Radiation-Absorbing Hydrogel–Melanin Blends for Ocular Devices

TRAIAN V. CHIRILA,*[,1] RICHARD L. COOPER,[1] IAN J. CONSTABLE,[1] and ROBERT HORNE[2]

[1]Department of Biomaterials, Lions Eye Institute, Nedlands, 6009, Western Australia, Australia and [2]Electron Microscopy Unit, Department of Pathology, Royal Perth Hospital, Perth, 6000, Western Australia, Australia

SYNOPSIS

Hydrophilic polymers and copolymers of 2-hydroxyethyl methacrylate, with low or high crosslinking density, were synthesized and then treated in aqueous medium with epinephrine (adrenaline) at neutral or acid pH, at room temperature, and in the presence of oxygen and light. During this treatment, a melanin is formed and uniformly dispersed in polymers. The resulting slightly colored hydrogels display radiation-absorbing properties in the ultraviolet and visible regions of the natural spectrum. This enhances significantly their value as materials for ocular devices (contact lenses, intraocular lenses) that should protect the retina of the patients without their natural lens from potential damage induced by ultraviolet and visible (violet and blue) radiation. The incorporation of common ultraviolet absorbers led to transmittances similar to that of the natural human lens, i.e., 30% or less at 450 nm, 40% or less at 500 nm, and no more than 50% at 700 nm. The two-phase morphology of the melanized hydrogels, as investigated by TEM, revealed a very fine structure comprising melanin domains of 1 to 2 nm in size. Although no proof for a network interpenetration could be provided, it is believed that the novel blends are true sequential interpenetrating polymer networks.

INTRODUCTION

For practical applications in ocular devices, i.e., aphakic contact lenses and artificial intraocular lenses for patients deprived of their natural lens, the acrylic hydrogels—essentially crosslinked polymers of 2-hydroxyethyl methacrylate (HEMA)—should ideally bring together a set of indispensable properties (biocompatibility, chemical stability, adequate mechanical properties, transparency and good optical resolution, and low specific weight) with radiation-absorbing characteristics similar to those of the natural intraocular lens.

Most of the intraocular lenses currently available for implantation are manufactured from poly (methyl methacrylate). Since its introduction as a material for intraocular lenses,[1] poly(methyl methacrylate) proved to be an ideal choice, if no notice is taken of the lack of flexibility and wettability. The acrylic hydrogels, however, possess these additional properties, which explains their success as materials for soft contact lenses. Although the concept of a soft and hydrophilic intraocular lens is as old as that of the soft contact lens,[2] the former was implemented only in 1977 when Epstein[3] designed and used a one-piece intraocular lens made of poly(2-hydroxyethyl methacrylate) (PHEMA). After some promising clinical results obtained by other investigators using PHEMA implants,[4–6] a commercial soft intraocular lens was eventually developed.[7,8]

There is an increasing body of experimental evidence[9–11] that long-wavelength (300–400 nm) ultraviolet (UV) and short-wavelength (400–500 nm) visible regions of the solar radiation are harmful to the human retina, even at relatively low level of daily exposure, through photochemical processes that are not completely understood. However, the natural lens protects the retina by absorbing most of the UV radiation between 300 and 400 nm. The absorption is enhanced and extends over the whole visible region as the lens grows older.[12–14] Therefore, any

* To whom correspondence should be addressed.

Journal of Applied Polymer Science, Vol. 44, 593–604 (1992)
 CCC 0021-8995/92/040593-12$04.00

material for ocular devices intended to act as substitutes for the natural lens should duplicate its radiation-absorptive characteristics.

Attempts to impart UV-absorbing properties to the polymers used for the manufacture of contact lenses and intraocular lenses are not new. Most of the poly(methyl methacrylate) intraocular lenses available now for implantation absorb UV radiation;[15-17] the highest cutoff wavelength reported is 400 nm. However, there is no hard intraocular lens available with absorption in the visible region of the solar radiation. The only currently available hydrogel intraocular lens does not have absorbing properties from 300 nm onward. Some manufacturers now claim UV-absorbing hydrogel contact lenses but no other details have been disclosed.

In order to obtain UV-absorbing polymers, compounds containing chromophores are incorporated either by physical mixing or by chemical bonding, e.g., copolymerization. Since the possible release of the absorber into the biological surroundings must be avoided, the latter procedure is preferred. Currently, two classes of compounds are used as UV absorbers in polymers for ocular devices: 2-hydroxybenzophenones and 2-(2′-hydroxyphenyl)-2*H*-benzotriazoles. There is a tendency to use predominantly benzotriazoles because they have higher molar extinction coefficients and steeper absorption edges toward 400 nm when compared with benzophenones.

Little work has been done on visible-radiation-absorbing ocular devices, notwithstanding the fact that the harmful effects of these radiations (especially 400–500 nm, known as the "blue light hazard" region) to the retina are better documented than those of the UV radiation. A few proposals for an "ideal" artificial intraocular lens have been advanced,[11,18,19] but never implemented.

During recent years some work has been done in this laboratory on UV-absorbing PHEMA.[20,21] Incorporation of the UV absorbers by chemical bonding appears to be the only suitable procedure for hydrogels: since the latter are highly water-permeable materials, the leaching of unbound molecules can occur without restraint.

As reported here, we have further developed HEMA-based hydrogels that absorb not only UV radiation but also a significant amount of the visible radiation. According to our procedure, hydrogels can be produced that duplicate in great detail the absorbing properties of the natural lens of the eye. The procedure is based on the incorporation of melanins into the tridimensional network of the hydrogels.

Melanins are biopolymers responsible for the black and some of the brown, red, and yellow pigmentation in animals and plants. The melanins that appear in man and other higher organisms were termed eumelanins and phaeomelanins. They are biosynthesized in specialized cells (melanocytes) from tyrosine and probably other 4-hydroxyphenylethylamines or β-(3,4-dihydroxyphenyl)ethylamines (catecholamines) in the presence of an enzyme (tyrosinase) and oxygen.

Melanins display a series of truly unique properties.[22-24] Thus, they are irregular polymers containing a variety of "mers," even when the precursor has been a single, definite compound. The mers are linked randomly through more than one type of bond. Any sample of melanin is a mixture of macromolecular species that differ not only in molecular weight but also in composition and structure. Finally, slight variations in the synthetic conditions induce marked structural changes. Another characteristic of melanins is their outstanding stability. For instance, melanin has been found in fossils about 180 million years old. Temperatures around 600°C do not cause significant degradation in melanins. Strong oxidizing agents and conditions are needed to degrade them into smaller fragments for analytical purposes. There is no enzyme known to depolymerize melanins. They are also virtually insoluble in all common solvents. A most interesting fact is that melanins can be synthesized in vitro in nonenzymic conditions from precursors such as catecholamines. (The in vitro synthesis of melanins from 4-hydroxyphenylethylamines still requires the presence of an enzyme.) Melanins have also the remarkable property to absorb strongly the UV and visible radiations. The absorption above 400 nm is smooth, displaying structureless bands, and decreases uniformly with wavelength.

The present work is concerned with the potential advantages brought about by some of the characteristics of melanins for the production of radiation-absorbing materials for aphakic ocular devices. We have found that the artificial melanogenesis within the matrix of water-swollen HEMA-based hydrogels results in hydrogels that absorb UV and visible radiation due to the melaninlike pigments entrapped in their network. The pigments do not leach out even after prolonged storage in water. 4-[1-Hydroxy-2-(methylamino)ethyl]-1,2-benzenediol [Fig. 1(a)], known also as adrenaline or epinephrine, was used as a precursor for melanin. In aqueous solution, within a large pH range, adrenaline undergoes successive oxidation and polymerization to a melaninlike pigment[25,26] via 2,3-dihydro-3-hydroxy-1-

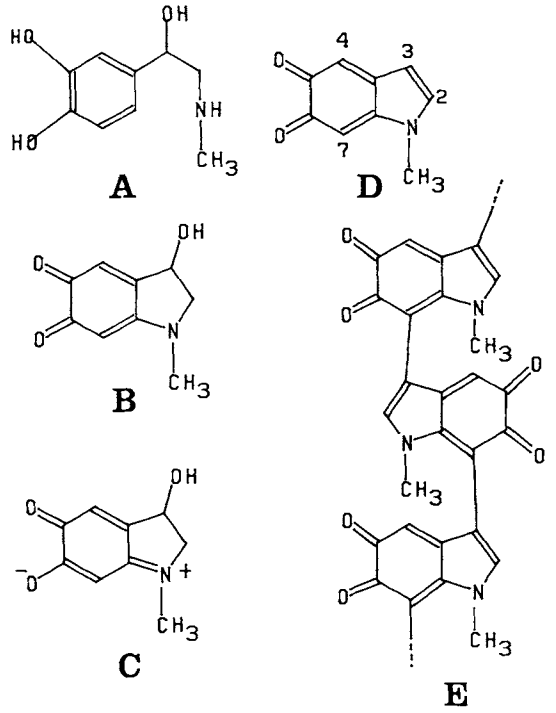

Figure 1 (a) Epinephrine. (b, c) Two possible structures for adrenochrome. (d) Indole-5,6-quinone monomer. (e) Simplified structure of adrenochrome-melanin.

methyl-1*H*-indole-5,6-dione, known also as adrenochrome, for which both *o*-quinonoid [Fig. 1(b)] and zwitterionic *p*-quinonoid [Fig. 1(c)] structures were proposed. Subsequent rearrangement and further oxidation lead to an indole-5,6-quinone [Fig. 1(d)], which through its highly reactive positions 4,7 and 2,3 polymerizes to give melanin. The product of epinephrine autoxidation is eventually an indole-type melanin, not known definitely to occur in nature, with a hypothetical structure shown in Figure 1(e). Although correct in principle, the latter is obviously an oversimplification. Repeating units such as 5,6-dihydroxyindoles, catecholamines, aminochromes, and pyrrol derivatives, various modes of binding between the units, branching and crosslinking, and carboxyl, carbonyl, and amino groups are also assumed in the indole-type melanins.[23,24]

The present study also attempts to include the novel materials within one of the known multicomponent polymer systems.

EXPERIMENTAL

Materials

2-Hydroxyethyl methacrylate (HEMA) was supplied by Ubichem Ltd., UK, with a purity of 99.8% w/w, an inhibitor (4-methoxyphenol) content of 18 ppm and a residual ethylene dimethacrylate content of 0.03% w/w. 2-Ethoxyethyl methacrylate (EEMA) was supplied by ICI with a purity of 99.4% w/w. Prior to its use as a monomer, EEMA was distilled under vacuum (5 mbar), using a 65-cm long Vigreux column, to remove most of the inhibitor [(2,4-dimethyl-6-*tert*-butyl)phenol].

As a crosslinking agent, ethylene dimethacrylate (EDMA), supplied by Tokyo Kasei Kogyo Co. with a purity of 97% w/w was used. As an initiator, 2,2-azo-*bis*-(2,4-dimethyl valeronitrile) supplied by Wako Chemical Industries Ltd., Japan, with a purity of 99.2% w/w was used.

Two different compounds were used as polymerizable UV absorbers. 4-(2′-Acryloyloxyethoxy)-2-hydroxybenzophenone (ABP) was supplied by American Cyanamid Co., and 2-[2-hydroxy-4-methacryloyloxy(2′-hydroxy-3′-propoxy)phenyl]-5-chloro-2*H*-benzotriazole (MBTR) was supplied by Tokyo Kasei Kogyo Co., with a purity of 96.6% w/w.

(±)Epinephrine (adrenaline) was supplied as a free base by Sigma Chemicals Co. with a purity of 95% w/w. A pharmaceutical solution of epinephrine hydrochloride (Epifrin, supplied by Allergan Pharmaceuticals Inc.) was also used in the melanization experiments; it has a concentration of 1% w/w in water, pH 3, and is stabilized against oxidation by added sodium metabisulfite and disodium ethylenediaminetetraacetate.

Polymerization

PHEMA and a copolymer of HEMA and EEMA were prepared in the shape of cylindric buttons in a molding unit designed in this laboratory. In an illustrative example, 0.5% w/w EDMA (crosslinking agent) and 0.1% w/w initiator were added to HEMA in a capped glass bottle. The mixture was homogenized in a shaker, then subjected to ultrasound until complete dissolution of the initiator, and finally a stream of high-purity nitrogen was bubbled through the liquid for 10 min. The homogeneous mixture was equally (2.5-mL portions) distributed into polypropylene molds fitted in the cavities of a two-piece molding unit. The unit was closed and sealed, and vacuum was created with a pump by evacuating the air for 5 min. High-purity nitrogen was then admitted through an inlet valve until the negative pressure was counterbalanced, and a slight overpressure was created. The molding unit was then placed in a water bath in which the temperature was controlled. A temperature program was run in the bath, consisting of six steps: It started at 30°C and

finished at 45°C, where the temperature was maintained stationary for 40 h. The total duration of the cycle was 50 h. On completion of the cycle, PHEMA buttons were removed from molds and cured for 18 h at 110°C in an oven.

Rectangular slabs (8 × 14 mm) and disks (14-mm diameter) with a thickness about 1 mm were cut by a diamond-tool lathe from the buttons and polished for further experiments.

Highly crosslinked PHEMA (5% w/w EDMA in the initial mixture) and copolymers of HEMA with 20% w/w EEMA were also synthesized by the same procedure. In their unhydrated state, all polymers were glassy and clear materials. When immersed in water, they swelled and became flexible gels. At equilibrium, the water uptake was about 38% w/w in the slightly crosslinked PHEMA, 29% w/w in the highly crosslinked PHEMA, and 29% w/w in the slightly crosslinked 80/20 w/w poly(HEMA-co-EEMA).

PHEMA containing bound UV absorbers, either ABP or MBTR, were also produced. To avoid the microphase separation of water during hydration, due to the presence of an increasing number of hydrophobic benzophenone or benzotriazole moieties in the polymer, which results in partial opacification, no more than 2% w/w of these absorbers were incorporated in polymers.

Melanization

Although epinephrine can be oxidized in aqueous medium over a wide pH range (3–12), not all of this range is suitable from a biomedical point of view. As the materials produced will be in direct contact with the eye tissue, either as contact lenses or as intraocular lenses, it is essential that no leachable deleterious compounds should be present in the materials. At high pH as provided by alkaline hydroxides, epinephrine dissolved instantly, and the formation of melanin was very fast. Since adrenochrome-melanin, like many other melanins, is soluble in alkaline hydroxides, its steady release from the hydrogels matrix would be expected. We have indeed noticed pigment release from these samples even after 9 months of storage in distilled water, despite frequent water exchange, that was interpreted as an indication of the presence of alkaline hydroxides. Concomitantly, the color of the hydrogel specimens faded gradually. Similar observations were made after using other alkaline agents to achieve a high pH, e.g., carbonates or phosphates. The melanization of hydrogels in alkaline media is therefore not recommended because the presence of dissolved pigment and/or alkaline agent cannot be avoided.

Consequently, we have used only acid and neutral epinephrine solutions in the experiments reported here. By immersing the polymer specimens in a solution of epinephrine and maintaining them in this medium for hours or weeks, during which the melanin formation progressed, they became colored (yellow to light brown) and acquired absorbing properties of both UV and visible radiations.

Besides solutions of epinephrine (0.01–5% w/w) freshly prepared prior to melanization experiments, we also used Epifrin, a commercial product that is in fact a 1% w/w aqueous epinephrine hydrochloride solution containing antioxidants. Due to a slower melanization rate, with a significant lag time induced by the presence of antioxidants, a better control of the process was possible. This also offered the chance to investigate properly the influence of the duration upon the radiation-absorbing properties of the melanized hydrogels. The methodology used in this study is described by two illustrative examples.

PHEMA containing 1% w/w ABP as a bound UV-absorbing agent was prepared as described in a previous section. A disk was cut from a button, finely polished, washed in hexane, dried in a vacuum oven, and finally hydrated for 2 days in distilled water. Two grams of epinephrine were dissolved in 11.5 mL 3.6% aqueous hydrochloric acid. The solution was neutralized with 1% ammonium hydroxide against litmus paper. After adding from a burette about 35 mL hydroxide, the neutralization was stopped and a pH 6.5 was measured. Within seconds, the solution became red. A polymer disk was immediately immersed in an open glass beaker containing 5 mL of neutralized solution and placed in a well-lit position. The polymer was kept in the solution for 3 days, then removed, and placed in distilled water.

In another experiment a hydrated specimen of PHEMA was immersed in 5 mL Epifrin, in an open glass beaker. Within 2–3 days, the solution became pink. After 8 days the color was dark red, and a few days later a sediment of black melanin particles was noticed.

It is important that the melanization process itself be completed so that little or no low molecular weight intermediates are present in the hydrogel. If released into the eye, these compounds may cause irritation and probably a toxic reaction. Therefore, the melanized hydrogels should be extensively washed in water. After melanization the hydrogels were rinsed with copious amounts of water, then stirred for four 2-h periods with fresh water. Addi-

tional 8–10 days of storage in distilled water, with 2–4 daily water exchanges, usually removed all absorbing intermediates as monitored by the absorption spectra of the aqueous media removed after storage.

Radiation Transmission Measurements

The transmittance versus wavelength curves of various polymers were recorded using an Ultrospec II-4050 (LKB Biochem Ltd., UK) ultraviolet/visible spectrophotometer. Prior to spectra recording, the polymer slabs were clamped in a holder designed for this purpose, which was fixed in the spectrophotometer.

Electron Microscopy

In order to obtain some preliminary information on melanized hydrogels as bicomponent polymer systems, we used transmission electron microscopy (TEM) of ultrathin polymer sections.[27-29]

From each button of polymer a cylindric core (1 mm in diameter) was cut using a diamond-tool lathe. The cores were washed in hexane, then dried in a vacuum oven for 8 h at 60°C and 230 mbar vacuum. Prior to TEM, each core was stored in a sealed vial.

Sections of 100 ± 10 nm thickness were cut from the polymer cores using an LKB 8800 Ultrotome III (LKB-Produkter AB, Sweden) ultramicrotome equipped with a diamond knife (Diatome Ltd., Switzerland). Absolute ethanol (BDH Chemicals, UK) was used as a bath fluid. The sections were transferred to a glass dish containing absolute ethanol, where they were teased flat. They were then placed onto uncoated 200-mesh copper grids and allowed to dry. The grids were viewed and photographed in a Philips 410 transmission electron microscope at an acceleration voltage of 80 kV.

Melanin is an electron-dense material, therefore no staining should be necessary. To check this assertion, some polymer samples were stained with osmium tetroxide prior to TEM. There was no difference between the electron micrographs of stained and unstained sections.

RESULTS AND DISCUSSION

Melanization and Transmission of Radiation

The conditions for melanization influenced significantly the radiation-transmitting properties of the end products. For instance, a higher initial concentration of epinephrine resulted in a faster rate of pigmentation, i.e., a certain desired transmittance was achieved much faster in 1% than in 0.05% w/w epinephrine; however, between two closer values such as 1 and 0.3% w/w, no difference in the process rate and final transmittance was noticeable.

The influence of duration on the transmission of radiation was difficult to study in solutions of epinephrine with no antioxidant additives, since the oxidative polymerization process virtually began as soon as epinephrine was added to the solution. Therefore, this dependence was studied in Epifrin, a commercially available epinephrine hydrochloride solution with added antioxidants. In Figure 2 the decrease of transmittance at selected wavelengths in near UV and visible (violet and blue) regions was represented as a function of the duration of melanogenesis. The drop in transmission was significant at lower wavelengths, and much attenuated at higher wavelengths.

At the thickness used in our experiments, even with the largest polymer specimens (17 mm diameter, 12 mm thickness), there was no difference in transmission whether the samples were fully hydrated or dried prior to melanization in identical conditions of concentration, pH, and temperature. With dried polymers, the minimum duration of the process was determined by the time required for the specimens to become fully swollen. At that stage melanogenesis was quite advanced, which could be noticed from the color of the polymer.

The influence of temperature on melanization was only marginally investigated and further research is needed for a comprehensive report. As expected, the higher the temperature, the faster the melanization process and the darker the pigmentation of final products.

Following their melanization, all polymers acquired absorbing properties that extended over UV and visible regions of the electromagnetic radiation. Shown in Figure 3(a) is the transmission spectrum of untreated PHEMA. A specimen of same size treated in Epifrin for 10 days displayed a spectrum shown in Figure 3(b). After this sample was stored in water for 28 days with daily water exchanges, its spectrum underwent some modifications [Fig. 3(c)]: transmittance was higher below 300 nm, but dropped at higher wavelengths. The phenomenon of attenuation of absorption in the middle UV and its enhancement in the near UV and visible regions as a results of prolonged storage and washing in water was noticed in most of our experiments. We presume that some low molecular weight intermediate compounds, which supposedly absorb strongly in the

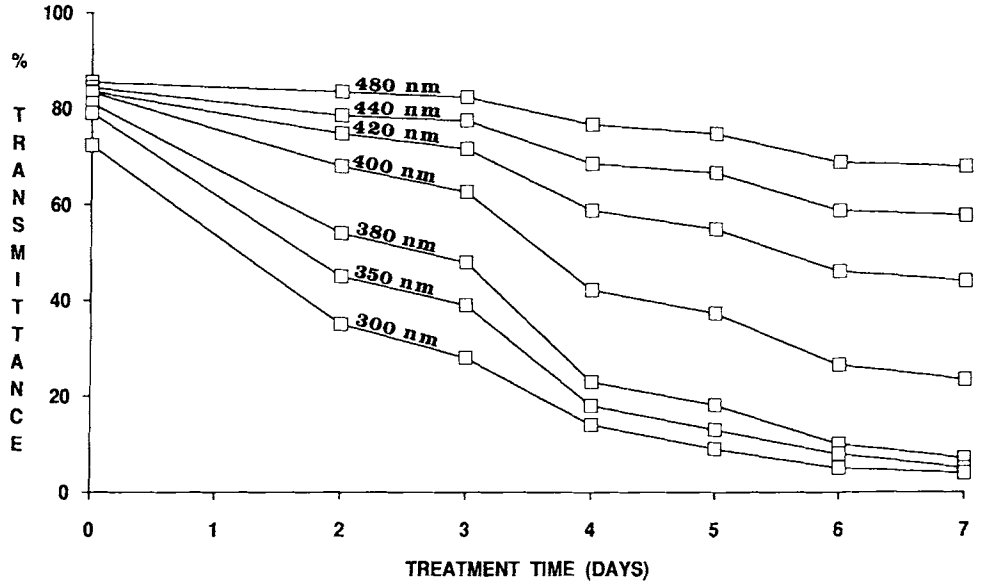

Figure 2 Transmission of melanized PHEMA at various wavelengths as a function of the duration of melanization process. Each polymer specimen (14 mm diameter, 4 mm thickness) was melanized in 5 mL Epifrin at pH 3 and room temperature. Spectra were recorded on polished, hexane-washed, and vacuum dried 0.8-mm thick specimens. Data for day 0 were taken from the spectrum of untreated PHEMA.

middle UV, were extracted in water; concomitantly, the melanogenesis advanced further, increasing absorption at longer wavelengths.

In Figure 3(d) the transmission spectrum of an identical PHEMA sample is shown; this specimen was treated in Epifrin for 45 days. Clearly, an increasing duration of the process leads to substantial enhancement of absorption.

When a UV-absorbing agent was incorporated by copolymerization in PHEMA, the transmission in

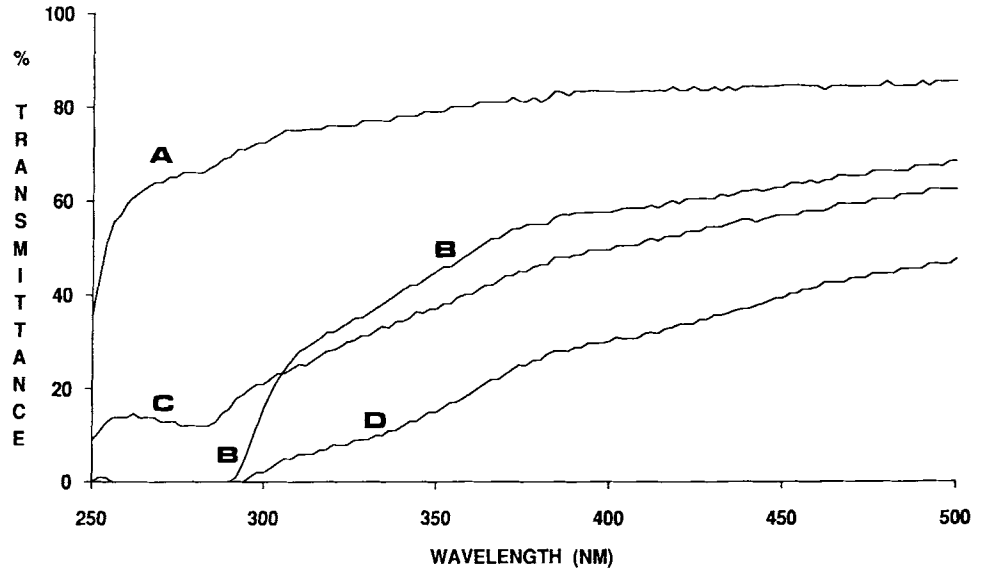

Figure 3 Transmission spectra of (a) untreated PHEMA and of a melanized PHEMA sample treated for 10 days, (b) before and (c) after a 28-day period of storage/washing in water. Further decrease in transmission is shown in the spectrum of a sample treated for (d) 45 days. Melanization was performed in 3 mL Epifrin at pH 3 and room temperature. Spectra were recorded on polished and hydrated 0.9-mm thick specimens.

the UV region was dictated by the presence of this agent. There was no enhancement of transmission in this region even after extensive washing in water. Shown in Figure 4 is the spectrum of a hydrated PHEMA sample containing 1% ABP and treated in neutralized epinephrine. There is little doubt that the cutoff wavelength at 376 nm is due to the presence of ABP, but it is the melanin that is responsible for the low transmittance at wavelengths above 400 nm.

In an attempt to obtain hydrogels with even better absorbing properties, we incorporated in PHEMA an absorber of the benzotriazole type, MBTR. After hydration, a specimen containing 2% w/w MBTR was treated in Epifrin and then washed. Its transmission spectrum is shown in Figure 5(a) and compared with the spectra[17] of a commercial nonabsorbing PMMA intraocular lens (Cilco SK21, Cooper Vision) [Fig. 5(b)] and of a commercial UV-absorbing intraocular lens (IOLAB, U706S) [Fig. 5(c)]. While our material clearly surpassed the nonabsorbing lens, the IOLAB lens displayed a better protection but only over a small domain at the end of the near UV. However, at higher wavelengths, in the violet and blue regions of light, the melanized hydrogel was distinctly superior to the commercial lens in terms of protection offered to the retina. When compared to the natural human lens, by employing the spectra reported[14] for a lens 25 years [Fig. 6(b)] and, respectively, 54 years old [Fig. 6(c)], our material [Fig. 6(a)] showed similar radiation-absorbing properties that definitely suggest its usefulness as a substitute for the natural lens.

Phase Morphology of Melanized Hydrogels

Phase separation is a phenomenon that occurs generally when two kinds of macromolecules are mixed. If we accept that melanins are true polymers with a certain level of crosslinking, then the problem of defining the two-phase hydrogel–melanin system becomes an important issue.

In spite of their relatively simple preparation, we found it somewhat difficult to classify correctly the melanized hydrogels within one of the known and well-defined classes of multicomponent polymer materials such as blends, grafts, blocks, or interpenetrating polymer networks (IPNs). Therefore, cautiously we regarded them so far as blends. However, it appears that melanized hydrogels are more than this. The blends do not have by definition any kind of bonding between their components, and the methods for their preparation are different from that employed to prepare melanized hydrogels. Indeed, chemical bonding can hardly be surmised between hydrogels and melanins. On further consideration the hydrogel–melanin "blends" comply formally with the accepted definition[30–32] of IPNs, i.e., (a) one polymer (melanin) is synthesized in the immediate presence of the other (hydrogel); (b) at

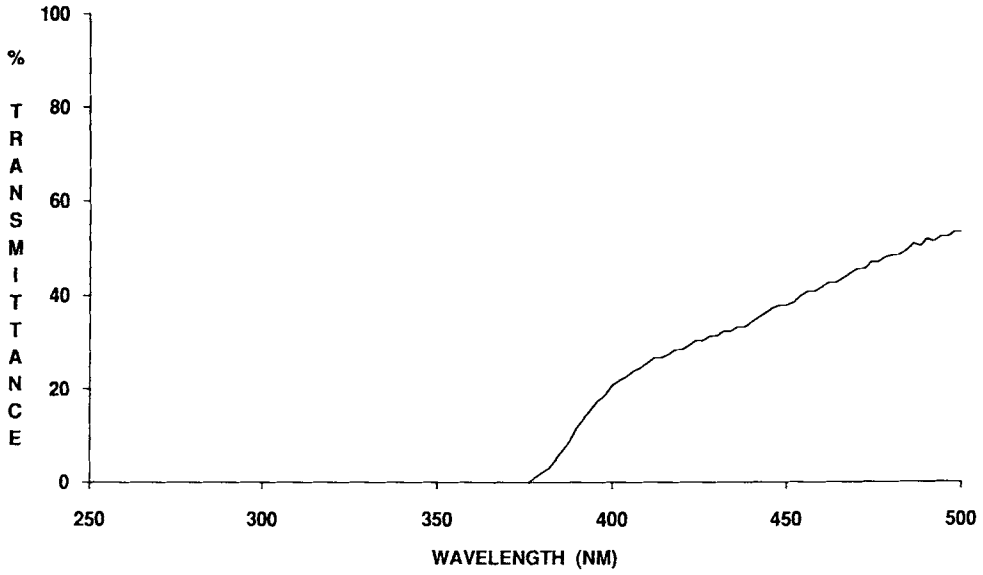

Figure 4 Transmission spectrum of melanized PHEMA containing 1% w/w ABP as a bound UV absorber. The 1-mm thick specimen was treated in neutral 4% epinephrine (pH 6.5) for 3 days at room temperature and water-washed for 6 days. Spectrum was recorded using a hydrated specimen.

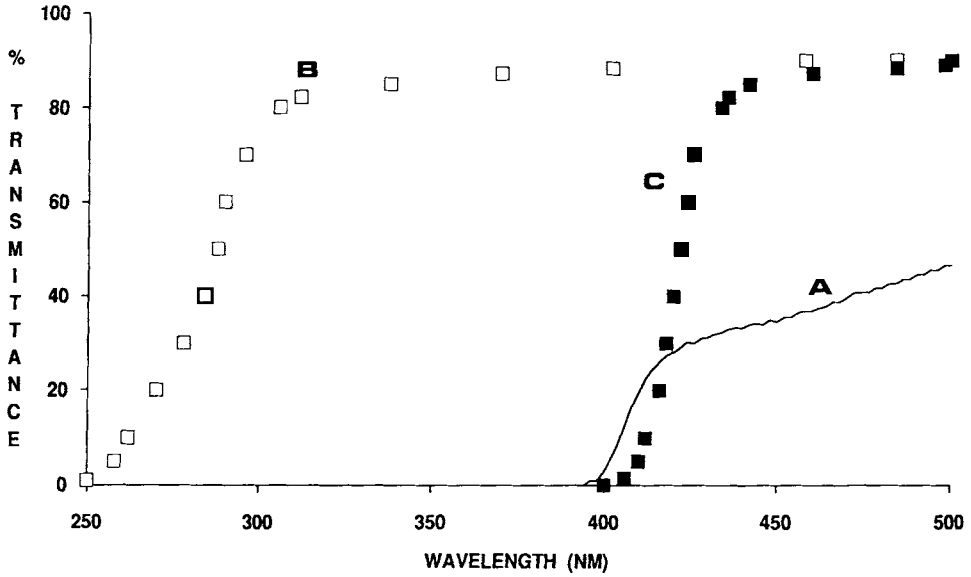

Figure 5 Transmission spectrum of melanized PHEMA containing 2% w/w MBT as an (a) bound UV absorber compared with spectra of (b) nonabsorbing and of (c) UV-absorbing PMMA intraocular lenses. Data for commercial lenses were taken from literature.[17] The 1.1-mm thick, extensively washed, hydrogel specimen was melanized in 10 mL Epifrin for 7 days at pH 3 and room temperature. Spectrum was recorded using a hydrated specimen.

least one polymer is crosslinked; (c) there is probably no covalent bonding, including grafting, between the two polymers; and (d) if there are accidental grafts, the crosslinks in each polymer outnumber them. Moreover, taking into detailed consideration the synthetic procedure employed to obtain the melanized hydrogels presently reported, they can be regarded as sequential IPNs in which polymer I is an acrylic hydrogel and monomer II is epinephrine dissolved in water. However, there are some aspects that do not fall into line with this description. Thus, the real monomer II is not epi-

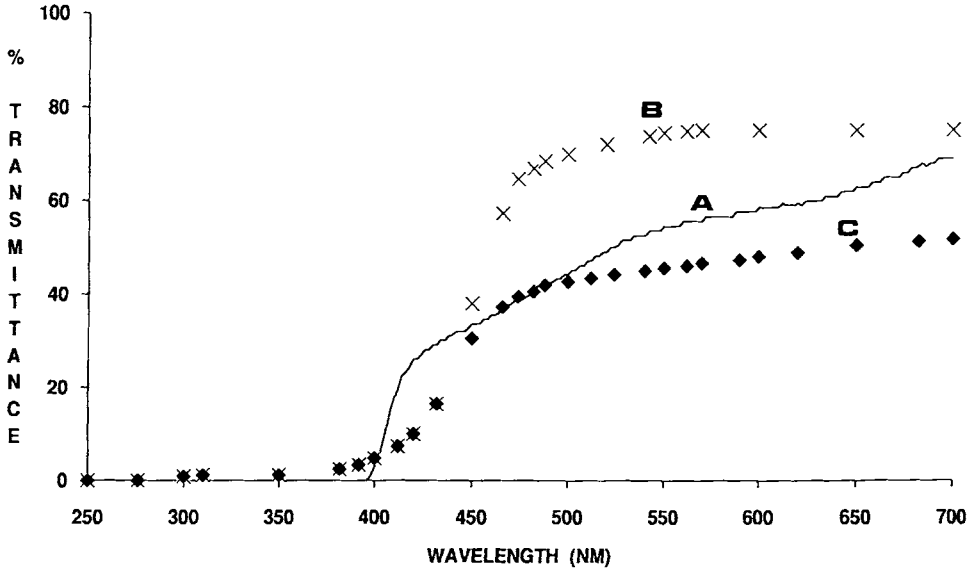

Figure 6 A comparison between the (a) transmission spectrum of the melanized hydrogel sample of Figure 5 and spectra of natural human lens at (b) age 25 and (c) age 54.[14]

nephrine, but a reactive indolequinone resulting from oxidation and rearrangement processes prior to polymerization. Also, usually when there is no lag time induced by added antioxidants, the polymerization of monomer II takes place simultaneously with its diffusion into the network of polymer I. In an ideal sequential IPN the polymerization of monomer II should commence after the polymer I was swollen to an equilibrium by monomer II or by a solution containing this monomer. Finally, since it is not clearly known how and to what extent the crosslinking does occur in melanins (polymer II), little can be assumed about the existence of permanent entanglements or true network interpenetration, which—at least in the limiting case of high compatibility between two crosslinked polymers—are salient features in IPNs. There is no doubt that melanins are crosslinked polymers; however, it is difficult to assent to the existence of catenation in mixtures of melanins with other polymers.

Against this background of a rather scholastic incertitude, we have used TEM to investigate the two-phase morphological features of melanized hydrogels. The micrograph in Figure 7 shows the microstructure of the melanized, slightly crosslinked PHEMA. There is no difference between this micrograph and those of highly crosslinked PHEMA (Fig. 8) or of 80/20 w/w poly(HEMA-co-EEMA) (Fig. 9), both melanized. All micrographs revealed the same very fine structure, apparently not influenced by the crosslinking density or by the nature of the acrylic component (polymer I). This morphology is characteristic of a nucleation and growth mechanism for the phase separation.[32] A similar morphology, with domains less than 10 nm in size, was found[33] in sequential IPNs of 72.2/27.8 w/w

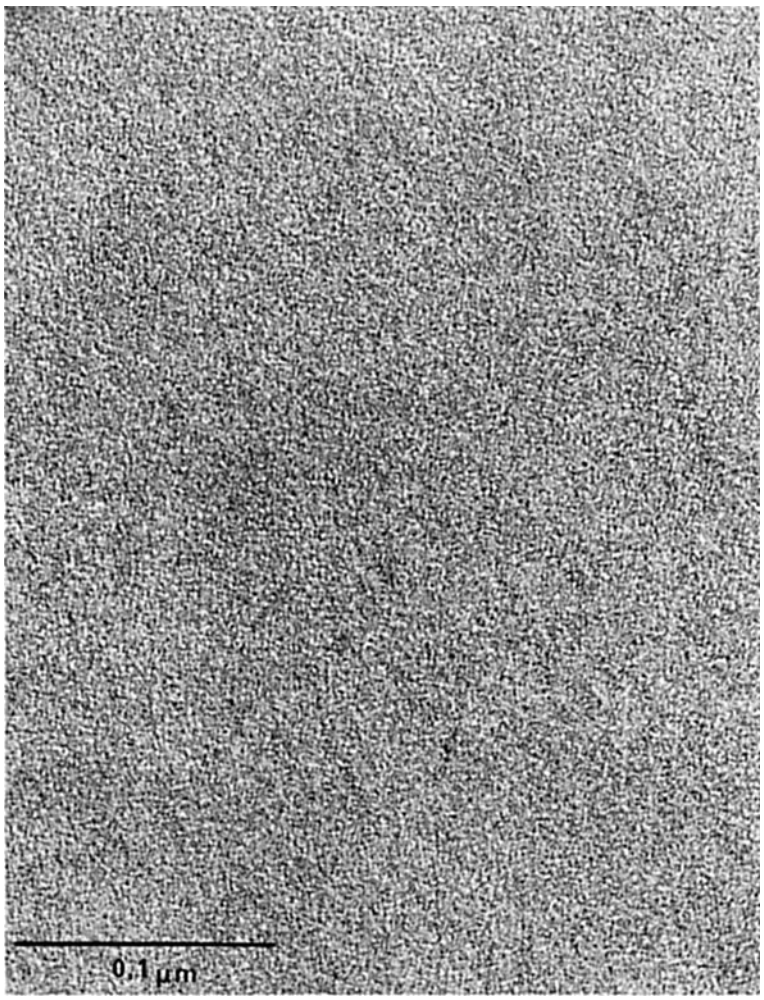

Figure 7 Electron micrograph of melanized PHEMA (0.5% w/w crosslinker). Melanization was performed in Epifrin at pH 3 and room temperature for 13 days.

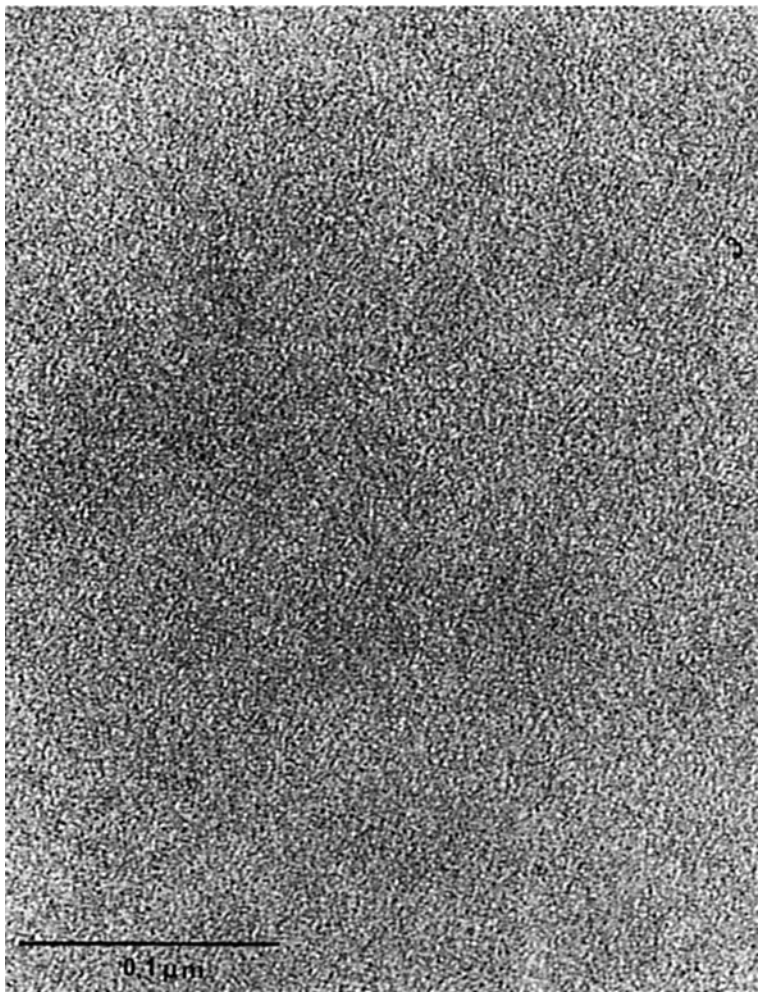

Figure 8 Electron micrograph of melanized PHEMA (5% w/w crosslinker). Melanization was performed in Epifrin at pH 3 and room temperature for 13 days.

and 23.2/76.7 w/w poly(ethyl acrylate)–poly(methyl methacrylate).

As a general rule in IPNs, the smaller the phase domain size as revealed by TEM, the higher the compatibility between the two components.[31] A cellular morphology would suggest low compatibility, but a fine structure with domain size less than 10 nm is usually interpreted as a proof for high compatibility. In Figures 7–9 no background matrix can actually be distinguished. All samples are composed of very fine structures in which melanin particles of 1–2 nm in size are seen. This would suggest phase continuity, therefore high compatibility between hydrogels and melanin. Perhaps the morphology revealed in our micrographs should not be discussed in terms of compatibility between hydrogels and melanin. A semiempirical equation for the phase domain size of polymer II in IPNs, developed by Donatelli, Sperling and Thomas,[34] predicts domain sizes of 5–10 nm provided that interfacial free energy, as a main variable, is close to zero. The important inference is that fine structures not necessarily caused by a high compatibility between components may be found in IPNs.

What we see in the micrographs of melanized hydrogels is just a very fine dispersion of melanin particles in PHEMA. The latter polymer is expected to be the predominant phase, although the micrographs cannot show precisely which phase does indeed predominate. Likely, the melanin particles are trapped in small regions within PHEMA. Because the particles are very small, they accommodate easily in any available space, therefore no effect of the network tightening through an increased crosslink

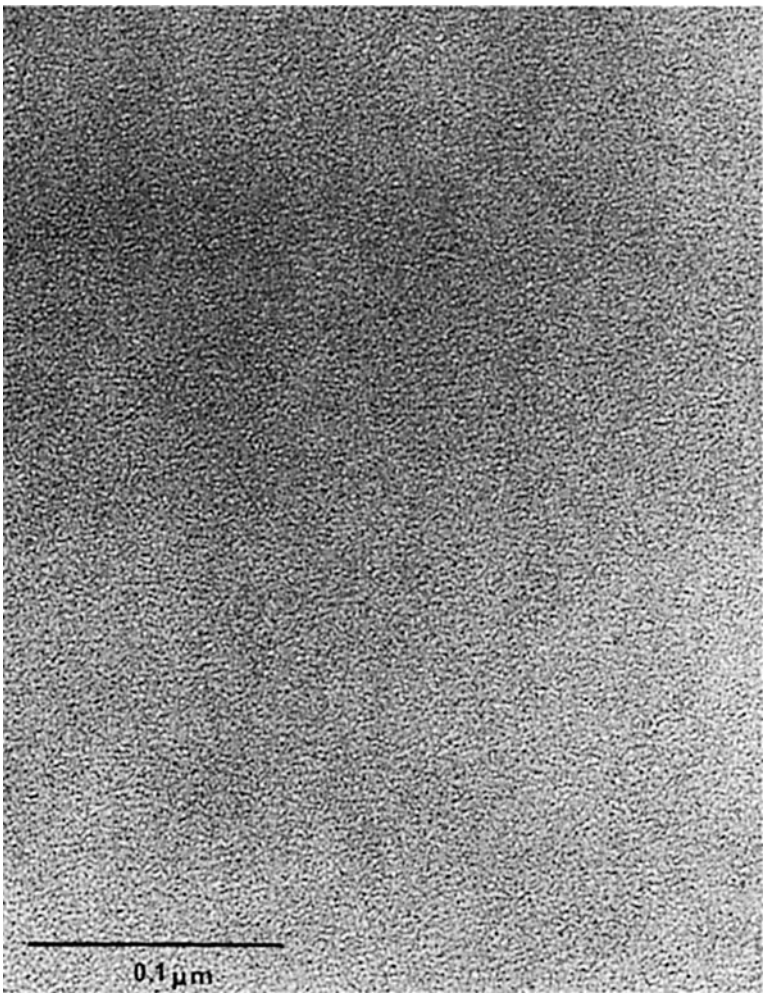

Figure 9 Electron micrograph of melanized 80/20 w/w poly(HEMA-*co*-EEMA). Melanization was performed in Epifrin at pH 3 and room temperature for 13 days.

density was noted on the domain size. Since the molecular entrapment is another form of interpenetration,[33] there is probably no reason why the melanized hydrogels should not be regarded as true IPNs.

Unfortunately, the transmission electron micrographs as such cannot provide any indication of network interpenetration.

With the possible exception of the polymer-modified natural leather, we believe the melanized hydrogels reported here are the first example of a polymer–biopolymer bicomponent system to be regarded as an IPN. Although the polysaccharide–rubber coprecipitates reported by Stephens and Reed,[35] in fact a continuation of Buchanan's work,[36,37] have been used as an example of semi-IPN of the first kind ("semi-I"),[38] it is unlikely that they are more than mechanical blends of two polymers.

CONCLUSIONS

In our quest for polymeric biomaterials for ocular devices capable of absorbing the ultraviolet and some of the visible radiation to the same extent as the natural crystalline lens, we found that PHEMA and copolymers of HEMA, after subjecting them to melanization in aqueous solutions of epinephrine, acquired such properties. Epinephrine undergoes oxidative polymerization to adrenochrome-melanin which, in the process, is entrapped as small particles within the hydrogel network.

Transmission electron micrographs of the melanized hydrogels reveal noncellular, fine structures regardless of the crosslinking density. This was interpreted as a very fine dispersion of melanin throughout the hydrogel. Since the melanin was

synthesized in the presence of the crosslinked hydrogel, we believe that the resulting blends should be regarded as IPNs.

This work was supported in part by grants from National Health and Medical Research Council of Australia (Grant No. 880049) and from IRD Board of the Commonwealth Department of Industry, Technology and Commerce of Australia (Grant No. 15001). The authors are grateful for the technical assistance of Albert V. Russo, Luis F. Aravena, Philippa L. Lord, and Sew H. Wee. The authors also wish to thank Cyanamid Australia Pty. Ltd. and Tokyo Kasei Kogyo Co. (M. Kumagai) for gifts of UV absorbers.

REFERENCES

1. H. Ridley, *Trans. Ophthalmol. Soc. U.K.*, **71,** 617 (1951).
2. O. Wichterle and D. Lim, *Nature,* **185,** 117 (1960).
3. E. Epstein, in T. R. Mazzocco, G. M. Rajacich, and E. Epstein, Eds., *Soft Implant Lenses in Cataract Surgery,* Slack Inc., Thorofare, NJ, 1986, pp. 1–10.
4. K. R. Mehta, S. N. Sathe, and S. D. Karyekar, *Am. Intra-Ocular Implant Soc. J.,* **4,** 200 (1978).
5. R. B. S. Packard, A. Garner, and E. J. Arnott, *Br. J. Ophthalmol.,* **65,** 585 (1981).
6. M. Yalon, M. Blumenthal, and E. P. Goldberg, *Am. Intra-Ocular Implant Soc. J.,* **10,** 315 (1984).
7. G. D. Barrett, Int. Patent Appl. PCT/AU84/00196 (1985).
8. G. Barrett, I. Constable, and A. Stewart, *J. Cataract Refract. Surg.,* **12,** 623 (1986).
9. W. T. Ham, Jr., J. J. Ruffolo, Jr., H. A. Mueller, and D. Guerry, *Vision Res.,* **20,** 1105 (1980).
10. J. Marshall, *Ophthalmic Physiol. Opt.,* **5,** 241 (1985).
11. R. W. Young, *Surv. Ophthalmol.,* **32,** 252 (1988).
12. F. S. Said and R. A. Weale, *Gerontologia,* **3,** 213 (1959).
13. E. A. Boettner and J. R. Wolter, *Invest. Ophthalmol.,* **1,** 776 (1962).
14. S. Lerman, *Am. J. Optom. Physiol. Opt.,* **64,** 11 (1987).
15. R. L. Lindstrom and N. Doddi, *J. Cataract Refract. Surg.,* **12,** 285 (1986).
16. H. M. Clayman, *J. Cataract Refract. Surg.,* **12,** 529 (1986).
17. M. A. Mainster, *Am. J. Ophthalmol.,* **102,** 727 (1986).
18. E. S. Rosen, in J. Cronly-Dillon, E. S. Rosen, and J. Marshall, Eds., *Hazards of Light; Myths and Realities,* Pergamon Press, New York, 1986, pp. 169–177.
19. D. H. Sliney, *Internat. Ophthalmol. Clinics,* **28,** 246 (1988).
20. T. V. Chirila, A. V. Russo, and I. J. Constable, *J. Cataract Refract. Surg.,* **15,** 504 (1989).
21. T. V. Chirila, G. D. Barrett, W. A. Fletcher, A. V. Russo, and I. J. Constable, *J. Cataract Refract. Surg.,* to appear.
22. T. B. Fitzpatrick, G. Szabo, and M. M. Wick, in L. A. Goldsmith, Ed., *Biochemistry and Physiology of the Skin,* Vol. II, Oxford University Press, New York, 1983, pp. 687–712.
23. G. A. Swan, *Prog. Chem. Org. Natural Prod.,* **31,** 521 (1974).
24. G. Britton, *The Biochemistry of Natural Pigments,* Cambridge University Press, Cambridge, 1983, pp. 222–239.
25. J. Harley-Mason, *J. Chem. Soc.,* **1950,** 1276.
26. J. D. Bu'lock, *J. Chem. Soc.,* **1961,** 52.
27. K. Kato, *J. Polym. Sci. B,* **4,** 35 (1966).
28. K. Kato, *Polym. Eng. Sci.,* **7,** 38 (1967).
29. A. J. Curtius, M. J. Covitch, D. A. Thomas, and L. H. Sperling, *Polym. Eng. Sci.,* **12,** 101 (1972).
30. J. A. Mason and L. H. Sperling, *Polymer Blends and Composites,* Plenum Press, New York, 1976, pp. 237–270.
31. D. A. Thomas and L. H. Sperling, in D. R. Paul and S. Newman, Eds., *Polymer Blends,* Vol. 2, Academic Press, New York, 1978, pp. 1–33.
32. L. H. Sperling, J. H. An, M. C. O. Chang, and D. A. Thomas, in M. Lewin, Ed., *Polymers for Advanced Technology,* VCH Publishers, New York, 1988, pp. 635–664.
33. V. Huelck, D. A. Thomas, and L. H. Sperling, *Macromolecules,* **5,** 340 (1972).
34. A. A. Donatelli, L. H. Sperling, and D. A. Thomas, *J. Appl. Polym. Sci.,* **21,** 1189 (1977).
35. H. L. Stephens and T. F. Reed, U.S. Patent 3,645,940 (1972).
36. R. A. Buchanan, C. R. Russell, and O. E. Weislogel, U.S. Patent 3,442,832 (1969).
37. R. A. Buchanan and C. R. Russell, U.S. Patent 3,480,572 (1969).
38. D. Klempner and L. Berkowski, in H. F. Mark, N. M. Bikales, C. G. Overberger, G. Menges, and J. I. Kroschwitz, Eds., *Encyclopedia of Polymer Science and Engineering,* Vol. 8, Wiley-Interscience, New York, 1987, p. 331.

Received February 6, 1991
Accepted April 17, 1991