroradiometer is an OL 752 UV-Visible Spectroradiometer. The OL 752 has extremely low stray light levels, high wavelength accuracy and repeatability, relatively narrow bandwidth, a high sensitivity, large dynamic range, and an integrating sphere input optics module specially modified for this application.

The xenon arc is focused on the end of a liquid light guide. A special fitting, which plugs into the integrating sphere in place of the standard quartz window, enables coupling of the liquid light guide directly to the sphere. Three equivalent openings in this attachment allow the liquid light guide to be positioned in three equivalent positions about the center of the sphere.

The sample (Transpore<sup>®</sup> tape or skin) is positioned on the bottom side of the aluminum block and held in place with Saran Wrap<sup>®</sup> plastic film. The top surface faces the light guide.

The transmission calibration is obtained with the Saran Wrap<sup>®</sup> and light guide in successive positions. Three measurements per sample (one for each site) are suggested. Calibrations are normally made from 280 to 400 nm. However, for some physical agents the range can be extended to 600 or even 700 nm. Calibrations are made at either 2 or 4 nm intervals.

The first measurement that needs to be made is to determine the transmittance of the skin or tape matrix without any product, and then the product needs to be applied. Weigh the matrix, then the product. For the small quantities required, this is the most difficult part. For the 20 mm diameter Testskin<sup>®</sup> epidermis, approximately 6 mg needs to be applied. Once applied, the product needs to be uniformly rubbed into the sample.

If skin is used, variations between samples require each piece to be measured as its own control. If Transpore<sup>®</sup> tape is used, a control spectrum for each piece may be unnecessary.

Weighing and applying the product appears to be the most difficult and operator sensitive part of the test. It is very easy to get too little or too much product on the skin or to rub it into the skin non-uniformly.

The treated sample is remounted at the input sphere of the OL 752 and the transmittance remeasured.

A spreadsheet (such as Quattro<sup>®</sup> Pro) can be used to analyze the data. Enter the appropriate ASCII transmittance files into the spreadsheet and convert the transmittance to absorbance. Calculate the absorbance of the product film without background matrix and analyze the results using any of several schemes. The absorbance of the product is then converted to a product transmittance, which is used in most analysis procedures.

## NOTE:

Since data over the entire spectrum is listed in the spreadsheets, convolutions require all spectra to cover the same wavelength range and at the same data intervals.

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Once the transmittance data is obtained, the product's potential can be analyzed for any of a number of action or risk spectra and any of a number of sources. A Berger-type solar simulator spectrum and several natural sunlight spectra can be used for source spectra; however, this is flexible. Care must be taken that the proper convolutions are performed. Predictive benefits come from performing the proper mathematical convolutions.

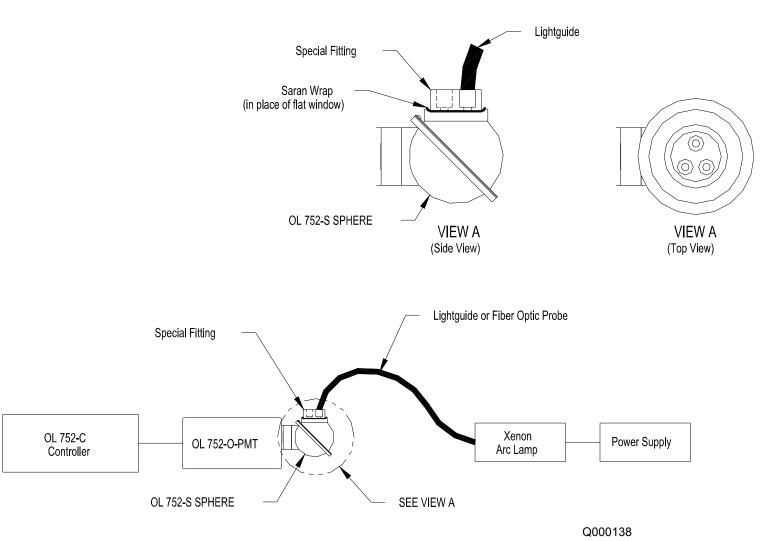
One major advantage to using a spectroradiometer is that the source can be measured with exactly the same precision as the transmittance data using the same measuring instrument. The data is saved in files, which can be easily read into the spreadsheet used for all calculations.

As noted, the critical features involve:

- 1. Using a spectrally adequate (intensity and spectral distribution) source.
- 2. Having the entrance optic so that the samples can be mounted and unmounted without destroying the instrument calibrations.
- 3. Uniformly applying the proper amount of product.
- 4. Analyzing the data properly.

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## SET-UP FOR IN VITRO SUNSCREEN TESTING

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