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Soy Protein Isolate/Poly(ethylene oxide) Films[1]

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ABSTRACT

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Films were prepared by casting and drying alkaline aqueous film-forming solutions of soy protein isolate (SPI). Four additional types of films were made by combining SPI with poly(ethylene oxide) (PEO) in SPI to PEO ratios of 19:1, 9:1, 4:1, and 1.5:1 (w/w). Glycerin was added to all film-forming solutions as a plasticizer at 60% of total solid weight. Addition of PEO decreased film tensile strength (TS) and increased elongation at break (E). TS values ranged from 1.4 to 3.9 MPa and E values ranged from 83 to 152%. Water vapor permeability of the films ranged from 3.0×10^{-9} to 4.0×10^{-9} g/m·sec·Pa. Scanning electron micrographs of film cross sections showed an increase in the inferior texture with increasing amounts of PEO in the films.

There is a growing concern over nondegradable plastic in the municipal solid waste (MSW) stream in the United States, as well as around the world. Plastic materials account for $\approx 7\%$ of the MSW; this number is expected to grow to $>10\%$ by the beginning of the next century (Thayer 1990). Plastic materials are indestructible in nature and resist rapid degradation. As a result, interest exists in developing degradable materials for single use items, such as utensils, garbage and grocery bags, plates, planting pots, and mulches, as an alternative to petroleum-based plastic products. Incorporation of biopolymers such as starches and proteins has shown promise in terms of enhancing degradation of plastic materials (Griffin 1974).

Plant proteins have the ability to form films that can be used in edible packaging applications. Edible films from biopolymers have been extensively reviewed by Kester and Fennema (1986), Guilbert (1986), Krochta (1992), and Gennadios et al (1994a). In general, protein films have poor moisture barrier properties due to the hydrophilic nature of their amino acids. Recent studies have concentrated on improving protein film mechanical and barrier properties (Brandenburg et al 1993, Gennadios et al 1993a, Shih 1994, Stuchell and Krochta 1994). Approaches more or less successfully employed to improve properties of soy protein films include treatment with alkali (Brandenburg et al 1993), alkylation with sodium alginate (Shih 1994), treatment with propylene glycol alginate (Shih 1994), and enzymatic treatment with horseradish peroxidase (Stuchell and Krochta 1994).

Several studies reported on incorporation of biopolymers, such as starch and protein, into extrusion blown polyethylene films (Otey et al 1974, 1977, 1980, 1987; Otey and Westhoff 1984; Ghorpade and Hanna 1993; Park et al 1993). Otey et al (1977, 1980, 1987) and Otey and Westhoff (1984) prepared starch-based compostible films containing polyethylene (ethylene-co-acrylic acid) for agricultural mulches. Dennenberg et al (1978) demonstrated the biodegradability by *Aspergillus niger* of a starch graft polymethylacrylate copolymer that exhibited excellent tensile properties. Park et al (1993) reported characteristics of zein-filled polyethylene compostible films, while Ghorpade and Hanna (1993) studied properties of extrusion-blown soy protein isolate/

polyethylene oxide/low density polyethylene films. Films combining protein with synthetic plastics show potential for production of compostible plastic materials. Degradation of protein by microorganisms can be easily achieved, thus rendering the remaining synthetic polymer vulnerable to photo- or thermal degradation.

This study was conducted with the objective of forming cast films from soy protein isolate (SPI) with various amount of poly(ethylene oxide) (PEO) and determining the effects of PEO addition on mechanical and barrier film properties. PEO was used in this study because of its high water solublity and to study interactions with protein molecules.

MATERIALS AND METHODS

Reagents

SPI (ARPRO 1100) was obtained from Archer Daniels Midland Corp. (Decatur, IL) and stored at 4°C before use. PEO was purchased from Scientific Polymer Products, Inc., (Ontario, NY). Glycerin was purchased from Fisher Scientific (Pittsburgh, PA).

Film Formation

Film-forming solutions were prepared by slowly adding 5 g of SPI to constantly stirred mixtures of 100 ml of distilled water and 3 g of glycerin. Glycerin was added as a plasticizer to overcome film brittleness and to obtain free-standing films. Solution pH was adjusted to 11.0 ± 0.1 with $1N$ sodium hydroxide. The solutions were incubated for 30 min in a 70°C constant temperature water bath. Solutions were strained through cheese cloth (grade 40, Fisher Scientific) upon removal from the water bath and cast on Teflon-coated glass plates. Films were peeled from the plates after drying at ambient temperature for ≈ 30 hr. Four additional types of films were made by combining SPI with PEO in ratios of 19:1, 9:1, 4:1, and 1.5:1 (w/w). These films are hereafter referred as 5, 10, 20, and 40%, respectively, based upon PEO concentration.

Moisture Content

Film moisture content (MC) was measured after conditioning films in an environmental chamber at 25°C and 50% rh for three days. These conditions were similar to those for conditioning film specimens before tensile testing. Samples of 400–500 mg were weighed in aluminum dishes and dried for 24 hr in an air-circulating oven at 105°C. MC was calculated in duplicate for each type of film as percentage weight lost during drying and reported on a wet basis.

Tensile Strength and Elongation at Break

Films were conditioned at 50% rh and 25°C for three days before testing. A universal testing instrument (model 5566, In-

stron Engineering Corp., Canton, MA) was used to determine tensile strength (TS) and elongation at break (E), according to ASTM Method D 882-88 (ASTM 1989). Film specimens (2.54 cm wide × 10 cm long) were cut. Five thickness measurements were taken along each specimen with a hand-held micrometer (B. C. Ames Co., Waltham, MA); the mean of the five measurements was used in TS calculations. The initial grip separation and cross-head speed were set at 5 cm and 50 cm/min, respectively. TS was calculated by dividing the maximum (peak) load necessary to pull the specimen apart by the original cross-sectional area of the specimen. E was calculated by dividing film elongation at rupture by the initial gauge length of the specimen and multiplying by 100. TS and E determinations for each type of film were replicated four times with individually prepared films as the replicated experimental units and six sampling units (specimens) tested from each film replicate.

Water Vapor Permeability

Film specimens (7 × 7 cm) were cut. Five thickness measurements were taken on each specimen: one at the center and four around the perimeter. The mean value was used as the specimen thickness in water vapor permeability (WVP) calculations. Before testing, all film specimens were conditioned at 25°C and 50% rh for two days. Four individually cast film specimens were tested from each type of film. WVP (g·m/m² ·sec·Pa) was calculated as:

$$WVP = (WVTR \times L)/\Delta p \qquad (1)$$

where WVTR was the measured water vapor transmission rate

$(g/m^2 \cdot sec)$ through a film specimen, L was the mean film thickness (m), and p was the partial water vapor pressure difference (Pa) across the two sides of the film specimen.

WVTR was determined gravimetrically using a modified ASTM Method E 96-80 (ASTM 1989). Film specimens were mounted on poly(methyl methacrylate) cups filled with distilled water up to 1 cm from the film underside. Design of the cups was described by Gennadios et al (1994b). The cups were placed in an environmental chamber set at 25°C and 50% rh. A fan was operated within the chamber creating an air velocity of 198 m/min over the surface of the cups to remove the permeating water vapor. Weights of the cups were recorded six times at 1 hr intervals. Steady state was reached after 1 hr. Slopes of the steady state (linear) portion of weight loss versus time curves were used to estimate WVTR. Because of the low water vapor resistance of protein-based films, actual rh values at the film undersides during testing were lower than the theoretical value of 100%. Actual rh values at the film undersides and film WVP values were calculated after accounting for the resistance of the stagnant air layer between the film undersides and the water surface in the cups (McHugh et al 1993, Gennadios et al 1994b). The mean of the initial and the final stagnant air gap heights was used in the calculations.

Color

Color values of the SPI-PEO films were measured (CR-300 Minolta Chroma Meter, Minolta Camera Co., Ltd., Osaka, Japan). This instrument is a tristimulus color analyzer with an 8-mm diameter measuring area. Film specimens were placed on a white standard plate (calibration plate CR-A43) and the HunterLab color scale was used to measure color: $L = 0$ (black) to $L = 100$
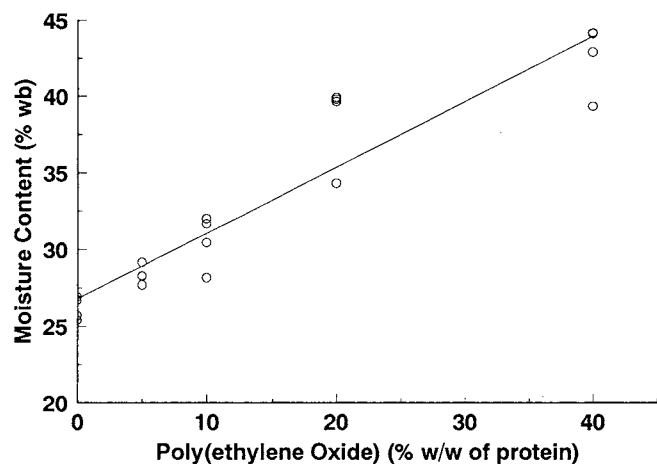

Fig. 1. Effect of poly(ethylene oxide) concentration on moisture content of soy protein isolate and poly(ethylene oxide) films stored at 50% rh and 25°C for three days. Linear regression ($R^2 = 0.88$) line was fitted to the data.
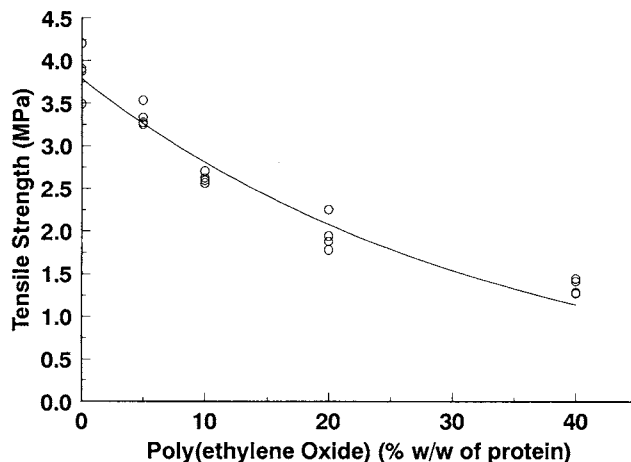

Fig. 2. Effect of poly(ethylene oxide) concentration on tensile strength of soy protein isolate and poly(ethylene oxide) films. Exponential regression ($r = 0.974$) line was fitted to the data.

TABLE I
Effect of Irradiation on Tensile Strength (TS) and Elongation at Break (E) of Soy Protein Isolate and Poly(ethylene Oxide) Films[a]

| | Poly(ethylene Oxide) Concentration (%w/w of protein) | | | | | | | | | |
| | 0 | | 5 | | 10 | | 20 | | 40 | |
Dosage[b]	TS[c]	E[c]	TS	E	TS	E	TS	E	TS	E
0	3.9±0.2	88.7±29.0	3.4±0.2a	151.3±3.4a	2.5±0.2a	162.4±16.1	1.9±0.1a	156.7±25.4	1.4±0.1a	158.8±18.2
5	4.1±1.1	76.2±12.7	3.4±0.5a	107.4±31.2bc	3.8±0.7b	114.1±43.8	2.9±0.5b	151.4±16.8	1.9±0.2b	170.2±17.1
10	4.2±0.9	72.0±21.6	4.2±0.5b	88.2±8.4c	3.9±0.3b	109.9±32.5	3.5±0.6b	136.1±64.6	2.3±0.4bc	139.6±43.8
20	4.3±0.6	89.3±29.8	4.1±0.2b	126.8±9.0ab	4.0±0.4b	124.2±12.0	3.4±0.1b	156.9±12.7	2.4±0.1c	136.1±25.0
30	4.2±0.6	90.8±10.5	3.9±0.1ab	90.5±25.2c	3.9±0.4b	126.0±37.2	3.3±0.1b	122.9±45.9	2.4±0.2c	124.6±36.3

[a] Values are means of four replicates ± standard deviation. Any two means in the same column followed by the same letter are not significantly ($P > 0.05$) different according to Duncan's multiple range test. Columns with no letters next to the means indicate that no significant ($P > 0.05$) difference among means was detected.
[b] Irradiation dosage (kGy).
[c] TS values in MPa. E values in %.

(white); $-a$ (greenness) to $+a$ (redness); $-b$ (blueness) to $+b$ (yellowness) (Francis and Clydesdale 1975). Total color difference (ΔE) was calculated as:

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{0.5} \qquad (2)$$

where: $\Delta L = L_{standard} - L_{sample}$; $\Delta a = a_{standard} - a_{sample}$; $\Delta b = b_{standard} - b_{sample}$. Standard values for the white plate were: $L = 96.86$, $a = -0.02$, $b = 1.99$. Measurements were taken in triplicate for each type of film.

Scanning Electron Microscopy

The effect of PEO addition on film microstructure was observed with a scanning electron microscope (JSM-6100 JEOL, Mountain View, CA). Small pieces of film were broken by bending, mounted vertically (to see the microstructure along the edge) on aluminum stubs, and coated with a layer of silver colloidal paste and gold-palladium alloy (15 nm) using a Blazer Union SCD-030 sputter coater. The films were dried and observed under the microscope using an accelerating voltage of 10 kV with a beam directed normal to the edge or at a 45° angle. The sharpest scanning electron microscopy (SEM) pictures were taken at a magnification of 10,000×.

Film Irradiation

Film samples (sheets of 18×25 cm) were sealed in polyethylene ziplock bags and exposed to 0 (control), 5, 10, 20, and 30 kGy of gamma irradiation at room temperature using a ^{60}Co irradiator (Nuclear Materials and Equipment Corporation, Apollo, PA). The dosage rate was 0.55 kGy/hr; corresponding exposure times required to achieve desired irradiation dosages were 10, 18, 36, and 55 hr.

RESULTS AND DISCUSSION

Moisture Content

The MC of SPI-PEO films conditioned at 50% rh and 25°C for three days increased as the amount of PEO in the film increased (Fig. 1). This suggested that PEO was more hydrophilic and ab-

sorbed more water than did SPI. Mean MC values ranged from $26.2 \pm 0.8\%$ for the 0% PEO film to $42.6 \pm 2.6\%$ for the 40% PEO film. The following linear regression model was satisfactorily fitted to the experimental MC data using the REG procedure in SAS (Freund and Littell 1991):

$$MC = 26.785 + 0.428C \qquad (3)$$
$$(R^2 = 0.88)$$

where MC was %wb and C was the PEO concentration in the film as %w/w of protein

Tensile Strength and Elongation

Film TS decreased with increasing amounts of PEO (Fig. 2). This was, at least, partially explained by the film MC data. As mentioned earlier, for films stored at conditions similar to those of film conditioning before tensile testing, MC increased with increasing amounts of PEO in the film. Water is known to have a plasticizing (weakening) effect on hydrophilic (protein) films (Gennadios et al 1993b, Gontard et al 1993).

Mean TS values ranged from 3.9 MPa for the 0% PEO film to 1.4 MPa for the 40% PEO film. The following exponential decay model was satisfactorily fitted ($r = 0.974$ between actual and predicted values) to experimental data using the NLIN procedure in SAS (Freund and Littell 1991):

$$TS = 3.786e^{-0.03C} \qquad (4)$$

where TS was in MPa and C was the PEO concentration in the film at %w/w of protein.

Mean values of E, a measure of a film's extensibility, for the different types of films are presented in Table I. Films containing 5–40% PEO had greater ($P < 0.05$) E by $68.7 \pm 13.2\%$, on average, than did the control SPI films (containing no PEO). However, no significant differences ($P > 0.05$) were detected among mean E values for 5, 10, 20, and 40% PEO films. The high E values for PEO-containing films was attributed partially to their higher MC, because water plasticizes hydrophilic films and improves film extensibility (Gennadios et al 1993b, Gontard et al 1993).

Park et al (1993) reported decreases in TS and E values with incorporation of increasing amounts of corn zein into extrusion-blown polyethylene films. Similar results were reported by Ghorpade and Hanna (1993) for soy protein isolate/polyethylene films. Efforts have been made to incorporate proteins into plastic materials to enhance degradation. The poor mechanical properties of such materials were partially attributed to lack of cross-linking between polymers and proteins. Lack of cross-linking between SPI and PEO in films could have contributed to the decrease of film TS with increasing amounts of PEO in the present study.

Water Vapor Permeability

WVP values, along with calculated actual rh conditions at the underside of films during testing of control and PEO-containing films, are reported in Table II. An increase in WVP was observed with PEO addition. Differences among means were significant at 90% level of confidence. It also was observed that the calculated

TABLE II
Water Vapor Permeability (WVP) and Calculated Actual Relative Humidity (rh) at Film Undersides for Soy Protein Isolate and Poly(ethylene Oxide) (PEO) Films

PEO Concentration (%w/w of protein)	WVP[a] ($\times 10^{-9}$ g·m/m²·sec·Pa)	rh Inside Cup[b] (%)
0	3.0±0.20	69.3±0.3
5	3.2±0.14	69.0±0.8
10	3.2±0.17	67.9±0.8
20	3.6±0.50	67.0±1.2
40	4.0±0.53	65.9±1.1

[a] Values are means of four replicates ± standard deviation.
[b] Actual rh values (means of four replicates ± standard deviation) at the underside of films calculated as described by Gennadios et al (1994b) to account for resistance of stagnant air layer between film and water surface in testing cups. rh outside cups was 50%.

TABLE III
HunterLab Color Values (L, a, and b) and Total Color Difference (ΔE) for Soy Protein Isolate and Poly(ethylene Oxide) Films[a,b]

Polymer[c]	L	a	b	ΔE
0	92.61±0.47a	−3.08±0.18a	16.93±0.46a	15.91±0.49a
5	92.83±0.31ab	−3.12±0.10ab	16.88±0.50a	15.73±0.50a
10	92.64±0.28a	−3.20±0.08b	17.86±0.51b	16.73±0.51b
20	93.16±0.15b	−3.44±0.03c	17.18±0.11a	16.00±0.12a
40	94.26±0.15c	−3.00±0.06a	14.14±0.46c	12.77±0.47c

[a] Film color was measured after placing films on a standard white plate with HunterLab color values of $L = 96.86$, $a = -0.02$, and $b = 1.99$.
[b] Values are means of four replicates ± standard deviation. Any two means in the same column followed by the same letter are not significantly different ($P > 0.05$) according to Duncan's multiple range test.
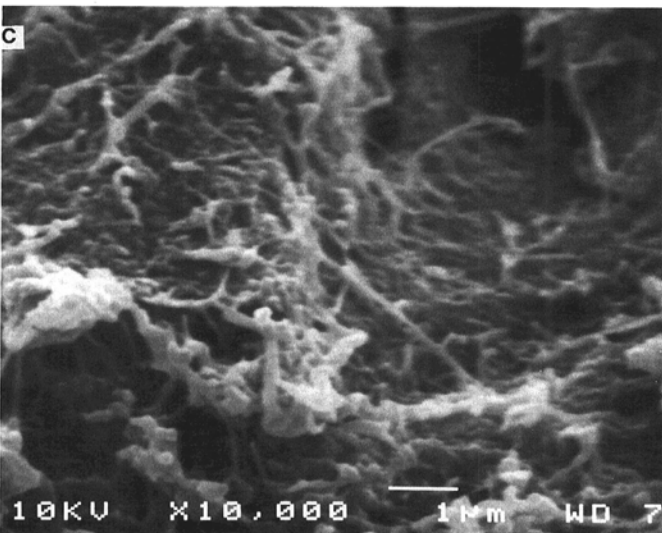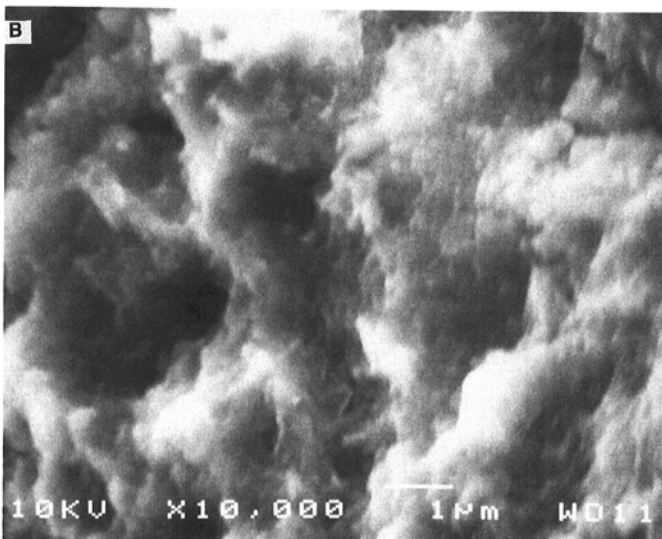[c] Values indicate poly(ethylene oxide) content in film (%w/w of protein).
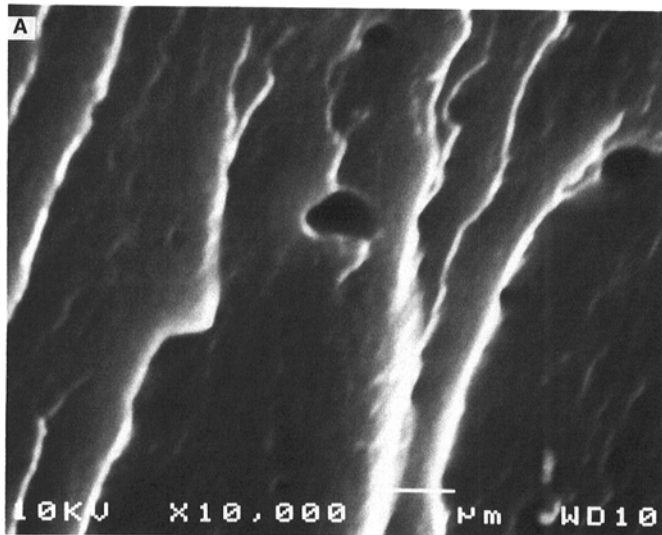
Fig. 3. Scanning electron micrographs of soy protein isolate and poly(ethylene oxide) films with poly(ethylene oxide) concentrations of 0% (**A**), 20% (**B**), and 40% (**C**) w/w of protein.

ences in WVP values of SPI-PEO films would have been greater under equal rh gradient conditions across the films. The increase of WVP values with increasing amounts of PEO in the films was attributed to the hydrophilicity of PEO.

Color

Film color can be an important factor in terms of consumer acceptance of both edible and inedible films. The *L, a,* and *b* HunterLab color values and total color difference (ΔE) for the different types of films are reported in Table III. The main observed difference was that films with higher concentrations of PEO had a lighter color ($P < 0.05$) as indicated by increased *L* values. The mean $+b$ (yellowness) values for films showed an increase from 16.93 for the control film to 17.86 for the 10% PEO film but dropped to 14.14 for the 40% PEO film. Similar changes were observed in *a* and ΔE values as film color measurements may be affected by film thickness. Our experience with the control films was that thicker films appeared more yellowish (higher $+b$ values).

Scanning Electron Microscopy

In an attempt to study microstructural changes in the films, SEM was used to visualize the surface of films. Film surfaces did not show any significant changes. A sharp blade was used to cut films to allow film cross sections to be visualized. The cut edges of the film cross sections showed no changes in film texture between film types due to sharp cut. Subsequently, films were broken in pieces by bending them and observing the broken edges under SEM. Figure 3 shows micrographs for control (A), 20% PEO (B), and 40% PEO (C) samples; B and C show significant differences as compared to A. Use of 40% PEO resulted in inferior texture, which explained the decreases in film TS values. Inferior texture in this case was formation of granular or coarse matrix inside the film than control films.

Irradiation Effect

Table I presents mean TS and E values for each type of film following prescribed levels of irradiation dosage. These data were statistically analyzed using the GLM procedure in SAS (Littell et al 1991). Significant mean values were compared using Duncan's multiple range test. E values were not substantially affected by irradiation for each type of film. Irradiation also did not affect the TS of the control SPI films. A significant ($P < 0.05$) increase was noticed in the TS of PEO-containing films due to irradiation (Table I).

CONCLUSIONS

TS and E of SPI-PEO films were affected by the amount of PEO in the films. TS decreased and E increased with increasing amounts of PEO. Film lightness (*L* values) increased with increasing amounts of PEO concentration, most likely due to the dilution effects of lightly colored PEO. Micrographs showed inferior texture formation at higher levels of PEO additions. Gamma irradiation of PEO-containing films at levels of 5–30 kGy resulted in small but significant increases in film TS.

ACKNOWLEDGMENTS

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actual rh at the undersides of films, and therefore the rh gradients applied across film specimens during testing, were not equal for all types of films. Consequently, the expected observed differ-

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