J. Cosmet. Sci., 51, 303–315 (September/October 2000)

A mathematical approach for the analysis of *in vitro* sun protection factor measurements

D. F. TUNSTALL, *Spring House, Fylingdales, Whitby, North Yorkshire, YO22 4 QQ, U.K.*

Accepted for publication August 11, 2000.

Synopsis

A mathematical analysis of the spectral UV mono-protection curves of formulations based on organic absorbers is described, which derives a thickness profile for formulations applied to artificial substrates. To illustrate the potential of the analysis, profiles for an octyl methoxycinnamate formulation at three absorber concentrations applied to Transpore® tape and Vitro-Skin® are used to detect the experimental errors observed in the mono-protection curves for these systems. The variations of sun protection factors with concentration are then explored. The wide-ranging potential benefits of this type of analysis are described.

INTRODUCTION

Considerable concern exists within the sunscreen market about the accuracy of *in vitro* sunscreen protection assessments. The tests for sun protection factor (SPF) assessment were developed by comparison with *in vivo* measurements, and equipment and test substrates evolved for organic-based UV absorbers within the constraints of having to match the *in vivo* data. The advent of mineral, or inorganic, UV attenuators introduced *in vitro* measurement inconsistencies.

It is accepted [e.g., Sayre (1)] that development of *in vitro* tests cannot be based on simple transmission spectra. Sayre *et al.* (2) had previously resorted to the use of hairless mouse skin. Diffey had shown that good correlation with *in vivo* results could be obtained by measuring the spectral transmittance of formulations applied to excised human cadaver skin. Diffey and Robson then developed a test (3) using a surgical tape (Transpore® tape, 3-M Corporation) designed to allow skin to "breathe," which became an adopted market *in vivo* test. O'Neill (4) showed that the non-uniformity of sunscreen distribution on the skin could satisfactorily account for the discrepancy between clinical results and simple spectrographic data. Brown and Diffey (5) subsequently showed that a substantial thinning of the formulation on skin to 6–10% of its theoretical uniform thickness was needed to achieve results comparable with their excised skin measurements. Spruce and Hewitt (6) have since shown that with Transpore® tape as a substrate, a small fraction of uncovered holes (i.e., in effect, zero thickness) occurs. A new substrate

designed to closely simulate skin has since become available (Vitro-Skin®, IMS Testing Group).

This paper analyzes these two substrates and develops a much better understanding than hitherto of the *in vitro* assessment of sunscreens. It describes an approach that allows a good approximation for the actual distribution of thicknesses of a formulation on substrates to be determined. It is demonstrated how powerful this kind of data can be for understanding the origins and errors of SPF results on different substrates.

EXPERIMENTAL

THE CALCULATION

The expected uniform thickness for an application of 2 μl/cm^2, the standard test application for sunscreen SPF assessment, is 20 microns. A model is adopted where a *range* of varying thicknesses, each at a fraction of unit area, is used to calculate the spectral transmittance of the system. At each wavelength the transmittance of each thickness is determined using a simple exponential transmission law [Beer-Lambert Law (7)]:

Table I
MPFs, SPFs, and UVA/UVB for 1 wt %, 2.5 wt %, and 10 wt % OMC Formulations on Two Substrates

	Transpore® tape			Vitro-Skin®		
Wavelength	1 wt %	2.5 wt %	10 wt %	1 wt %	2.5 wt %	10 wt %
290	4.76	10.78	37.69	2.12	4.97	12.25
295	4.99	11.64	40.22	2.16	5.31	14.57
300	5.11	12.06	41.04	2.18	5.42	15.81
305	5.16	12.25	41.82	2.17	5.43	16.44
310	5.01	11.88	42.04	2.11	5.28	16.58
315	4.54	10.43	39.27	1.99	4.83	15.7
320	3.79	8.12	33.84	1.79	4.06	13.93
325	2.88	5.34	23.71	1.54	3.04	10.64
330	2.11	3.21	12.35	1.31	2.13	6.47
335	1.64	2.08	5.73	1.16	1.57	3.49
340	1.38	1.55	2.99	1.08	1.29	2.04
345	1.24	1.31	1.93	1.04	1.16	1.44
350	1.17	1.18	1.49	1.02	1.09	1.18
355	1.13	1.12	1.29	1.01	1.06	1.07
360	1.11	1.09	1.19	1.01	1.04	1.02
365	1.1	1.08	1.14	1	1.02	0.99
370	1.1	1.07	1.11	1	1.02	0.98
375	1.09	1.06	1.1	1	1.01	0.97
380	1.09	1.06	1.09	0.99	1.01	0.97
385	1.09	1.06	1.09	0.99	1	0.97
390	1.09	1.06	1.08	0.99	1	0.97
395	1.08	1.05	1.08	0.99	0.99	0.96
400	1.08	1.05	1.08	0.99	0.99	0.96
SPF	3.72 (0.89)	6.38 (0.83)	12.41 (1.78)	1.89 (0.09)	3.82 (0.22)	7.86 (0.12)
UVA/UVB	0.17	0.16	0.21	0.11	0.14	0.18

$$\text{Transmittance, } T = e^{-c \cdot E \cdot x}$$

where c is the unit weight extinction coefficient, E is the concentration of absorber in suspension, and x is the thickness of suspension.

The total transmittance of the system is then assumed to be

$$Ts = \sum fn \cdot Tn$$

where Ts is the total transmittance, Tn is the transmittance of surface fraction fn at thickness xn, and $xn \geq 0$. There can be any number of values of xn, but preliminary curve-fitting experience suggests a maximum of five to be adequate.

The following conditions must also apply:

$$\sum fn = 1$$
$$\sum fn \cdot xn = 20$$

The "fractions x thicknesses" must add up to 20 to preserve the quantity of applied formulation. At first sight it appears this must have an infinite range of solutions, but if three concentrations of absorber covering a relatively wide range of SPF are used in otherwise identical formulations, there is surprisingly little, if any, leeway for "multiple" answers. The fact that most absorbers have a wide range of absorption factors across the UVB/UVA wavelength range further assists the procedure. This approach is immensely difficult to apply to formulations containing inorganic, light-scattering materials such as titania and zinc oxide, for which simple exponential transmission laws do not apply.

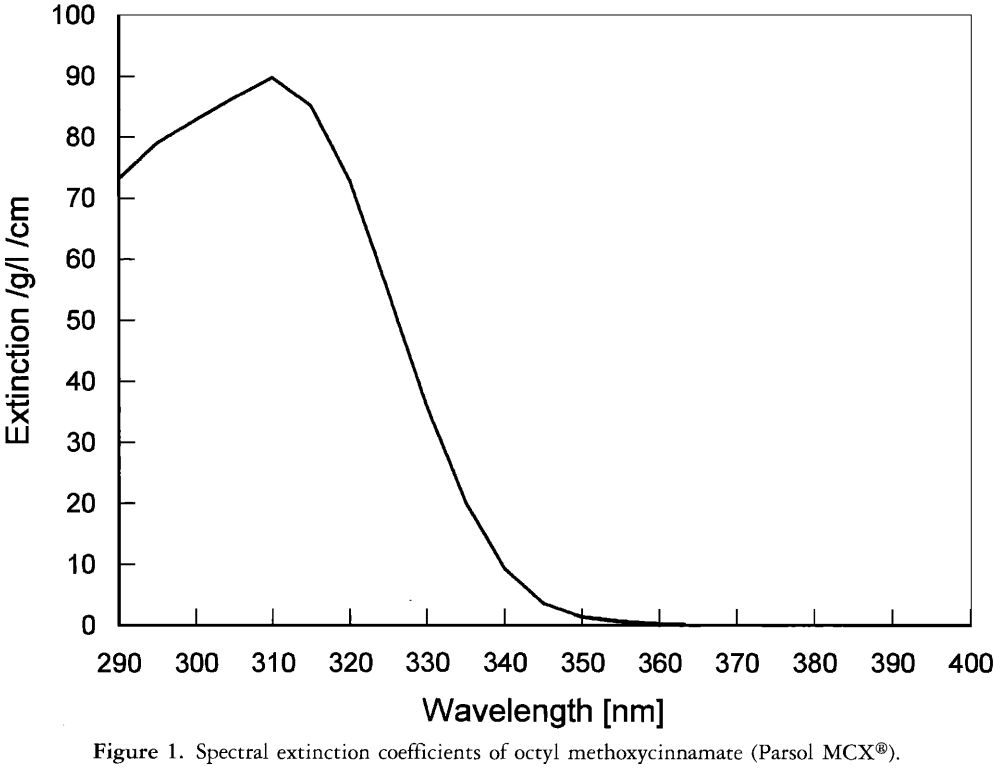

Figure 1. Spectral extinction coefficients of octyl methoxycinnamate (Parsol MCX®).

Thus it can at present be applied only to formulations containing non-scattering absorbers, and to formulations that do not retain a "milky" appearance on substrates. However, this still leaves plenty of scope for investigating the actual distribution of formulations on substrates, thereby assessing the test suitability of the substrates. Formulations containing more than one absorber can, if necessary, be accommodated, since the absorption coefficients are additive within the exponential law.

EXPERIMENTAL DETAILS

Simple formulations containing 1 wt %, 2.5 wt %, and 10 wt % of octyl methoxycinnamate [OMC-Parsol MCX® (Givaudan)] were applied to Transpore® tape and Vitro-Skin® and their spectral protection curves assessed on a Labsphere UV-1000S spectrophotometer. Application of the formulations to the substrates was carried out by experienced operators.

The variation of transmittance over the 290–400 nm wavelength range was measured, from which SPF can then be calculated (3). Multiple scans were carried out on each system. Sun protection factors and UVA/UVB ratios were calculated for each sample.

Averages of the spectral scans are listed in Table I, together with the average of the SPFs obtained on each sample (standard deviations in brackets) and the average UVA/UVB ratios. The samples were not subjected to any specific period of drying time prior to

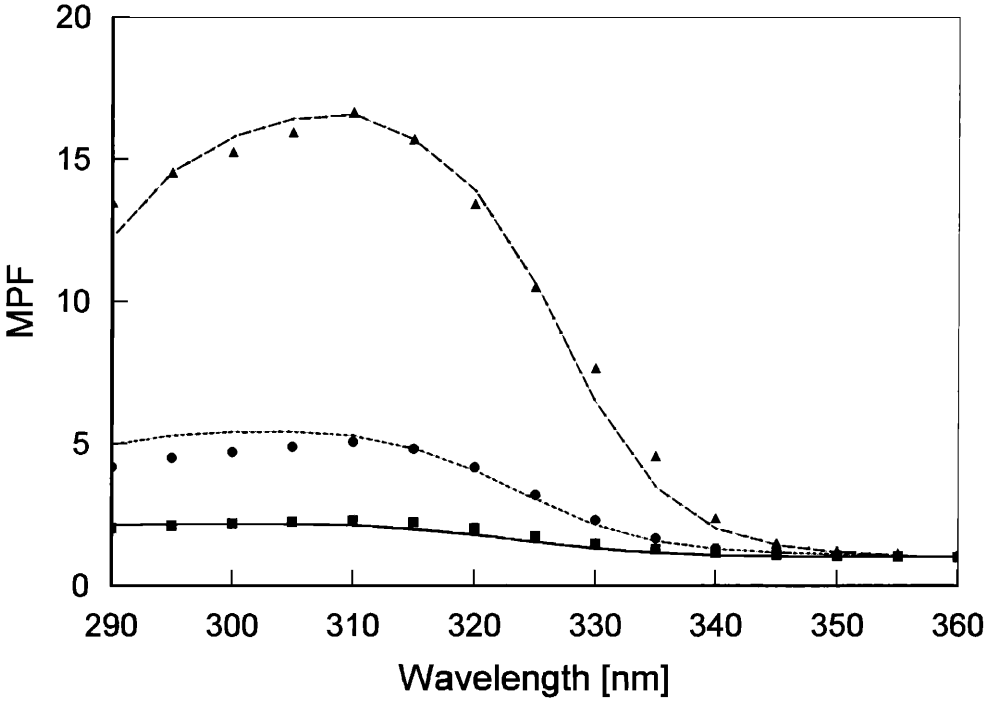

Figure 2. Comparison of measured and calculated mono-protection factors for OMC on Vitro-Skin® substrate. 1 wt % OMC, measured ———, calculated ■; 2.5 wt % OMC, measured -------, calculated ●; 10 wt % OMC, measured – – – –, calculated ▲.

measurement, i.e., they were measured immediately after application to the substrates. This introduces a source of possible variation into the data, especially for the Transpore® tape. It was determined separately that allowing the Transpore® tape samples to dry for ten minutes prior to measurement reduced the average SPF values by 15%.

ANALYSIS OF THE RESULTS

The analysis has been applied using an ad hoc trial-and-error approach, with the final fit to the measured data being determined by least squares error assessment. The transmittance calculations use absorption coefficients measured for a dilute solution of OMC in ethanol, shown plotted in Figure 1.

The equation evolved for the Vitro-Skin® substrate is

$$T_S = 0.187\,(10^{-0.55 \cdot c \cdot E/1000}) + 0.75\,(10^{-5 \cdot c \cdot E/1000}) + 0.063\,(10^{-256 \cdot c \cdot E/1000})$$

where T_S is total transmittance at wavelength λ.

This is more convenient if abbreviated to the following form:

$$\text{Thickness profile} = 0.187[0.55] + 0.75[5] + 0.063[256] \quad (1)$$

There is no simple relationship between the thicknesses. The number of thicknesses needed for this substrate is surprisingly small.

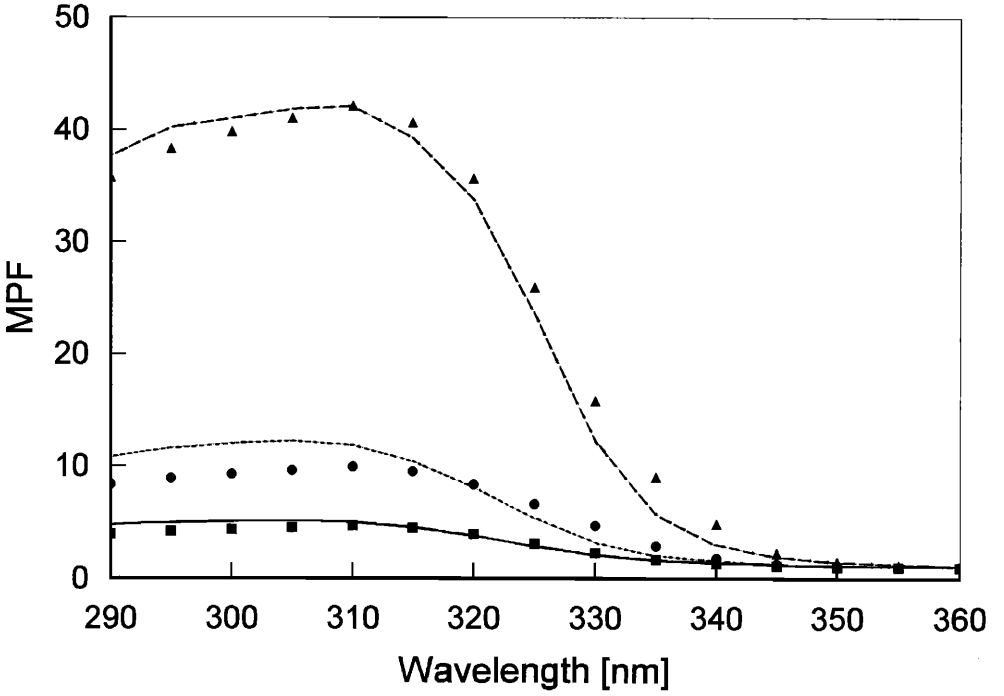

Figure 3. Comparison of measured and calculated mono-protection factors for OMC on Transpore® tape substrate. 1 wt % OMC, measured ——, calculated ■; 2.5 wt % OMC, measured -------, calculated ●; 10 wt % OMC, measured – – – –, calculated ▲.

The fit to the experimental results is illustrated in Figure 2, where the reciprocal of transmittance, the mono-protection factor (MPF), is plotted against wavelength. The fit is very reasonable, considering the assumptions and constraints applying to the system.

The profile developed for the Transpore® tape is

$$\text{Thickness profile} = 0.02[0] + 0.22[2] + 0.64[13] + 0.12[94] \quad (2)$$

The fit, shown in Figure 3, is not as good as for the Vitro-Skin®, despite the occurrence of an extra term in the equation. However, the number of terms needed is still small, considering that the formulations probably rarely actually form very many regions of parallel-faced areas on the substrates. Both profiles contain a significant fraction of areas $\leq 10\%$ of the theoretical thickness, as deduced by Brown and Diffey (5) from excised skin measurements. A schematic of the profiles is shown in Figure 4.

SUBSTRATE VARIABILITY

The uniqueness and sensitivity of the thickness profiles can be demonstrated by considering the variations across the sets of scans measured on individual samples. This provides an analysis for the source of the variations in SPF found for the two substrates. The most important difference between the two substrates is the emergence of a fraction of uncovered area in the Transpore® tape, agreeing with the microscopic examination by Spruce and Hewitt (6).

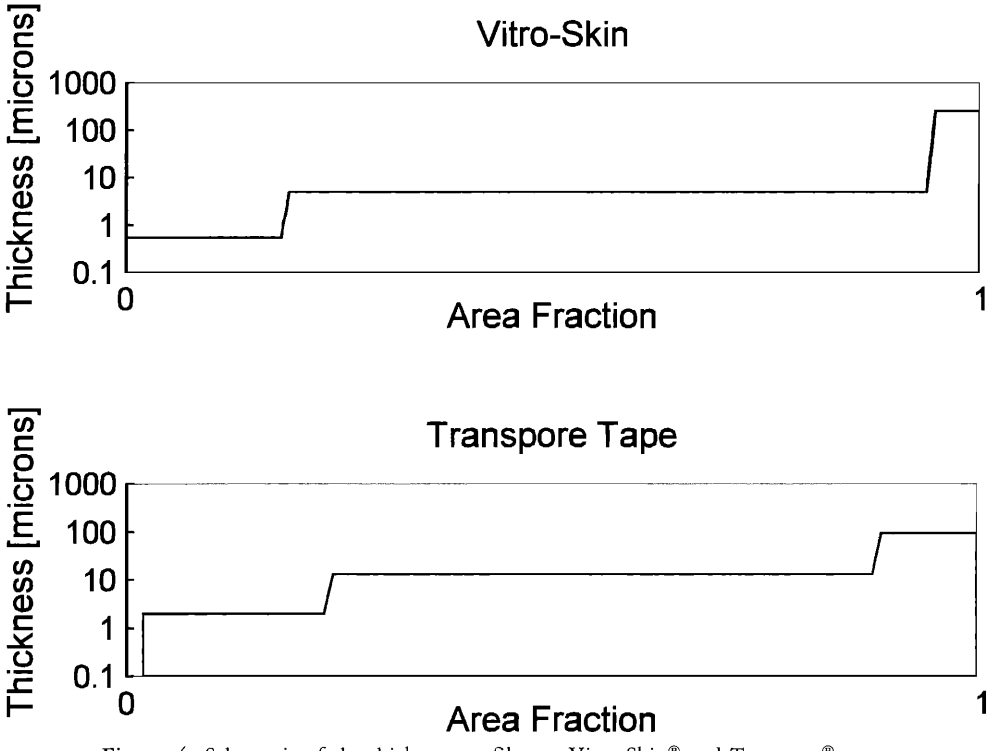

Figure 4. Schematic of the thickness profiles on Vitro-Skin® and Transpore® tape.

Vitro-Skin®. By altering the fractions in equation 1 for the 10% OMC formulation on Vitro-Skin®, it can be shown that the only source of variation that can produce the variability in the spectral MPF curves for this system is a change of about ±0.008 in the 0.55-micron-thickness fraction. This is shown in Figure 5, where the high and low MPF curves from the multiple scans (not tabulated in detail) are plotted. The high and low calculated curves are given by Profile 1, with the 0.55-micron fraction changed from 0.187 to 0.180 and 0.196, i.e., roughly ±4%. Variation of any of the other thickness fractions has no impact on the basic calculated curve. This shows that the 10% formulation has spread very evenly across the Vitro-Skin® substrate, and that, as expected at the highest concentration of absorber, the MPF curve is totally dominated by the areas of thinnest formulation.

A similar analysis of the 2.5% OMC formulation shows that a greater variation has occurred in the thinnest fraction. It is evident from Figure 6 that at this concentration there is greater experimental variation across the multiple scans. The high and low calculated curves were obtained by using 0.150 and 0.192 in place of the 0.187 fraction, i.e., −19%, +4%. Changing the 0.55-micron thickness has only marginal effects. The high curve can also be obtained by reducing the 0.55 fraction to 0.164, i.e., −11%, and either increasing the 5-micron *thickness* to 5.5 microns or reducing the *fraction* from 0.75 to 0.6. However, both of these changes at 5 microns are major compared to altering the 0.55 fraction. At 1% OMC (Figure 7) the calculated best fit curve for the three concentrations matches the high-variation MPF curve. The low curve can be simulated by increasing the 0.55-micron fraction from 0.187 to 0.21, i.e., +14%, and reducing the

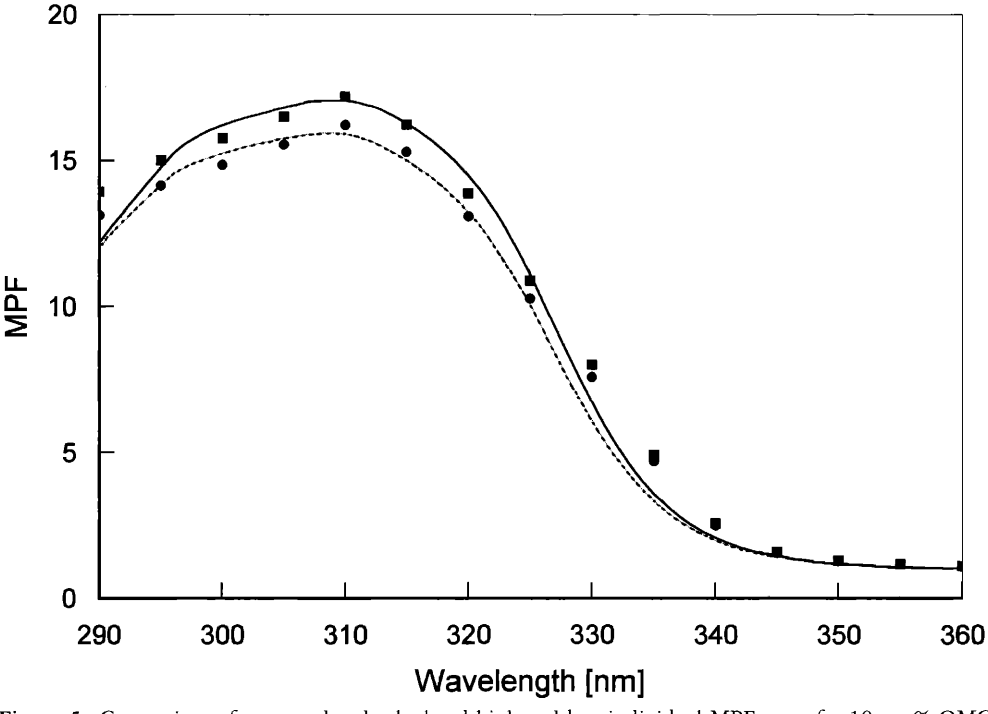

Figure 5. Comparison of measured and calculated high and low individual MPF scans for 10 wt % OMC on Vitro-Skin® substrate. High, measured ——, calculated ■; low, measured -------, calculated ●.

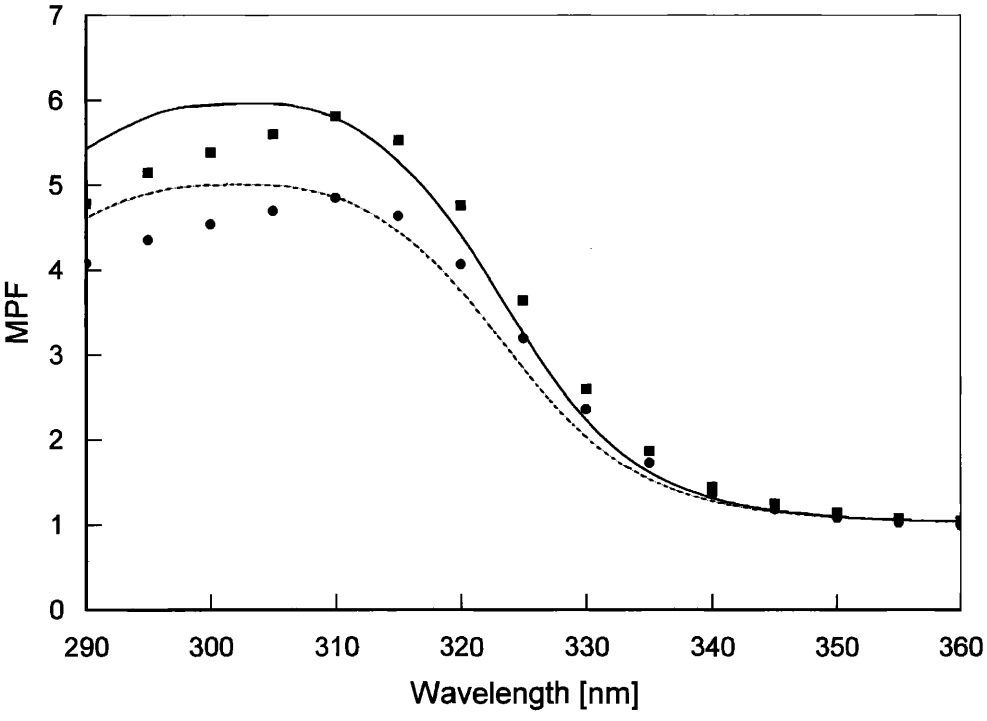

Figure 6. Comparison of measured and calculated high and low individual MPF scans for 2.5 wt % OMC on Vitro-Skin® substrate. High, measured ——, calculated ■; low, measured -------, calculated ●.

5-micron thickness to 4 microns. Increasing the 5-micron fraction is not possible because of the "Sum(Fractions) = 1" requirement.

Transpore® tape. The Transpore® tape MPF curves vary much more dramatically than those obtained on Vitro-Skin®, as shown in Figure 8, where the 10% OMC high and low curves are plotted. As with the Vitro-Skin® substrate, the variations at 10% OMC can only be accounted for by varying the thinnest areas, in this case the zero-thickness fraction. The calculated curves in Figure 5 were obtained by varying the 0.02 fraction in profile 2 from 0.012 to 0.039, i.e., -40%, to +95%. Although this amounts to a spread of only 2.7% of the total surface, it produces a dramatic change in the MPF curve. This is obviously the major source of variability in measurements on Transpore® tape, leading to the much larger standard deviations in SPF for Transpore® tape compared to Vitro-Skin® (Table I). Variations of ±100% in the 2-microns fraction could only account for 25% of the experimental spread. Analysis of the 2.5% and 1% OMC data follow the same procedure, but it is not necessary to include the whole process again since the preceding analysis demonstrates how the calculations are applied.

DISCUSSION

The detailed analysis above of the measured MPF curves illustrates how the thickness profiles can be used to determine the source of the variations in the curves, relating the curve spreads to fluctuations in fractions of area and thicknesses. The analysis also

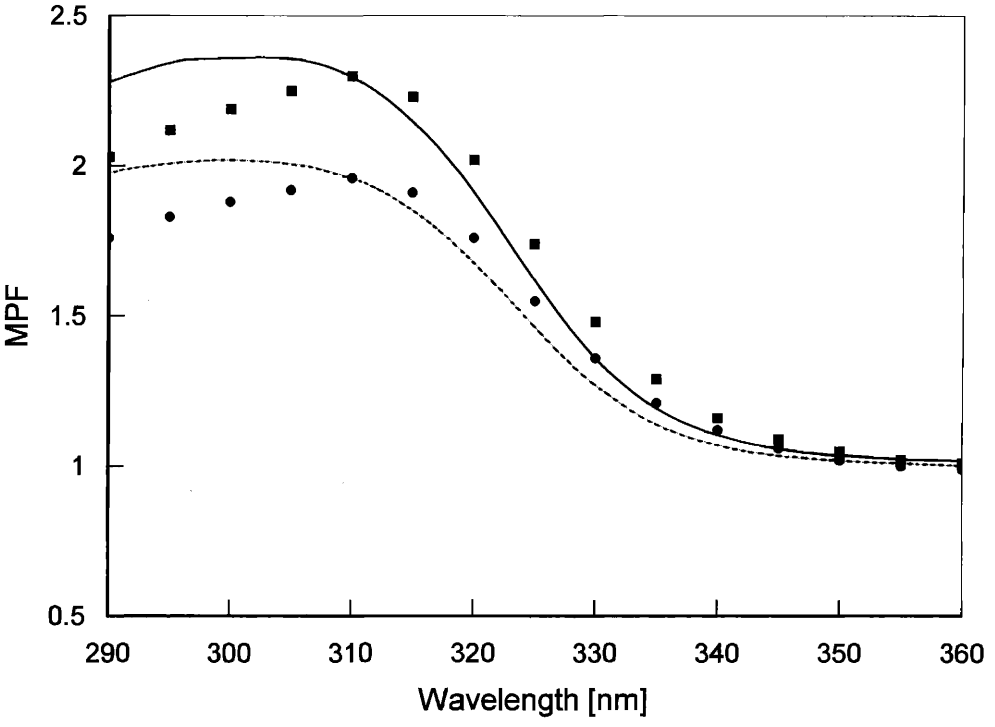

Figure 7. Comparison of measured and calculated high and low individual MPF scans for 1 wt % OMC on Vitro-Skin® substrate. High, measured ——, calculated ■; low, measured -------, calculated ●.

highlights the sensitivity of the MPF curves at high concentrations of absorber to the fractions at the smallest thicknesses. This allows the fractions at small thicknesses to be quite accurately defined and provides a useful point to commence initial analysis of the data to find the complete thickness profile.

By routine application of the analysis, experience could be built up about how particular formulations behave on the substrates. Improved accuracy could be achieved by selecting more appropriate absorber concentrations or perhaps using four or five concentrations instead of three. Vitro-Skin® produces much more uniform data than Transpore® tape by virtually eliminating the possibility of producing small areas of uncovered substrate. It produces a very simple thickness profile that does not permit wide fluctuations in thicknesses or their surface fractions. Both substrates develop SPF values similar to that of skin by providing a "sink" for a large fraction of the formulation in relatively deep crevices in their surfaces, Transpore® tape taking up 56% of the formulation in this fashion and Vitro-Skin® 81%.

The change in SPF noted for the Transpore® tape after allowing the formulations to dry implies a modification of the profiles on drying. If the profile had remained unchanged, then the concentration change of the absorber on drying would not have altered the MPF curve. There is scope here for assessing the effects of the drying process on the thickness profiles.

The transmission equations permit a detailed analysis to be made of the variation of SPF with concentration of active ingredient. These calculations are presented in Figure 9,

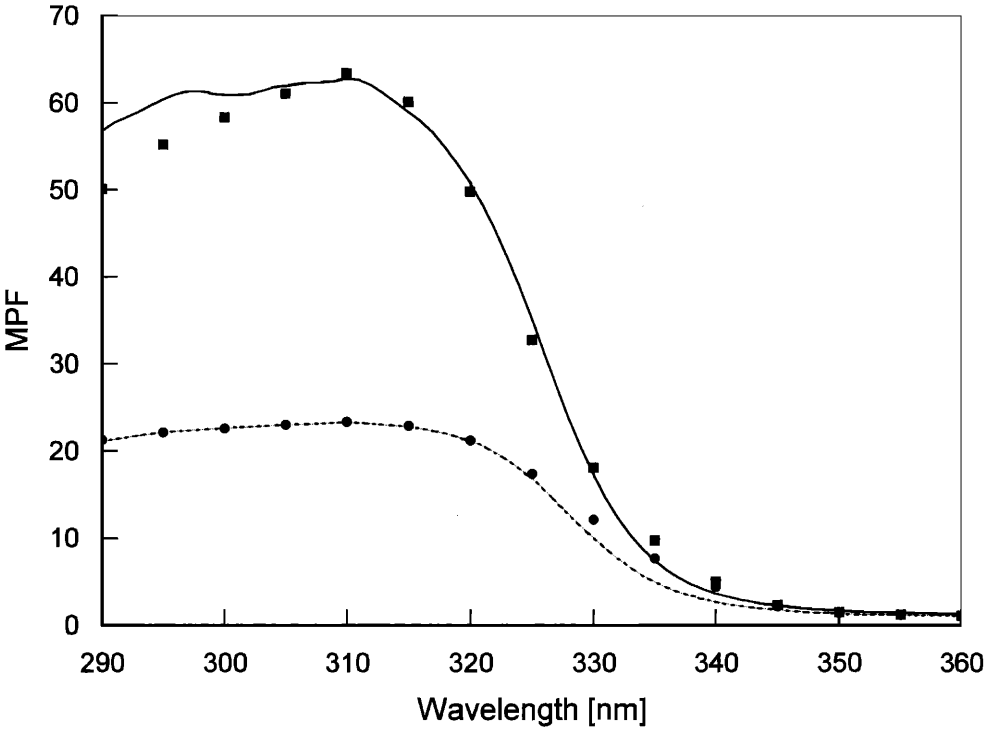

Figure 8. Comparison of measured and calculated high and low individual MPF scans for 10 wt % OMC on Transpore® tape substrate. High, measured ——, calculated ■; low, measured -------, calculated ●.

where it can be seen that the Transpore® tape curve flattens out at high absorber concentrations, producing a range of SPFs that agrees roughly with the Vitro-Skin® data. This is a direct result of the presence of a small fraction of uncovered substrate. The Vitro-Skin® data are displaying the characteristic, fortuitous linearity of SPF variation with concentration that is generally noted in sunscreens. The plot also contains the measured SPF values, showing excellent agreement between calculated and measured SPFs.

A distinctive recurring deviation of the calculated curves from the measured ones occurs mainly at the two lower concentrations in the 290–310 nm wavelength regions (see, for instance, Figures 6 and 7). The calculated curve shapes parallel that of the measured absorption properties of OMC (Figure 1). It would appear that the absorption characteristics of OMC are slightly different in the formulation from those applying to its alcohol solution. It is known that the solvent can influence the absorption characteristics of organics (9). It is also possible that at these lower wavelengths other components in the formulation have significant absorption properties. These effects are demonstrated in Figure 10, where the absorption curves for OMC in alcohol and mineral oil, a component in the formulation, are compared with an absorption curve obtained from a very thin film of the 1% OMC formulation. It is clear that the thickness profile analysis could be improved if a reliable thin film method could be established for measuring the absorption characteristics of the actual formulations. However, separate derivation of the profiles using all three absorption curves shows that the curve differences in Figure 10 have only relatively minor impact on the profile.

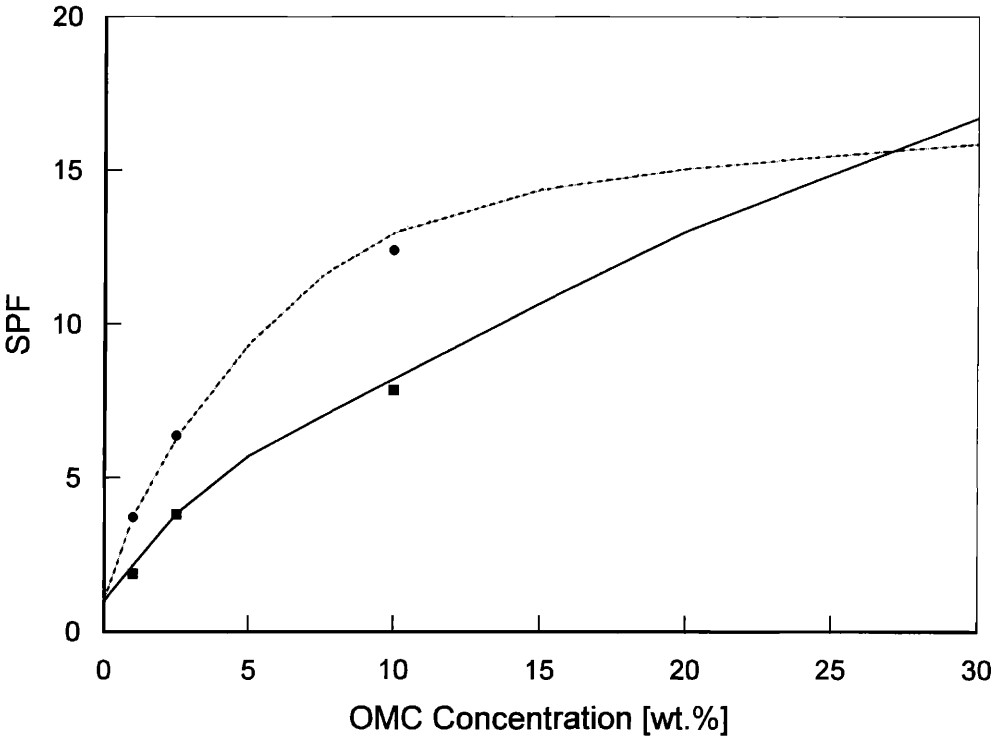

Figure 9. Comparison of measured and calculated SPFs against OMC concentration. Vitro-Skin®, calculated ——, measured ■; Transpore® tape, calculated -------, measured ●.

Analysis of excised skin data is now required to determine which substrate behaves most similarly to human skin. Rhodes and Diffey (8) have already shown that sunscreen application on human skin at the macro scale is a very hit-and-miss affair. Analysis of organic-based formulations on excised skin would complete the picture down to the micro scale.

The thickness profile analysis will also permit comparisons of the surface profiles of different skin types. It will allow different formulations to be analyzed for their detailed "spreading" qualities, related to their viscosity properties, and will thus provide underlying reasons for the occurrence of varying SPFs from different formulations containing identical absorber concentrations.

Although it has been shown that it is possible to derive an equation for MPF at a fixed application rate, thereby allowing calculation of the MPF curves at all concentrations at that rate, it is not possible to calculate the effects of varying the application rate itself. Each rate of application will have its own thickness profile, requiring the full analysis of formulations at varying concentrations. Only in this way will the relationship that eluded Brown and Diffey (5) be explained.

CONCLUSIONS

A calculation method has been applied to measured spectral mono-protection factors for

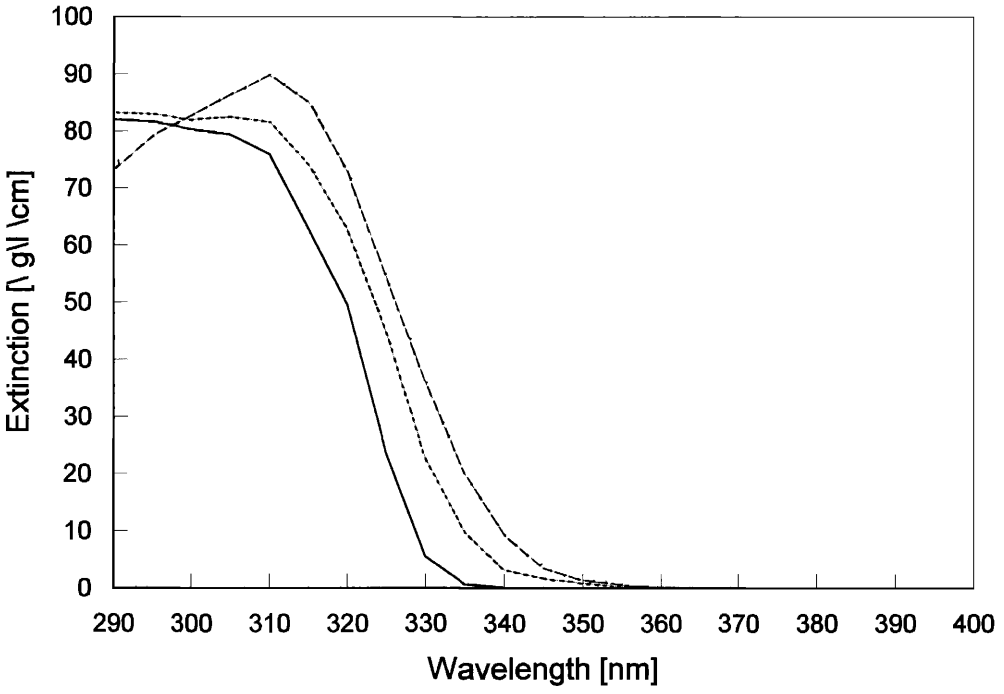

Figure 10. Comparison of the spectral absorption curves of OMC in ethanol and mineral oil with an absorption curve for the 1% OMC formulations. Ethanol – – – –; mineral oil ——; 1% OMC formulation -------.

a formulation containing varying amounts of OMC supported on Transpore® tape and Vitro-Skin® substrates. Relatively simple thickness profiles were evolved that simulate the widely varying thicknesses of formulation which occur on these substrates. Excellent agreement has been obtained between measured and calculated SPFs. The wide variability between individual spectral mono-protection curves obtained on Transpore® tape compared with Vitro-Skin® can be explained. The profiles allow calculation of the SPFs for all concentrations of absorber in the formulation on the two substrates. The variability of the spectral absorption curves for OMC in different solvents is illustrated, but it can be shown that such variation has minor impact on the thickness profiles.

ACKNOWLEDGMENTS

The author is indebted to Uniqema (ICI), Solaveil, for the preparation of the formulations and the use of their laboratories and staff, and to Labsphere for making staff and equipment available for the measurements.

REFERENCES

(1) R. M. Sayre, Correlation of *in vivo* tests, *in vivo* SPF predictions. A survey of published studies, *Cosmet. Toiletr.,* **108,** 111–114 (1993).

(2) R. M. Sayre, P. P. Agin, G. J. LeVee, and E. Marlowe, A comparison of *in vivo* and *in vitro* testing of sunscreening formulations, *Photochem. Photobiol.,* **29,** 559–566 (1979).
(3) B. L. Diffey and J. Robson, A new substrate to measure sunscreen protection factors throughout the ultraviolet spectrum, *J. Soc. Cosmet. Chem.,* **40,** 127 (1989).
(4) J. J. O'Neill, Effect of skin irregularities on sunscreen efficiency, *J. Pharm. Sci.,* **73,** 888–891 (1984).
(5) S. Brown and B. L. Diffey, The effect of applied thickness on sunscreen protection: *In vivo* and *in vitro* studies, *Photochem. Photobiol.,* 44, 509–513 (1986).
(6) S. R. Spruce and J. P. Hewitt, *In vitro* SPF: Methodology and correlation with *in vivo* data, *Euro Cosmetics,* 14–20 (June 1995).
(7) A. Beer, *Ann. Physik Chem.* (J. C. Poggendorff), **86,** 78 (1852).
(8) L. E. Rhodes and B. L. Diffey, Quantitative assessment of sunscreen application technique using *in vivo* fluorescence spectroscopy, *J. Soc. Cosmet. Chem.,* 47, 109–115 (1996).
(9) N. J. Turro, *Molecular Photochemistry* (W. A. Benjamin Inc., New York, 1967), p. 45.