

T E C H N I C A L N O T E

a discussion of

optical geometry

and measurement effects

A stylized sun graphic consisting of a white semi-circle on the left and several dark grey triangular rays on the right, positioned behind the main title.

SPF Analysis of Sunscreens

using the Labsphere

UV-1000S Ultraviolet

Transmittance Analyzer



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PART ONE - MEASUREMENT OF DIFFUSE TRANSMITTANCE

1.1 Introduction

The Labsphere UV-1000S Ultraviolet Transmittance Analyzer is designed for predicting the Sun Protection Factor (SPF) of cosmetic sun care products, in vitro. The UV-1000S operates by measuring the diffuse transmittance of a carefully prepared sample as a function of wavelength in the ultraviolet spectrum. This technical note serves to answer questions about the instrument design and how it compares to other instruments. **Part One** discusses the requirements for an optical system to measuring diffuse transmittance. **Part Two** details how this data is used to calculate SPF and other figures of merit for sunscreens. **Part Three** discusses the recommended methods of sample preparation to get meaningful and consistent results.

The UV-1000S takes advantage of an optical device known as an integrating sphere and its ability to collect light transmitted in all directions after passing through a sample substrate. The integrating sphere in practice can be used as either a diffuse light collector or as a diffuse light source, utilizing the principle of optical reciprocity.

The following illustrates diffuse transmittance measurements, the direct applicability of the Labsphere UV-1000S and the design advantages of employing a UV flashlamp and diffuse illumination.

1.2 Diffuse Transmittance Measurements of Sunscreens

Many sunscreens are translucent materials which diffuse incident light. A ray of light incident onto a sunscreen sample will often be scattered. Light that is not transmitted is reflected or absorbed. For translucent samples, the radiation intensity is strongest in proximity to the regular transmitted direction as shown in Figure 1.

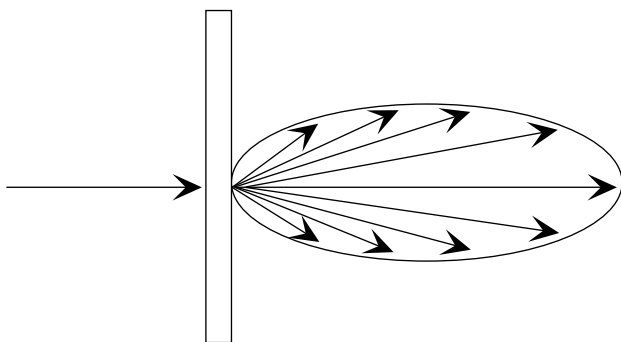


FIGURE 1

More opaque samples will produce an intensity pattern that approaches a uniform, hemispherical distribution. The ratio of the total transmitted light to the total incident light is known as the transmittance, a measurable quantity. Total hemispherical transmittance is measured by the use of an integrating sphere to collect the light scattered at all angles.

The interior walls of the integrating sphere are coated with a white, highly reflective material. In Figure 2, light from an external source is collimated and strikes the sample surface at normal incidence. A photodetector responds proportionally to the internal illumination produced on the sphere wall. A baffle prevents direct illumination of the detector after scattering from the sample. The incident beam flux is recorded initially without the sample in place to determine the measurement baseline.

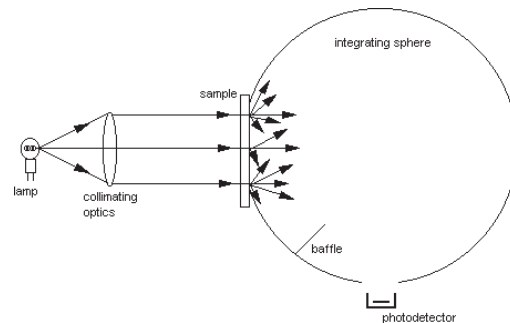


FIGURE 2

1.3 Reciprocity of Illuminating and Viewing Geometry

The geometry depicted in Figure 2 is known as normal/hemispherical, which refers to the illuminating/viewing conditions. It is often abbreviated as $0^\circ/h$ or more commonly $0^\circ/d$ (diffuse) geometry. The Helmholtz Reciprocity Principle^{1,2} states that the loss of light flux within a ray bundle will not be changed if the direction of travel is reversed. As applied to measuring instruments, the results will not change if the geometry of the illuminating and viewing beams are interchanged.³ Optical engineers often make full use of this principle in ray tracing calculations.

Consider the light scattering depicted in Figure 1. Instead of an incident ray, consider an observer's field-of-view (FOV). The sample scattering extends the FOV to multiple points in space beyond the sample. The scattered FOV is specific to the sample's scattering profile. As shown in Figure 3.

$$R = \frac{\int_{290\text{nm}}^{\lambda} A_{\lambda} \cdot d\lambda}{400\text{nm}} \cdot \frac{\int_{290\text{nm}}^{\lambda} A_{\lambda} \cdot d\lambda}{290\text{nm}}$$

FIGURE 3

In practice, illuminating a material sample over the entire hemisphere is the reciprocal method for determining transmittance for a given FOV and any scattering profile. Therefore, the light source and photodetector in Figure 2 can be reversed in order to construct a diffuse/normal (d/0°) measuring instrument as shown in Figure 4.

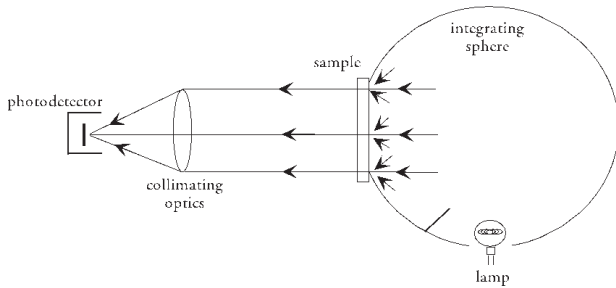


FIGURE 4

It can appear to those not completely familiar with geometrical optics that diffuse illumination will produce a different measurement result than collimated illumination since so many more incident rays and angles are transmitted through the sample. This is not true. The collimated viewing system with hemispherical illumination only accepts the incident rays which are reciprocal to the radiation pattern produced by an identically collimated incident beam.

1.4 Radiometric Advantage of d/0°

The major advantage of the d/0° system as compared to the 0°/d is in radiometric system efficiency. In Figure 2, the amount of light which is collected from the lamp is a function of the f-number (f/#) of the collimating optics. Those familiar with photography understand that f/# expresses the light gathering power of a camera lens. Lower values of f/# are required for slower speed film. The total flux collected from the lamp in Figure 2 can be expressed as;

$$\Phi_{0^\circ/d} = I * \frac{\pi}{4 f/\#^2} \quad (\text{Watts}) \quad \text{Eq. 1}$$

where *I* is the intensity of the lamp in units of Watts/sr. The second half of the equation expresses the collected solid angle as a function of f/# in units of steradians (sr). If the same lamp is placed inside the integrating sphere, the total flux from the lamp is collected;

$$\Phi_{d/0^\circ} = I * 4 \pi \quad (\text{Watts}) \quad \text{Eq. 2}$$

where 4π expresses the solid angle subtended by a sphere. The ratio of Equation 2 to Equation 1 quantifies the efficiency improvement of the d/0° design. This is equal to:

$$\frac{\Phi_{d/0^\circ}}{\Phi_{0^\circ/d}} = 16 * f/\#^2 \quad \text{Eq. 3}$$

Therefore, even for a highly efficient f/1 lens, the d/0° geometry would be 16 times more efficient. When one applies this analysis to diffraction grating spectrophotometers, which are generally f/3 and larger, the d/0° system offers greater than two orders of magnitude improvement in radiometric efficiency.

1.5 Optical Design of the UV-1000S

There are several reasons for improving system efficiency by using the d/0° design. One of the most important engineering reasons is known as the signal-to-noise ratio which often determines the dynamic range of a spectrophotometer. Labsphere's UV-1000S utilizes the efficiency offered by d/0° for several reasons. Improved signal-to-noise ratio is the first. The efficiency of most optics and photodetectors is poor in the ultraviolet spectral region.

Although UV rich light sources are available to compensate for the component efficiency, the amount of UV and thermal exposure on the sample must be minimized. Excessive exposure from the lamp may actively induce unwanted changes in the optical properties being measured.

The optical design of the UV-1000S is depicted below in a side view. The integrating sphere moves up and down for sample insertion. The sample is sandwiched between the sphere port window and the collimating lens. The diameter of the viewing beam is 10 mm. The lamp used inside the integrating sphere is a xenon flashlamp. The lamp supplies sufficient energy for the instrument's spectral range of 250 nm - 450 nm while minimizing the sample exposure during its microsecond pulse interval.

The instrument utilizes two photodiode array spectrographs for instantaneous spectrum acquisition. One measures the spectral transmittance of the sample while the other references the spectral power distribution of the illuminant to compensate for flash-to-flash variation and the effect of the sample's reflectance on the sphere illumination. A complete spectrum is obtained using three flashes, and data is processed and displayed within five seconds.

1.6 Summary - Part One

Labsphere's UV-1000S meets the geometric requirements for measuring diffuse transmittance. The $d/0^\circ$ geometry, which uses diffuse illumination, was selected over the reciprocal $0^\circ/d$ geometry in order to maximize system dynamic range while minimizing light exposure on the sample.

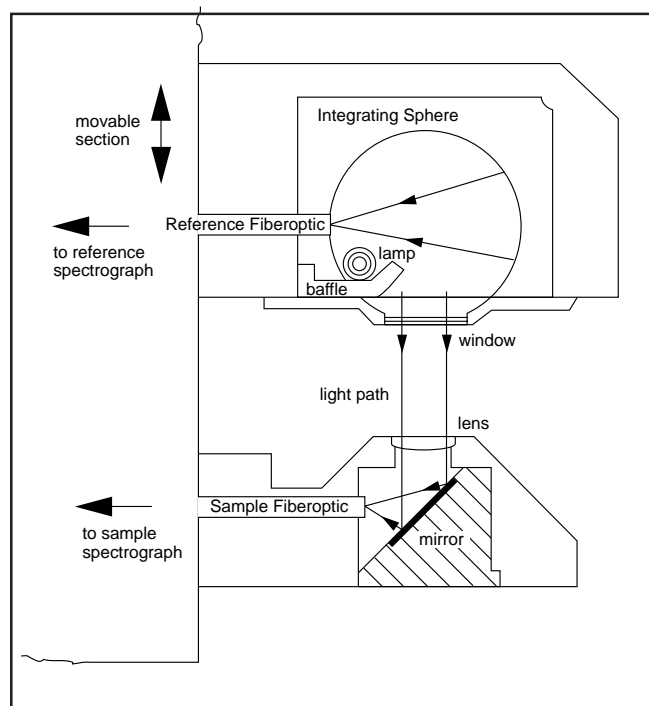


FIGURE 5

PART TWO - IN VITRO SPF AND UVA ANALYSIS

2.1 Calculation of In Vitro SPF

SPF by definition is determined *in vivo* as the increase in exposure time required to induce erythema, i.e. - SPF 4 means four times longer to induce erythema. The most common *in vitro* technique involves measuring the spectral transmittance at UV wavelengths from 280 nm to 400 nm⁴. The *in vitro* SPF is calculated as follows:

$$SPF = \frac{\int_{280\text{ nm}}^{400\text{ nm}} E_{\lambda} \cdot S_{\lambda} \cdot d\lambda}{\int_{280\text{ nm}}^{400\text{ nm}} E_{\lambda} \cdot S_{\lambda} \cdot T_{\lambda} \cdot d\lambda} \quad \text{Eq. 4}$$

where;

- Eλ = CIE erythema spectral effectiveness
- Sλ = solar spectral irradiance
- Tλ = spectral transmittance of the sample (as measured on the UV-1000S)

The two standardized functions, Eλ and Sλ, are illustrated in Figure 6.

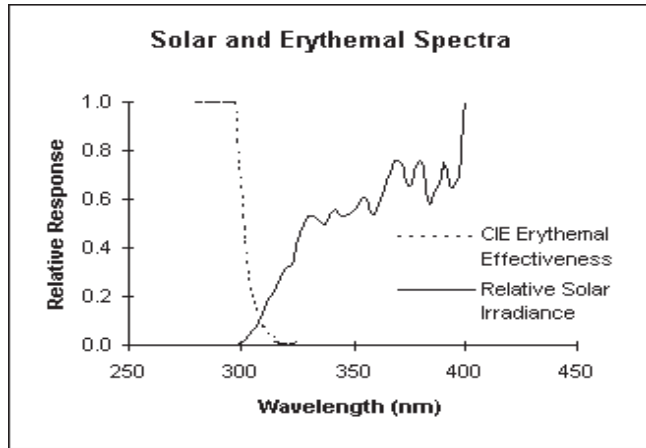


FIGURE 6

The two functions describe the relative sensitivity of erythema to individual wavelengths and the spectral distribution of sunlight as it reaches the earth's surface. The data are meant to be typical and based on physical measurements previously published in scientific journals.

A more revealing plot is the product Eλ x Sλ, which appears in both the numerator and denominator of Eq. 4. In Figure 7, the determination of SPF is weighted most heavily by a sample transmittance in the UVB portion of the spectrum, with maximum weighting near 305 nm.

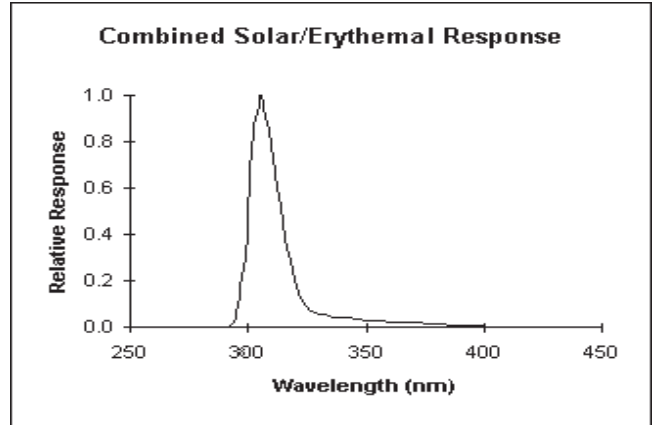


FIGURE 7

2.2 Average Transmittance for UVA and UVB

The ultraviolet spectrum, as it reaches the earth's surface, is electromagnetic radiation of wavelengths ranging from 250 nm to 400 nm. The spectral region is often broken down further into two parts which are of primary concern to human health issues. The UVA spectrum is just below the visible spectrum with a wavelength range of 315 nm to 400 nm. The UVB region, which is sometimes referred to as the erythema region, incorporates wavelengths of 280 to 315 nm.

The transmittance spectrum of a sunscreen in either region is averaged in order to produce one value which describes the amount of UVA or UVB blocking. The average transmittance in each region is given by:

$$T(UVA)_{AV} = \frac{\sum_{315\text{ nm}}^{400\text{ nm}} T_{\lambda} \times \Delta\lambda}{\sum_{315\text{ nm}}^{400\text{ nm}} \Delta\lambda} \quad \text{Eq. 5}$$

and;

$$T(UVB)_{AV} = \frac{\sum_{280\text{ nm}}^{315\text{ nm}} T_{\lambda} \times \Delta\lambda}{\sum_{280\text{ nm}}^{315\text{ nm}} \Delta\lambda} \quad \text{Eq. 6}$$

where; Δλ = measured wavelength interval.

Consequently, the percent blocking for UVA or UVB, respectively, is calculated as follows:

$$= 100\% - T(UVA)_{AV} \quad \text{Eq. 7}$$

$$= 100\% - T(UVB)_{AV} \quad \text{Eq. 8}$$

where;

T(UVA) or T(UVB) is expressed as a percentage.

2.3 UVA Effectiveness - The Boots Star System

A recent concern with the SPF rating system for sunscreens is that it is based on erythema as an endpoint. Therefore, active ingredients that serve primarily as UVB blockers substantially improve a product's SPF. There is a need to add a product label system that describes the UVA protection offered in addition to the SPF.

Boots the Chemist, the largest producer of sunscreens in the UK, has developed a label system that uses a four star rating based on spectrophotometric analysis⁵. The sunscreen samples are prepared according to a substrate method (see Part Three).The labeling system is already in use within the UK.

The spectral transmittance values, Tλ, are converted to spectral absorbance values Aλ = -log(Tλ). A term called the *UVA ratio* is calculated which is the ratio of the total absorption in the UVA to that in the UVB:

$$\frac{\alpha_{UVA}}{\alpha_{UVB}} = \frac{\int_{320\text{nm}}^{400\text{nm}} A_{\lambda} \cdot d\lambda}{\int_{290\text{nm}}^{320\text{nm}} A_{\lambda} \cdot d\lambda} \quad \text{Eq. 9}$$

The method specifically calls for the Aλ data to be measured in 5 nm increments and for the integrals to be solved using Simpson's Rule for area approximation. The limits of the UVA and UVB spectral regions also vary slightly from those previously noted. This sort of discrepancy for UVA and UVB is frequently encountered within scientific publications.

The star rating, and its associated claim for UVA protection, is determined from the measured UVA ratio:

TABLE 1

UVA Ratio	Star Category	Category Descriptor
0.0 to < 0.2	–	too low for UVA claim
0.2 to < 0.4	*	MODERATE
0.4 to < 0.6	**	GOOD
0.6 to < 0.8	***	SUPERIOR
≥ 0.8	****	MAXIMUM

2.4 UVA Effectiveness - Critical Wavelength

A proposed alternative to the Boots Star System evaluates the uniformity of a sunscreen product's absorbance spectrum⁶. The accompanying descriptor merely answers the question "does the product offer any UVA protection?" The result is based on a number called the *critical wavelength* which is determined spectrophotometrically from the absorbance spectrum. The technique is not as sensitive to sample preparation as the *in vitro* SPF or Boots Star measurements, since it only depends on the relative values of spectral absorbance and not the absolute values.

Similar to the Boots Star method, a sample is prepared according to a substrate technique, its spectral transmittance is measured, Tλ, and converted to spectral absorbance values Aλ = -log(Tλ). A ratio is calculated as follows which determines the total absorption in incremental wavelength bands and compares it to the total UV absorption. The ratio recorded for each wavelength, λ is:

$$R = \frac{\int_{290\text{nm}}^{\lambda} A_{\lambda} \cdot d\lambda}{\int_{290\text{nm}}^{400\text{nm}} A_{\lambda} \cdot d\lambda} \quad \text{Eq. 10}$$

The critical wavelength, λ_c, is the first value where the ratio R ≥ 0.9. The potential descriptors are:

TABLE 2

λ _c	Level of Protection
340 nm ≤ λ _c < 370 nm	SOME (UVA/UVB)
λ _c > 370 nm	MORE (broad-spectrum)

Another stipulation to using this method is to first evaluate the photostability of a sunscreen formula containing UVA absorbers. The samples must be pre-irradiated, using a solar simulator light source before the spectral transmittance is measured. The pre-irradiation exposure dosage is measured in units of MED (minimal erythema dose) and is equal to one third of the SPF value for the particular formulation under test. The photostability concerns for certain sunscreen formulas support the use of a flashlamp in the Labsphere UV-1000S. Pre-irradiating samples must be done with a continuous source whose spectrum and exposure are closely monitored. The light source of the analyzing spectrophotometer should not be used for sample irradiation.

2.5 Summary - Part Two

The spectral transmittance of a sunscreen in the ultraviolet spectral range can be used to predict an *in vitro* SPF value based on standard erythema and solar data. The Boot's Star and critical wavelength methods for categorizing the effectiveness of UVA absorbers are also performed from spectrophotometric data. Any pre-irradiation of samples to evaluate their photostability, needs to be performed with a controlled dose from a solar simulator. The flash lamp used in the UV-1000S does not expose samples to excessive light doses, keeping the spectrophotometric analysis independent of any photostability issues.

PART THREE - SAMPLE PREPARATION

3.1 Sample Preparation

Many regulatory agencies, such as the US Food and Drug Administration (USFDA) and The European Cosmetic Toiletry and Perfumery Association (COLIPA), mandate *in vivo* testing on human subjects, using an erythema endpoint to determine the SPF of a topical sunscreen. The *in vivo* tests are costly and time-consuming and may not be practical for routine product evaluation.

The UV-1000S is designed to make the evaluation of SPF a simple and routine analytical procedure performed within the formulation laboratory. Although *in vivo* testing is mandatory to make a product label claim for SPF, an investment in the UV-1000S will insure that only one *in vivo* test will have to be performed for each particular formulation.

The measurement of an *in vitro* SPF can be performed by measuring the diffuse transmittance in the ultraviolet spectrum of a carefully prepared sample. There are two objectives in a sample preparation method. The first is to simulate the application conditions used for *in vivo* testing, both the applied quantity and substrate interaction. This would produce a reliable *in vitro* SPF value that would positively predict the result of a subsequent *in vivo* test. The second objective is for the method to be consistent enough to generate reproducible results sample-to-sample for the same sunscreen formulation.

3.2 Ultraviolet Spectrophotometry

Most spectrophotometric techniques for transmittance measurements rely on preparing samples to a uniform and known thickness so that the optical path length through the sample is standardized. Many samples are dissolved with special solvents and placed into 10mm path length cuvettes. The cuvettes for ultraviolet spectrophotometry are usually made from quartz (fused silica) which is transparent to ultraviolet wavelengths.

Some have proposed using a thin film method, in which a small amount of sunscreen is sandwiched between two quartz plates and compressed into a capillary film. The method may be acceptable for certain non-viscous sunscreens or cosmetics but a material that is highly viscous cannot be made to spread thinly or evenly to make this method viable. Testing at Labsphere has shown that this method is highly inconsistent with any of the sunscreens we have evaluated. It is difficult to generate a uniform film thickness due to the mechanical non-uniformity of even the best quartz plates.

In addition, this method does not allow for proper drying or the breakdown of sunscreen emulsions for proper measurement. Reasonable results have been obtained using quartz plates with a metal shim spacer between the plates. This allows for accurate pathlengths from sample to sample, however, the following methods have proven more accurate and reproducible in practice.

3.3 Substrate Methods

In contrast, sample preparation of sunscreens for *in vitro* SPF attempts to mimic the application technique used for *in vivo* testing. After all, the *in vivo* tests are also sensitive to the optical path length of UV radiation through the applied sunscreen. The *in vivo* technique intends to simulate both end-user application and a standard applied thickness. A biological endpoint (erythema) is used to measure the effect of UV absorbers and blockers.

The recommended amount of sunscreen to apply in both FDA and COLIPA *in vivo* methodologies is $2\text{mg}/\text{cm}^2$ or $2\ \mu\text{L}/\text{cm}^2$. Most sunscreens have a specific gravity of almost unity. The area of application is measured and then the corresponding amount of sunscreen is measured using a pipette (volume) or weighed by loss.

The ideal substrate for *in vitro* SPF needs to be fairly transparent to the ultraviolet and simulate the porosity and texture of human skin, the *in vivo* substrate. Suitable *in vitro* substrates range from human epidermis and mice epidermis to sausage casings and natural lamb condoms. However, Labsphere has tested, and recommends three readily available substrates: 3M Transpore™ Tape, Vitro-Skin™, and Polyvinyl Chloride Film.

Transpore Tape

Transpore tape is a surgical tape manufactured by the 3M Company. The tape is readily available and inexpensive. The adhesive side makes it easy to apply to a quartz microscope slide for a rigid working surface. Although the tape is discarded for each prepared sample, the quartz slides can be washed and used again.

The use of this substrate was first evaluated by Diffey and Robson⁷. It was selected for its uneven topography that distributes the sunscreen in a way similar to human skin. The substrate was tested using 15 different sunscreens. In almost all cases, good correlation resulted when comparing the *in vitro* SPF with the published value for each formulation. The measurement reproducibility was very good and comparable to that obtained during *in vivo* testing.

The main advantages of the Transpore tape are its low cost, ready availability, and ease of use. There are a few disadvantages that need consideration when using Transpore tape: (a) the tape will not absorb formulations that use alcohol or oil as a vehicle; (b) pore size can vary at the beginning and end of each roll therefore, it is recommended to discard the first two feet at the start and end of each roll; (c) the pore size can vary batch-to-batch – it is advisable to screen at least 4 rolls to determine the suitability of a larger batch (screening involves measuring a standard sunscreen formula of known SPF); (d) complex formulations using multiple organic active ingredients have shown poor correlation⁸. This may be due to a swelling of the tape's substrate or a solvation of some of the tape's adhesive.

Vitro-Skin

Vitro-Skin, a registered trademark of IMS Inc., is a synthetic skin substitute that has been widely used for *in vitro* analysis of sunscreens. Once hydrated, Vitro-Skin has a texture very similar to human epidermis. In addition, the hydrated material seems to help sunscreen emulsions break down in much the same way as human skin. Published data by the producer of Vitro-Skin indicates that the substrate gives excellent correlation with *in vivo* SPF measurements.

The primary advantages of Vitro-Skin are its topographical similarity to human skin and the ability to break down emulsions. The apparent disadvantages are: (a) a relatively high cost per sample- approximately \$1.50 per test compared to pennies for other methods; (b) an overall low UV transmittance, especially at low wavelengths; (c) the need to hydrate the substrate starting the day before testing; (d) a relative short lifetime of the hydrated Vitro-Skin.

This being said, it has been the author's experience that if all other tests fail to give accurate results, Vitro-Skin is usually the substrate that gives the most consistent correlation with published *in vivo* measurements for commercial sunscreens.

Polyvinyl Chloride Film

Polyvinyl chloride or polyvinylidene chloride film, available under the commercial tradename Saran Wrap[®], is a highly transmissive material in the UV-Visible portion of the spectrum. While the film does not have the texture of human skin, it is very easy to form uniform dispersions of sunscreen products on the material. Commercially available polyvinyl/vinylidene chloride films are also extremely uniform in their material properties from roll to roll, and throughout each individual roll.

The primary advantages of polyvinyl chloride films are its availability, very high UV transmittance, ease of sample preparation, and low per sample cost. The disadvantages are: (a) the texture does not approximate that of human skin; (b) literature references to using polyvinyl chloride film as a substrate for SPF measurement are only anecdotal; (c) certain materials that claim sun protection such as lip balms or liquid cosmetics may not disperse well on the films.

3.4 Sample Preparation Methods

Three methods for sample preparation are presented – **Transpore Tape, Vitro-Skin and Polyvinyl Chloride Film**

3.4.1. Transpore Tape Sample Preparation

Transpore tape is placed in a single layer on clean 2mm thick quartz slides. Typically, the first and last two feet of a roll are not used for sample preparation. The size of the slide or tape is not important, but it is suggested that an area of at least two square inches (12.5cm^2) be used to enable measurement over at least five non-overlapping spots. A minimum of five samples should be prepared for each sunscreen to be measured. At the same time, a reference sample of Transpore Tape should be prepared. It is essential that the reference slide be from an area of the Transpore Tape roll close to that from which the samples slides were prepared.

To prepare the sunscreen samples, a small micropipettor ($100\mu\text{L}$ or smaller) or fine needle syringe is used to transfer the sample. For a 2 square inch (12.5cm^2) sample size, the slide is placed on an analytical balance and $2\text{mg}/\text{cm}^2$ of the samples are distributed on the sample plate by dotting the sunscreen on the slide and noting the weight. It is our experience that there should be at least twenty dots per slide (Figure 8). The sunscreen coated slide is then removed from the balance and the sunscreen distributed over the entire surface by slowly and deliberately rubbing the surface of the slide with a cot coated finger for 20-30 strokes (approximately 20 seconds). The sample should then be put aside to dry and let the emulsion break for at least twenty minutes before measurements begin. Failure to allow this dry-down period will result in inaccurate SPF measurements.



Spotting pattern of sample on Transpore tape slide

FIGURE 8

Other methods of Transpore Tape sample preparation have been suggested, including support on photographic film (polyvinyl acetate) or as a free standing tape in a film holder. It is our experience that the use of quartz slides allows for a more uniform distribution of sunscreen and in general gives more repeatable results than other methods described in the literature.

3.4.2. Vitro-Skin Sample Preparation

Vitro-Skin is hydrated by the method suggested by the manufacturer. For each sample, a 1" x 2" section of Vitro-Skin should be cut (for use with 35mm slide holders). Prepare at least five samples for each sunscreen to be tested. At the same time, a reference sample should also be prepared. This reference can be used for all Vitro-Skin based measurements made from the same sheet for samples run during the same time period.

The Vitro-Skin is then placed rough side up on an analytical balance and a small micropipettor or syringe with a fine blunt needle is used to dot the sample over the center area of the Vitro-Skin. Care should be taken not to puncture the Vitro-Skin during the application process, nor should sunscreen be placed on the outer 5mm edge, which is used to hold the sample in its frame. Once the sunscreen sample has been accurately weighed, the Vitro-Skin sample is transferred, sunscreen coated side up, to a foam block-used to simulate the flexibility of human flesh- and gently rubbed in with a cot covered finger for 20 to 30 seconds. The rubbing motion should be circular at first, then slowly back and forth, as one would apply sunscreen to one's body.

Once the sunscreen has been spread, the samples should be allowed to dry for 20 minutes to let the emulsion break down before beginning measurements. Failure to allow this dry-down period will result in inaccurate SPF measurements.

3.4.3. Polyvinyl Chloride Film

Polyvinyl Chloride film samples are prepared by pulling a piece of film taut across a small wood or plastic embroidery hoop (a 3 inch hoop is ideal). Turn the hoop with the film over and draw or trace a circle of the size you desire to measure directly in the center of the film. We found that a 1 3/8 inch circle, which requires 19mg of sunscreen is an effective size. Place the entire hoop including the film on an analytical balance and place the required amount of sunscreen ($2\text{mg}/\text{cm}^2$) in the center of the circle. Unlike the two previous techniques, we have found that spotting in many places does not increase the efficiency of spreading. With a cot covered finger, vigorously rub using a circular motion until the sunscreen has spread evenly. Rub until the sunscreen begins to feel sticky.

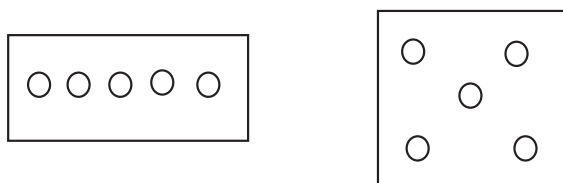
Holding the sample to a light source, assure that there are no lines or streaks, then allow to dry for twenty minutes. A mild heat source such as a 100 watt light bulb at a distance of between 6 to 8 inches aids drying. While the sample is drying, prepare a reference film using a similar hoop with a layer of PVC film. Place a second layer of film over the first layer, assuring it is smooth across the measurement area. This will serve as the reference for all PVC film measurements. After the sunscreen on the prepared PVC film has dried for twenty minutes, cover the area of measurement with a second smaller piece of PVC film and assure it is smooth. The samples are now ready to measure.

3.5 Alternate Methods of Sample Preparation

Labsphere and others have evaluated additional methods of sample preparation. These methods include spreading the sunscreen on quartz slides either as a “sandwich” or uncovered, using sandblasted or similarly roughened quartz slides to give some texture. While these methods use a highly transmitting substrate, our experience is that it is difficult to prepare films that are uniform across the surface or from sample to sample. Other methodology such as lambskin condoms, sausage casings, and other natural materials rely on materials that are both quite inconsistent in transmittance and in piece to piece consistency. For that reason, we recommend the three methods that have been described in the section above.

3.6 Summary - Part Three

When measuring sunscreens, it is important to determine the quality of the sample that has been prepared. Typically, this is accomplished by running multiple samples. We recommend five samples per sunscreen be tested at multiple spots on the sample. A typical measurement pattern for a rectangular and square samples are illustrated below.



*Typical measurement patterns
for rectangular and square samples*

FIGURE 9

The quality of sample preparation can be achieved very easily on the UV-1000S by running multiple spots and evaluation of the coefficient of variance (COV). The COV is a measurement of average variation from the mean SPF value for each spot measured. A low COV ($\leq 5\%$) is an indication of a well prepared sample; a high COV ($\geq 10\%$) indicates that the sample is either not well prepared or the emulsion has not fully broken.

Testing of samples is performed by running a baseline on the reference media, then successively running each of the prepared samples. Typically, three scans per spot are performed with five spots being evaluated on each sample. With the diode array technology and rapid measurement capability of the UV-1000S, this typically means measurement times of under two minutes for the sample. Thus a large number of samples can be analyzed in a short time period.

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