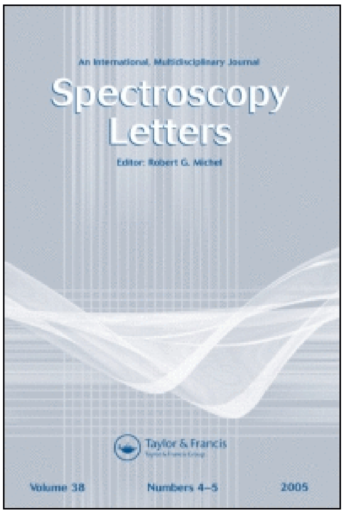

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

THE USE OF STABLE FLUORESCENT ARTIFACTS FOR CALIBRATION OF BRIGHTNESS LEVELS IN POLYOLEFIN FILMS AND PLASTIC SHEETS USING A PRECISE SET OF FLUORESCENT CALIBRATION STANDARDS

Jerome J. Workman[a]; Debbie Graverson[a]; Robert C. Pilecky[a]; Art Springsteen[b]
[a] West Research & Engineering, Kimberly-Clark Corporation, Neenah, WI, U.S.A. [b] Avian Technologies LLC/Avian Group USA, Wilmington, OH, U.S.A.

Online publication date: 13 June 2002

To cite this Article Workman, Jerome J. , Graverson, Debbie , Pilecky, Robert C. and Springsteen, Art(2002) 'THE USE OF STABLE FLUORESCENT ARTIFACTS FOR CALIBRATION OF BRIGHTNESS LEVELS IN POLYOLEFIN FILMS AND PLASTIC SHEETS USING A PRECISE SET OF FLUORESCENT CALIBRATION STANDARDS', Spectroscopy Letters, 35: 2, 245 — 256

To link to this Article: DOI: 10.1081/SL-120003810
URL: http://dx.doi.org/10.1081/SL-120003810

SPECTROSCOPY LETTERS, 35(2), 245–256 (2002)

THE USE OF STABLE FLUORESCENT ARTIFACTS FOR CALIBRATION OF BRIGHTNESS LEVELS IN POLYOLEFIN FILMS AND PLASTIC SHEETS USING A PRECISE SET OF FLUORESCENT CALIBRATION STANDARDS

Jerome J. Workman, Jr.,[1,*] Debbie Graverson,[1]
Robert C. Pilecky,[1] and Art Springsteen[2]

[1]Kimberly-Clark Corporation,
West Research & Engineering, 2100 Winchester Road,
Neenah, WI 54956, USA
[2]Avian Technologies LLC/Avian Group USA,
P.O. Box 822/91 Worthington Rd, Wilmington,
OH 45177-0822, USA

ABSTRACT

A specific set of materials and methods are used to create uniform fluorescent calibration standards over an extremely wide range of fluorescence intensities. These standards can be formulated for a specific optical brightness (OB) level and can be formulated using a variety of optical brighteners and fluorescent materials. These materials are used to create calibration standards suitable for multiple instrument types for

*Corresponding author. E-mail: jworkman@kcc.com

standard brightness measurements of films or plastic sheets. Such standards can be custom made to any optical brightness suited to the unique measurement characteristics of the wide variety of fluorescent measuring instruments or meters. The fluorophore or brightening agent is prepared using a series of concentrations to bracket the desired film or plastic brightness level(s). Once bracketed, the appropriate fluorophore or brightener concentration is added to the base material in the correct concentrations resulting in a precise level of optical brightness for each calibration standard comprising a linear set of reference standards.

Key Words: Fluorescent calibration standards; Optical brightener (OB); Polyolefin

INTRODUCTION

Unlike paper measurements, which demand the use of a fluorophore that 'exactly' duplicates the excitation and emission spectrum of the optical brightener in paper, the calibration of a series of instruments for measurement of optical brightness in polyolefin films and plastic sheets can be accomplished by use of a number of standards that meet the following criteria:

1. Stability of artifact standards over a long period of time under ambient storage conditions;
2. Ability to establish linearity of the sensor by use of a calibrated and gradient series of artifacts that cover a range of brightness that cover the expected range of the products to be measured.
3. Use of these artifact standards as transfer standards to establish linearity of similar instruments.

MATERIALS AND METHODS

A fine powdered optical brightener or fluorophore is thoroughly mixed into a PTFE fine powder having a particle size diameter within 0.1 to 5 times the fluorophore. The materials are thoroughly mixed; the mixing time is predetermined by testing the final standard reproducibility between test tiles made from a specific batch of material. The deviation cannot exceed more than 2 percent relative OB reading from batch to batch for a pre-specified fluorophore or brightener concentration when using a pre-specified fluorescent meter. The PTFE is sintered to produce a final OB or fluorescent standard tile block and is tested for optical quality and values using any OB or fluorescent surface measurement detector. These measurements are compared to measurements made of polyolefin films or plastic sheets containing an optical brightener and the levels of brightness of the films are recorded. The optical brightness (OB) standards so produced are compared to previous measurements made using a pre-specified detector system and are assigned OB values based upon new or pre-established measurement scales. These scales can be arbitrary or absolute.

The standard practice for providing materials for optical brightener standards has been to mix fluorophores or optical brighteners with highly viscous plastic resins and allow these resins to cure for the production of reference brightness tiles. The procedure is time consuming and results in microinhomogeneity of the standards making them non-reproducible from batch to batch. Fluorophores and optical brightener compounds have extremely high extinction coefficients, absorptivities, designated as ϵ in the ultraviolet region for these compounds are typically greater than 50,000–100,000 $L*mol^{-1}\ cm^{-1}$. The high photon efficiency of these materials requires that standards be completely homogeneous in composition even at the micro-level. Thus the traditional methods fail to produce a series of consistently reproducible optical brightness standards.

The practical requirement of such standards in industry is to be able to produce multiple sets of calibration standards at three linear concentration levels which can be used with any instrument developed for measuring brightness or fluorescence. Typical brightness measurement instruments may consist of difference angles between illumination and detection such as: 90-degress (typical fluorescence measurements), 45-degress (typical fluorescence solid measurements), normal angle incidence and detection (low-cost instruments), diffuse or specular reflectance (research-grade spectrophotometers), and others. Each instrument will give different values for brightness and will demonstrate differences in their respective instrument responses versus the fluorophore or optical brightener content of a set of measurement standards. Such measuring instruments will also have variable response to microhomogeneity of the fluorescing compound contained within the standard material substrate. Thus a set of calibration standards of exacting homogeneity and precise brightness is required for this measurement application. Note: Each specific instrument type and each specific application requires a unique set of calibration standards to ensure accurate an linear brightness measurement of films and plastic sheets.

RESULTS

The raw data and test protocol from a round-robin test of the standards is given in Tables 1a through 1d. Statistical methods used for these data are given in detail in references 1–7. Four sets of three tiles each (12 tiles total) comprising three levels of optical brightener were collected into sets, the high-level tiles were composed following the procedures described within the preceding Materials and Methods section. The zero and mid-level tiles were purchased as standard items from Avian Technologies of NH and Ohio (Part Nos. AT-FS09-KCC-CSTM, no OB; and AT-FS10-KCC-CSTM, mid-level OB). The three nominal optical brightener levels were none (0.00), mid-level (approximately 40 OB units), and high-level (approximately 73 OB units). Three different operators measured the four sets of three tiles, each using a separate instrument (each calibrated twice, with two replicate measurements per calibration). Analysis of the measurement data provided statistics as shown in Tables 2–5. The mean value for each row of data comprised of three instruments/operators, two calibration sets of two replicate measurements each ($n = 12$ measurements per tile). Thus the mean value (column 3, Table 2) indicates the average value for each tile for the 12 measurements per tile. The Grand Mean (column 4, Table 2) indicates the average of three instrument/operators, 4 measurements each, for each of the combined 3 OB level tile sets (i.e., the Grand Mean of 48 measurements per OB level). The precision (Prec.*, column 5, Table 2) indicates the standard deviation of the 12 measurements for each tile. The pooled precision (P-Prec.**, column 6, Table 2) represents the standard deviation of the 48 measurements per OB level).

The results of the accuracy statistics are shown in Table 2. The accuracy (Acc.+, column 7, Table 2) indicates the standard deviation of each set of 4 measurements for each tile from the original Certified (OC) OB values for each tile. This statistic indicates the 1 sigma deviation expected for each individual tile measurement from the original Certified OB level for each tile. Thus this is a measure of the overall deviation for each individual

Table 1a. Data Collection Protocol from Round-Robin Test of the Standards

Testing should be performed in the following manner for each Instrument/Operator:

1. Calibrate detector using tile A to a value of 74.
2. Test sequentially all 12 tiles A-L (Round 1). Then repeat this step testing A-L (Round 2).
3. Turn the adjustment screw until value for tile A is less than 60 or greater than 88.
4. Repeat steps 1 and 2 resulting in 4 total measurements per tile.

Table 1b. Instrument I/Operator I Raw Data from Round-Robin Test of the OB Standards

Tile	Round 1	Round 2	Recalibrate	Round 1	Round 2	Mean
A	74	74		74	74	74
B	0	0		0	0	0
C	0	0		0	0	0
D	41	40		40	40	40.25
E	41	41		41	41	41
F	74	73		73	72	73
G	73	73		72	72	72.5
H	0	0		0	0	0
I	41	40		40	40	40.25
J	74	74		73	74	73.75
K	0	0		0	0	0
L	40	40		40	40	40

Table 1c. Instrument II/Operator II Raw Data from Round-Robin Test of the OB Standards

Tile	Round 1	Round 2	Recalibrate	Round 1	Round 2	Average
A	74	74		74	74	74
B	0	0		0	0	0
C	0	0		0	0	0
D	36	36		36	36	36
E	37	37		37	37	37
F	73	73		73	73	73
G	73	73		73	73	73
H	0	0		0	0	0
I	37	37		37	37	37
J	74	74		74	74	74
K	0	0		0	0	0
L	37	36		36	36	36.25

tile for each original certified OB level. The smaller this number, the closer is the tile in comparison to its specified OB value. The pooled accuracy (P-Acc.++, column 8, Table 2) indicates the pooled standard deviation of all 48 measurements for each OB level based on the original Certified OB values of each tile. This deviation indicates the comparative accuracy of measurements made with each tile set. The smaller this number, the more similar

Table 1d. Instrument III/Operator III Raw Data from Round-Robin Test of the OB Standards

Tile	Round 1	Round 2	Recalibrate	Round 1	Round 2	Average
A	74	74		74	74	74
B	0	0		0	0	0
C	0	0		0	0	0
D	37	36		37	36	37
E	38	38		38	37	38
F	73	74		73	73	73
G	74	74		73	74	74
H	0	0		0	0	0
I	39	39		38	38	39
J	75	75		74	74	75
K	0	0		0	0	0
L	38	38		38	38	38

Table 2. Measurement Statistics Using Original Certified Values

Sample	Original Certified'	Mean	Grand Mean	Prec.*	P-Prec.**	Acc.+	P-Acc.++
B	0.00	0.00	—	0.00	—	0.00	—
C	0.00	0.00	—	0.00	—	0.00	—
H	0.00	0.00	—	0.00	—	0.00	—
K	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D	39.0	37.58	—	0.44	—	2.77	—
E	40.0	38.58	—	0.29	—	2.60	—
I	40.0	38.58	—	0.44	—	2.29	—
L	39.0	38.08	38.21	0.29	0.37	2.08	2.45
A	74.0	74.0	—	0.00	—	0.00	—
F	72.0	73.08	—	0.55	—	1.37	—
G	72.0	73.08	—	0.44	—	1.45	—
J	74.0	74.08	73.56	0.44	0.42	0.58	1.04

'Original Certified Tile OB levels.
Mean values for 3 instruments and operators, 2 calibrations, 2 replicates each calibration (n = 12).
Grand Mean for 4 tiles at each nominal OB level, 3 instruments, 2 calibrations, 2 replicates per calibration (n = 48).
Prec.*/ Precision each tile (n = 12).
P-Prec.**/ Pooled Precision (each OB level) (n = 48).
Acc.+/ Accuracy each tile vs. OC (n = 12).
P-Acc.++/ Pooled Accuracy (each OB level) vs. OC (n = 48).

Table 3. Measurement Statistics Using Adjusted New Certified Values

Sample	Original Certified'	New Certified''	Acc.+	P-Acc.++
B	0.00	0.00	0.00	—
C	0.00	0.00	0.00	—
H	0.00	0.00	0.00	—
K	0.00	0.00	0.00	0.00
D	39.0	38.0	2.29	—
E	40.0	39.0	2.08	—
I	40.0	39.0	1.67	—
L	39.0	38.0	1.80	1.97
A	74.0	74.0	0.00	—
F	72.0	73.0	1.37	—
G	72.0	73.0	1.45	—
J	74.0	74.0	0.58	1.04

'Original Certified Tile OB levels.
''New Certified Tile OB levels.
Acc.+/ Accuracy each tile vs. NC (n = 12).
P-Acc.++/ Pooled Accuracy (each OB level) vs. NC (n = 48).

Table 4a. Systematic Error Testing for Instrument I/Operator I (Each Tile)

Sample	New Certified''	Measured*	t-Test for Bias	t-Critical for Bias	Difference	Bias
B	0.00	0.00	0	3.182	0	NO
C	0.00	0.00	0	3.182	0	NO
H	0.00	0.00	0	3.182	0	NO
K	0.00	0.00	0	3.182	0	NO
D	38.0	40.25	9.0	3.182	+2.25	YES
E	39.0	41.0	801	3.182	+2.00	YES
I	39.0	40.25	5.0	3.182	+1.25	YES
L	38.0	40.0	800	3.182	+2.00	YES
A	74.0	74.0	0	3.182	0	NO
F	73.0	73.0	0	3.182	0	NO
G	73.0	72.5	1.73	3.182	−0.50	NO
J	74.0	73.75	1	3.182	−0.25	NO

*/ Average of Instrument I/Operator I; 2 calibrations, 2 replicates each calibration (n = 4).

Table 4b. Systematic Error Testing for Instrument II/Operator II (Each Tile)

Sample	New Certified''	Measured*	t-Test for Bias	t-Critical for Bias	Difference	Bias
B	0.00	0.00	0	3.182	0	NO
C	0.00	0.00	0	3.182	0	NO
H	0.00	0.00	0	3.182	0	NO
K	0.00	0.00	0	3.182	0	NO
D	38.0	36.0	799	3.182	−2.0	YES
E	39.0	37.0	799	3.182	−2.0	YES
I	39.0	37.0	799	3.182	−2.0	YES
L	38.0	36.25	7.00	3.182	−1.75	YES
A	74.0	74.0	0	3.182	0	NO
F	73.0	73.0	0	3.182	0	NO
G	73.0	73.0	0	3.182	0	NO
J	74.0	74.0	0	3.182	0	NO

*/ Average of Instrument II/Operator II; 2 calibrations, 2 replicates each calibration ($n = 4$).

Table 4c. Systematic Error Testing for Instrument III/Operator III (Each Tile)

Sample	New Certified''	Measured*	t-Test for Bias	t-Critical for Bias	Difference	Bias
B	0.00	0.00	0	3.182	0	NO
C	0.00	0.00	0	3.182	0	NO
H	0.00	0.00	0	3.182	0	NO
K	0.00	0.00	0	3.182	0	NO
D	38.0	36.5	5.196	3.182	−1.5	YES
E	39.0	37.75	5.00	3.182	−1.25	YES
I	39.0	38.5	1.73	3.182	−0.50	NO
L	38.0	38.0	0	3.182	0	NO
A	74.0	74.0	0	3.182	0	NO
F	73.0	73.25	1.00	3.182	+0.25	NO
G	73.0	73.75	3	3.182	+0.75	NO
J	74.0	74.5	1.73	3.182	+0.50	NO

*/ Average of Instrument III/Operator III; 2 calibrations, 2 replicates each calibration ($n = 4$).

Table 4d. Combined Systematic Error Testing for Instruments/Operators I–III (All Tile Measurements)

Sample	New Certified''	Measured*	t-Test for Bias	t-Critical for Bias	Difference	Bias
B	0.00	0.00	0	2.201	0	NO
C	0.00	0.00	0	2.201	0	NO
H	0.00	0.00	0	2.201	0	NO
K	0.00	0.00	0	2.201	0	NO
D	38.0	37.58	0.714	2.201	−0.42	NO
E	39.0	38.58	0.787	2.201	−0.42	NO
I	39.0	38.58	1.00	2.201	−0.42	NO
L	38.0	38.08	0.178	2.201	+0.08	NO
A	74.0	74.0	0	2.201	0	NO
F	73.0	73.08	0.56	2.201	+0.08	NO
G	73.0	73.08	0.432	2.201	+0.08	NO
J	74.0	74.08	0.56	2.201	+0.08	NO

*/ Average of Combined Instrument/Operator I-III; 2 calibrations, 2 replicates each calibration ($n = 12$).

Table 5. Linearity Statistics for Calibration Test Samples (Certified Values vs. Average Measured Values)

Data	n	Range	Slope*	Intercept*	RSD**	R2***
Original certified	36	0–74	0.992	0.541	3.4/1.27	0.998
New certified	36	0–74	0.998	0.142	2.7/0.995	0.999

(n) represents 4 tiles of 3 OB levels each, for 3 instruments (average values for 2 calibrations, 2 replicates used).
*/ Certified (x) vs. measured (y).
**/ Relative Standard Deviation (percent error)/Residual Standard Deviation (in OB units).
***/ Coefficient of Determination (full range).

are the comparative OB levels within a tile set, and the more uniform and precise are the OB level measurements.

Following statistical assessment the original Certified OB values for each tile (column 2, Table 3) were corrected to *new* Certified (NC) OB values (column 3, Table 3). These corrected values were adjusted to the nearest integer number for each tile based on the mean or average (column 3,

Table 2) of the 12 initial measurements for each tile. Accuracy based on the new Certified OB values for each tile is shown as the standard deviation of each set of 12 measurements as Acc.+, column 4, Table 3. The pooled accuracy for all 48 measurements for each OB level based upon the new Certified calibration values is given as P-Acc.++ column 5, Table 3. The similarity in these accuracy statistics as compared to those calculated by using the Grand Mean indicate that the new Certified values are more accurate based on the measurement data in Table 1, particularly for the mid-level tiles.

The following Eqs. (1) and (2) are used for calculations of the standard deviations for precision and accuracy (σ), and pooled precision and accuracy (σ_P) parameters, respectively for each set of calculations given by the different columns in Tables 2 and 3[1–6].

$$\sigma = \left\{ \frac{1}{n-1} \sum (X_i - \overline{X}_i)^2 \right\}^{\frac{1}{2}} \tag{1}$$

$$\sigma_P = \left\{ \frac{\sigma_1^2 + \sigma_2^2 + \cdots + \sigma_n^2}{n} \right\}^{\frac{1}{2}} \tag{2}$$

All measurements of the 0.00 OB level tiles indicated precisely zero level OB unit measurements for all tiles and all measurements. The results for the approximately 40 OB level tiles indicates that an expected 1 sigma accuracy of 1.97 OB units irrespective of calibration tile, instrument number, or calibration. The results for the approximately 73 OB level tiles indicates an expected 1 sigma accuracy of 1.04 OB units irrespective of calibration tile, instrument number, or calibration. These results are excellent for the high-level and zero-level tiles and marginally acceptable for the mid-level tiles, noting that the adjustment of the detector systems employed is limited to incremental settings of ± 1.0 OB units.

A t-test statistic was used for determining the presence of systematic errors[3], and indicates that for individual instruments there is significant systematic error (bias) for the measurements of the mid-level tiles near 40 OB, Tables 4a through 4c. For the high-level tiles, near 73 OB, there is no systematic error (bias). There is absolutely no error in the zero tiles (0.0 OB), as they all yielded measurements of 0.00 OB units. Note: There is no systematic error (bias) indicated when all instrument data are combined as shown in Table 4d. These data indicate the greater variability apparent in the commercial mid-level tiles near 40 OB as compared to the new design tiles at 73 OB.

Linearity of the averages of four measurements for each tile, for three instruments/operators, at three different OB levels ($n = 36$ measurements) is shown in Table 5. These data are compared as the regressed numbers for Certified (x) OB values versus measured (y) OB values for both the *original* and *new* Certified OB values. The linearity statistics are improved by using the new Certified values resulting in an intercept closer to zero (0.142 vs. 0.541) and a coefficient of determination of 0.999 versus 0.998 indicating excellent linearity over the entire range.

CONCLUSIONS

The combination of a new method to manufacture accurate fluorescence standard OB tiles combined with appropriate statistical analysis of the measured data yields precise certified values for comparison of brightness levels. With this new process of manufacturing and certifying OB standards, accurate, precise, and customizable OB measurements are possible even when an arbitrary standard measurement system is employed.

The final results for the tiles indicates that 1 sigma accuracy for the three levels of OB tiles are 0.0 OB units (for the zero-level OB tiles), 1.97 OB units (for the mid-level OB tiles), and 1.04 OB units (for the high-level OB tiles). These results are irrespective of calibration tile, instrument number, or calibration. Note that the adjustment of the detector systems used in this application is limited to incremental settings of ± 1.0 OB units. The statistics for the new Certified values resulted in an intercept near zero (0.142) and a coefficient of determination of 0.99997, indicating excellent linearity over the entire range. There is no systematic error (bias) indicated when all instrument data are combined as shown in Table 4d. The greater variability apparent in the commercial tiles near 40 OB as compared to the new design tiles at 73 OB, indicates that the new design tiles are more precise and accurate even when at high significantly higher relative OB levels.

ACKNOWLEDGMENTS

Appreciation is expressed to those individuals involved in defining the problem for calibration of optical brightener sensors to an accurate, reproducible, yet arbitrary scale. These individuals include: Kim Ellefson, Jim Smolinski, Mike Hellweg, Greg Blincoe, Patti Konkol, and Chris Kundert of Kimberly-Clark Corporation.

REFERENCES

1. Workman; Mark, H. Chemometrics in Spectroscopy: A Blueprint for Collaborative Laboratory Studies – Part 1. Spectroscopy **2000**, *15* (1), 16–25.
2. Workman, J.; Mark, H. Chemometrics in Spectroscopy: ANOVA Test Comparisons for Laboratories and Methods – Part 2. Spectroscopy **2000**, *15* (2), 28–29.
3. Workman, J.; Mark, H. Chemometrics in Spectroscopy: Testing for Systematic Error in a Method – Part 3. Spectroscopy **2000**, *15* (5), 28–29.
4. Workman, J.; Mark, H. Chemometrics in Spectroscopy – Ranking Test for Laboratories and Methods – Part 4. Spectroscopy **2000**, *15* (6), 26–27.
5. Workman, J.; Mark, H. Chemometrics in Spectroscopy: Computations for Efficient Comparison of Two Methods – Part 5. Spectroscopy **2000**, *15* (7), 16–19.
6. Youden, W.J.; Steiner, E.H. Statistical Manual of the AOAC; 1 ed.; Association of Official Analytical Chemists; Washington, D.C., 1975.

Received August 7, 2001
Accepted December 15, 2001