Absorption Spectra of Corneas in the Far Ultraviolet Region

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Purpose. To study the corneal absorption in the far ultraviolet (UV) region between 260 and 190 nm.

Methods. Thirty-four corneal samples of thickness near 20 μm were obtained from 18 porcine corneas and six human corneas with a microtome-cryostat. The authors conducted absorbance measurements of the sectioned corneal samples supported by two UV optical windows from 350 to 190 nm using a dual-beam spectrophotometer. Three whole porcine corneas were used to study the effect of freezing on the absorbance from 350 to near 290 nm.

Results. The absorption spectra of porcine and human corneas from 350 to 190 nm were measured and three segments in the spectrum between 260 and 190 nm have been identified. The linear absorption coefficients were determined to be 2300 ± 330 (cm^{-1}) at 210 nm and 2410 ± 370 (cm^{-1}) at 193 nm for the porcine corneas and 2320 ± 470 (cm^{-1}) at 210 nm and 2340 ± 450 (cm^{-1}) at 193 nm for the human corneas.

Conclusions. A "window of ablation" in the far UV region between 220 and 190 nm has been identified in which short laser pulses of similar durations and different wavelengths may be interchangeable to ablate the corneal surface with similar characteristics. Invest Ophthalmol Vis Sci. 1997;38:1283–1287.

Nanosecond ultraviolet (UV) laser pulses have been used clinically to correct refractive errors of the eye through surface ablation of the cornea. The strong corneal absorption of the far UV radiation at the wavelength of 193 nm has made the ArF excimer lasers the current choice for precision ablation of the cornea. Although extensive studies have been performed on corneal ablation by excimer lasers,[1–3] investigations continue to search for the next generation of laser systems that produce short UV pulses efficiently and safely. Among these efforts, experimental results have been published on corneal ablation with the harmonics of fundamental outputs from short-pulsed solid-state lasers. For example, the fifth harmonic of output from Nd^{+} doped lasers has been used to ablate the corneal surface at wavelengths near 210 nm.[4,5]

Although the corneal absorption at 193 nm has been measured previously,[2] the measurement of corneal absorption between 190 and 230 nm has not been reported. This makes it difficult to directly compare corneal ablation with laser pulses near 210 nm to the results by ArF lasers at 193 nm because the ablation process strongly depends on the absorption in this region. In an earlier investigation of corneal ablation with picosecond laser pulses at 211 and 263 nm, we have suggested that the corneal absorption in this spectral region may be divided further into three segments based on the analysis of collateral tissue damage zones.[5] We have concluded that the corneal absorption is relatively weak from 266 to 248 nm,[2] increases steeply from 248 to 213 nm, and remains strong from 213 to 193 nm.[5] In this report, we present a detailed study of the far UV absorption spectrum of the porcine and human corneas from 350 to 190 nm and its dependence on tissue conditions.

METHODS. Samples Porcine and human corneas were used for the absorbance measurements. Institutional guidelines regarding use of tissue and organs were followed. Fresh porcine eye globes were obtained from a local slaughterhouse and the School of Medicine, East Carolina University. This arrangement was approved by the University Animal Care and Use Committee. All procedures involving animals were performed in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Twenty-three sectioned corneal samples from 18 porcine corneas and three whole porcine corneas were used for the study reported here. All eye globes were removed from the animals immediately after death and stored on ice until the corneas were excised from the globes. Absorbance measurements of porcine corneas were performed within 12 hours postmortem, except for those samples used for studying the effect of freezing time. Eleven corneal samples from five fresh human corneas and one preserved human cornea also were used. Both the human eye globes and preserved cornea were provided by the North Carolina Eye and Human Tissue Bank. The absorbance of the fresh human corneas was measured within 33 hours postmortem and the preserved human cornea at 1 week postmortem.

Sectioning of the Corneas Because of the strong absorption of far UV radiation by the cornea, the sample thickness needs to be approximately 20 μm to keep the absorbance within the scale of the spectrophotometer. To obtain a thin and contiguous corneal sample of uniform thickness, we used a microtome-cryostat (model 4551; Ames, Naperville, IL) to section a frozen cornea at −18°C. Corneas were excised from refrigerated eye globes, stored in saline solution, and frozen in the cryostat for 15 minutes or longer before sectioning. The superficial layers of the cornea, including the epithelium, were removed until a uniform slice from the corneal stroma was obtained. To keep

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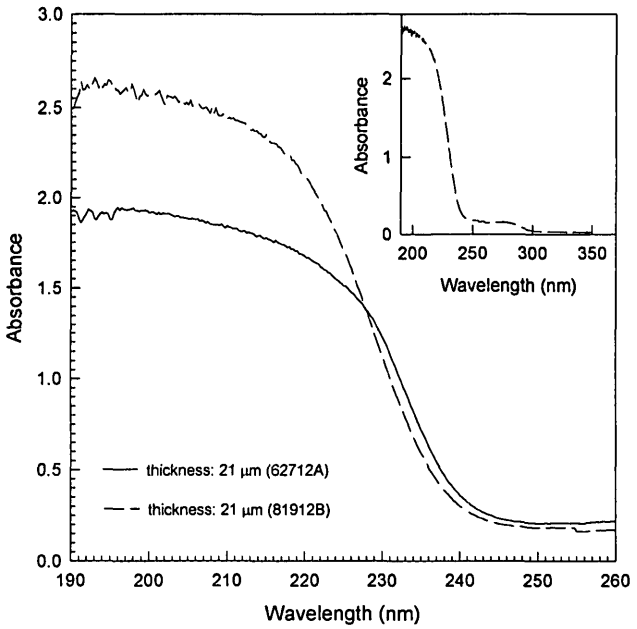

FIGURE 1. The dependence of absorbance on the wavelength for two porcine corneal samples between 260 and 190 nm. (*inset*) The dependence of absorbance on the wavelength for one porcine corneal sample between 350 and 190 nm.

the structure of the thin corneal sample intact and total absorbance low, two rectangular optical windows made of UV grade fused silica (type 7940; Corning, Corning, NY) were used as a sample holder to support the sample between the windows. The sample holder has a total thickness of 12 mm and a low thermal expansion coefficient, which helps to protect the corneal sample in the course of thawing and transferring to the spectrophotometer. The absorbance of the sample holder increases slowly as the wavelength decreases in the far UV region, which was measured to be <0.01 at 260 nm and 0.011 at 190 nm after the surface reflection contribution was deducted. The thickness of a corneal sample was determined through the measurement of the thickness difference of the sample holder with and without the sample at room temperature using a micrometer of 3 μm (0.0001 inch) resolution. The micrometer has a ratchet stop mechanism that enabled us to apply a consistent measuring pressure on the tissue between measurements. Furthermore, the thickness measured by the micrometer was verified by the setting of knife advancement in the microtome. We estimated that the uncertainty in the thickness measurement is <20%.

Absorbance Measurement The absorbance measurements of the corneal samples in the UV region from 350 to 190 nm were carried out at room temperature with a dual-beam UV-visible-IR spectrophotometer (model 17D; Varian Associates, Palo Alto, CA). The spectrophotometer can be used to measure absorbance from 0 to 3 in five scales between 2500 and 186 nm in wavelength. The UV wavelength reading was calibrated with a standard UV mercury lamp and the absorbance reading with a dye solution of known absorbance. Both were found to be within the manufacturer's specifications. Two apertures of 4 mm in diameter were used at the front and back surfaces of the sample holder to collimate the incident and transmitted light beams. The output signal from the spectrophotometer was digitized and averaged by a personal computer with an analog/digital board. The total absorbance of the sample and the sample holder in the sample chamber was measured, and the corneal absorbance was obtained by subtracting the absorbance of the sample holder from the total absorbance.

RESULTS. Figure 1 shows the absorbance of two sectioned samples of two porcine corneas as a function of wavelength from 260 to 190 nm. One full spectrum of the absorbance is plotted in the insert of Figure 1 from 350 to 190 nm. We also have measured the absorbance of human corneal samples as a function of wavelength from 260 to 190 nm, as shown in Figure 2, with one full spectrum from 350 to 190 nm displayed in the insert of Figure 2. One of the human corneal samples in Figure 2 had been placed in a preservation solution (Optisol; Chiron Vison, Irvine, CA) for 1 week before the measurement, and it exhibited a light pink color after it was removed from the preservation cell. From Figures 1 and 2, one can see that the absorbance readings have a larger fluctuation in the short wavelength region below 195 nm than that in the long wavelength region. This was expected

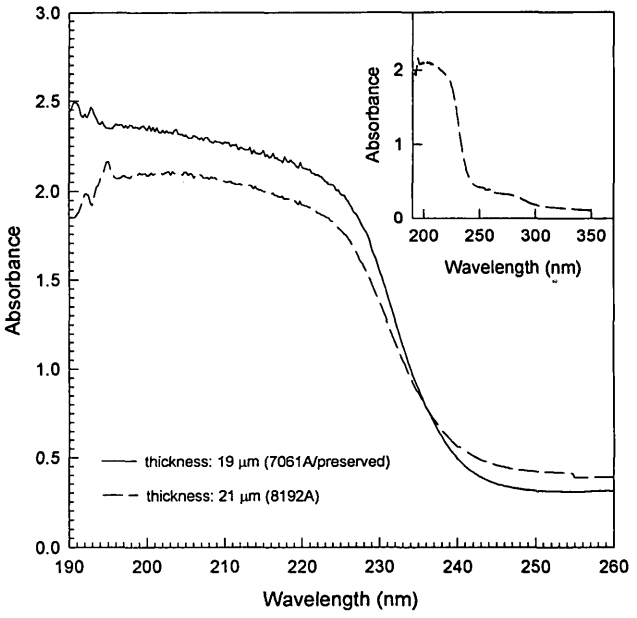

FIGURE 2. The dependence of absorbance on the wavelength for two human corneal samples between 260 and 190 nm. (*inset*) The dependence of absorbance on the wavelength for one human corneal sample between 350 and 190 nm.

because of the relative large noise presented in the weak signal. This is caused by the strong absorption of the corneal sample and the low light intensity near the short wavelength end of the emission spectrum of the light source.

Although the absorbance fluctuates from sample to sample, it is clear from our measurements that the dependence of the corneal absorption on wavelength is rather consistent with each other between 260 and 190 nm for both the porcine and human corneas. All the spectra of absorbance show that the corneal absorption increases significantly when wavelength decreases from 240 to 220 nm. Based on this steep increase, the corneal absorption in the far UV region between 260 and 190 nm can be delineated into three segments with clear boundaries at 240 and 220 nm: a weak absorption segment between 260 and 240 nm, a steeply increasing segment between 240 and 220 nm, and a high absorption segment below 220 nm.

Because the scattering of UV radiation by the cornea is much weaker than its absorption, we can use Lambert's law to find the transmittance T of a corneal sample as:

$$T = \frac{I}{I_0} = (1 - R)^2 (1 - R')^2 e^{-\alpha d}, \qquad (1)$$

where I_0 and I are the light intensity at the front and rear surfaces of the sample holder, R and R' are the Fresnel reflectivity of the glass–cornea and glass–air interfaces, respectively, α is the linear absorption coefficient of the cornea, and d is the thickness of the corneal sample. The corneal absorbance A was measured through the dual-beam configuration of the spectrophotometer and obtained by subtracting the absorbance of the sample holder (with transmittance T') from the total absorbance. Therefore, A is given by:

$$A = \log_{10}\left(\frac{T'}{T}\right) = 0.434\alpha d - 2 \cdot \log_{10}(1 - R). \qquad (2A)$$

If $-2 \cdot \log_{10}(1 - R) << 0.434\,\alpha d$, then equation 2A becomes

$$A \cong 0.434\alpha d. \qquad (2B)$$

Because the refraction index of the cornea in the far UV region is unknown, the reflection loss contribution to the measured absorbance cannot be calculated. We estimated from the refraction index of water that R is smaller than 0.1 in this spectral region. Thus, equation 2B should only be used to accurately calculate the linear absorption coefficient α from the measured A in the segment of strong absorption between 220 and 190 nm, in which the absorbance is well above 1. The results of thickness measurements and the calculated α of porcine cornea, from equation 2B, at two wavelengths of 210 and 193 nm are listed in Table 1. We determined the linear absorption coefficient α to be 2300 ± 330 (cm^{-1}) at 210 nm and 2410 ± 370 (cm^{-1}) at 193 nm from 23 samples of 18 porcine corneas. Similar results for human corneas are listed in Table 2. We determined α to be 2320 ± 470 (cm^{-1}) at 210 nm and 2340 ± 450 (cm^{-1}) at 193 nm from 11 samples of six human corneas. The standard deviations in the absorption coefficients for porcine corneas are consistent with our estimation of the uncertainty in the thickness measurement, which is expected to be the dominant source of errors. The large standard deviation in α for human corneas may be attributed to the large statistical fluctuation associated with the small number of samples used in our study.

TABLE 1. Thickness and Linear Absorption Coefficients of Porcine Cornea Samples

Sample Number	*Thickness (μm)*	*α (cm^{-1}) at 210 nm*	*α (cm^{-1}) at 193 nm*	*Postmortem*/ Freezing Time (hours)*
62711B	20	2350	2430	1.5/1.2
62711C	23	2010	2120	1.5/9.0
62712A	21	1990	2120	1.3/1.5
62731B	25	1790	2080	3.3/3.2
71121A	26	2590	2300	5.2/0.6
71122C	19	2940	3010	7.2/5.6
71131A	27	1970	1940	7.2/7.5
72911A	18	2340	2510	1.5/0.3
72911B	18	2520	2670	1.5/10.5
72912A	26	2350	2630	2.4/0.4
72912B	23	2580	2680	2.4/1.2
72923A	21	2140	2260	5.2/0.7
72936A	12	2710	2630	9.0/0.7
81923A	15	2700	3180	5.6/1.0
81912B	21	2750	2910	2.8/2.8
81923B	20	2480	2800	5.2/2.0
81924B	21	1930	2100	7.6/5.0
81924C	24	2130	2190	7.6/5.9
11011A	30	2190	2160	2.0/0.5
11021B	21	1710	1660	4.3/1.5
11022A	15	2600	2510	4.3/2.7
11031A	16	2020	2220	6.8/1.2
11032A	28	2230	2280	6.8/2.2

* Postmortem time is defined as the period between animal death and the beginning of corneal freezing.

To investigate the effect of freezing on the absorbance measurement, we have conducted two sets of tests with porcine corneal samples. The absorbance of three whole porcine corneas from 350 to near 290 nm was measured before and after freezing and results from one of them are shown in Figure 3. Although the absorbance spectra differ in the long wavelength region, they converge toward 290 nm when the absorbance readings approach the maximum of the scale. The effect of freezing time on the absorbance

TABLE 2. Thickness and Linear Absorption Coefficients of Human Cornea Samples

Sample Number	*Thickness (μm)*	*α (cm^{-1}) at 210 nm*	*α (cm^{-1}) at 193 nm*	*Postmortem Time*
7061A	19	2750	2920	1 week (preserved)
7061B	28	2090	2180	1 week (preserved)
7062A	15	2030	2120	33 hours
7062B	20	1670	1720	33 hours
7062C	15	3210	3140	33 hours
8191A	17	2760	2680	28 hours
8192A	21	2250	2120	30 hours
0301A	21	2540	2420	28 hours
0301B	24	1680	1680	28 hours
0302A	21	2220	2390	28 hours
0302B	28	2290	2380	28 hours

of porcine corneal sample also has been studied with the variation of freezing time from 15 minutes to 10 hours (Table 1). No significant change because of the length of the freezing time was found in the spectral region from 260 to 190 nm. We were unable to measure directly the effect of freezing on corneal absorbance in the far UV region because of the technical difficulty in quality sectioning fresh corneas.

DISCUSSION. To our knowledge, we have reported here for the first time a study of the complete spectrum of far UV absorbance of the cornea between 260 and 190 nm. We found that the corneal absorption between 260 and 190 nm can be divided into three segments. Moreover, the average value of the linear absorption coefficient of the corneal samples remains approximately the same as the wavelength decreases from 210 to 190 nm. These conclusions are consistent with our previous suggestion on the corneal absorption based on the analysis of collateral tissue damage in the corneal ablation with short laser pulses in the far UV region.

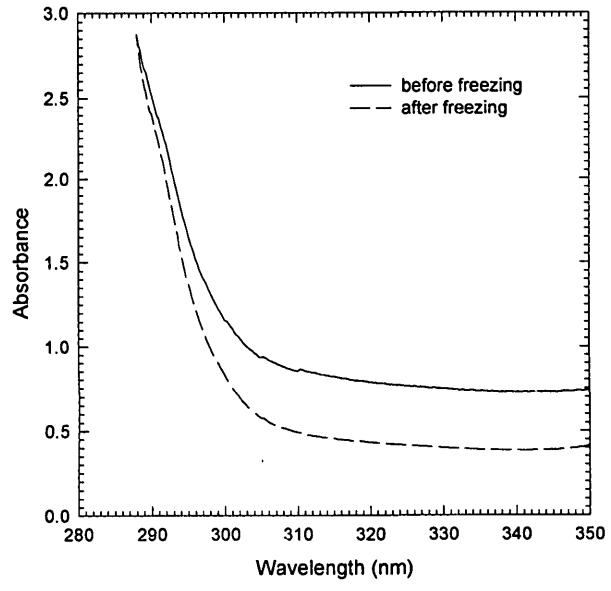

FIGURE 3. The dependence of the absorbance of one whole porcine cornea before and after freezing.

We noticed that our measurement of the linear absorption coefficient of cornea at 193 nm is approximately 15% less than that measured by Puliafito et al.[2] In examining the effect of freezing on corneal absorption with three whole porcine corneas, we found a decrease in average thickness from 810 to 720 μm, which may be attributed to dehydration in the freezing and thawing processes. If a similar reduction in thickness occurred in the sectioned samples, our determination of the linear absorption coefficient from the measured absorbance may overestimate the coefficient by approximately 10%. In addition, the porcine eyes obtained from the slaughterhouse were removed from scalded pigs, whereas the porcine eyes used for the last five samples in Table 1 were obtained from the School of Medicine without scalding. No effect of scalding can be identified in our measurement of the far UV absorbance of porcine corneas.

Because the effect of freezing on the absorbance measurement has not been studied directly in the far UV region between 260 and 190 nm, there is some uncertainty in correlating our results to those of the clinical investigations of corneal ablation. Previous studies indicated that among the major corneal components, only collagen has shown a steep rise in the UV absorption spectrum below 240 nm,[3,6] which is similar to our results reported here. Corneal collagen consists of small amino acids linked in long molecular chains by peptide bonds. Considering that the freezing involves only the phase transition between water and ice, it is reasonable to assume that freezing has a minimal effect on corneal collagen. Furthermore, nuclear magnetic resonance and other measurements suggested that the fraction of water bound to the glycosaminoglycans is <1% of total corneal water.[7] Thus, any change in corneal absorption caused by freezing may be insignificant compared with the major source of uncertainty in determination of sample thickness.

By proposing a model of plasma ablation assisted by chromophore absorption, we have suggested pre-

viously that the threshold of laser ablation of the cornea depends on both linear absorption coefficient of the cornea and duration of the laser pulses.[5] Moreover, we have pointed out that the size of the collateral tissue damage zone depends primarily on the linear absorption coefficient of the cornea. Therefore, we may conclude from this report that short laser pulses with similar pulse duration and a wavelength in a "window of ablation" between 220 and 190 nm can have similar parameters of ablation threshold, rate, and the size of collateral damage zone in the surface ablation of the cornea. This indicates that either the fifth harmonic generation of the output at the fundamental wavelength $\lambda_0 < 1.1$ μm or the fourth harmonic generation of the output with $\lambda_0 < 880$ nm from a short-pulsed ($\leq$10 nsec) solid-state laser can provide UV pulses in the window of ablation to achieve similar results of corneal ablation as the ArF excimer lasers do at 193 nm. Because fourth harmonic generation can be accomplished with only two nonlinear optical crystals, this approach may provide a fairly simple method to replace toxic excimer lasers with solid-state laser systems with, among others, Cr:LiSAF[8] or Ti:sapphire[9] as the gain medium.

Key Words

cornea, corneal ablation, corneal absorption, corneal surgery, far UV absorption

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