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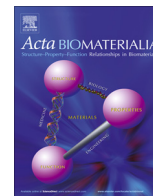
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Fluorescent solute-partitioning characterization of layered soft contact lenses



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ABSTRACT

Partitioning of aqueous packaging, wetting, and care-solution agents into and out of soft contact lenses (SCLs) is important for improving wear comfort and also for characterizing lens physico-chemical properties. We illustrate both features of partitioning by application of fluorescent-solute partitioning into DAILIES TOTAL1[®] (delefilcon A) water-gradient SCLs, which exhibit a layered structure of a silicone-hydrogel (SiHy) core sandwiched between thin surface-gel layers. Two-photon fluorescence confocal laser-scanning microscopy and attenuated total-reflectance Fourier-transform infrared spectroscopy (ATR-FTIR) characterize the lens and assess uptake profiles of six prototypical fluorescent solutes. Comparison of solute uptake in a SiHy-core prototype lens (i.e., O₂OPTIX[™]) validates the core SiHy structure of DAILIES TOTAL1[®]. To establish surface-layer charge, partition coefficients and water contents are obtained for aqueous pH values of 4 and 7.4. Solute fluorescence-intensity profiles clearly confirm a layered structure for the DAILIES TOTAL1[®] lenses. In all cases, aqueous solute partition coefficients are greater in the surface layers than in the SiHy core, signifying higher water in the surface gels. ATR-FTIR confirms surface-layer mass water contents of 82 ± 3%. Water uptake and hydrophilic-solute uptake at pH 4 compared with that at pH 7.4 reveal that the surface-gel layers are anionic at physiologic pH 7.4, whereas both the SiHy core and O₂OPTIX[™] (lotrafilcon B) are nonionic. We successfully confirm the layered structure of DAILIES TOTAL1[®], consisting of an 80-μm-thick SiHy core surrounded by 10-μm-thick polyelectrolyte surface-gel layers of significantly greater water content and aqueous solute uptake compared with the core. Accordingly, fluorescent-solute partitioning in SCLs provides information on gel structure and composition, in addition to quantifying uptake and release amounts and rates.

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1. Introduction

Partitioning of packaging, wetting, and care-solution agents in soft contact lenses (SCLs) is a well-explored avenue for improving and maintaining on-eye lens performance [1–6]. Similarly, partitioning of tear components in SCLs, such as proteins, salts and lipids, can affect lens behavior during wear [1,7–11]. Further, solute partitioning is critical for possible use of SCLs as a drug-delivery vehicle [12–15]. Thus, understanding how solutes partition and transport in hydrogels is an important challenge [13,16–20].

Although not as common, solute partitioning may be adopted to characterize hydrogel structure. Watkins et al. [21] used fluorescent-solute loading to visualize multi-laminated hydrogels. Furthermore, by employing non-interacting solutes of known shape

and size, Walther et al. [22,23] ascertained the pore-size distribution (i.e. mesh-size distribution) of parent hydrogels. We apply this philosophy to understand the structure of DAILIES TOTAL1[®] lenses, which, according to trade and patent literature, consist of a 33% water-content SiHy core that transitions through an interpenetrating anchor region to outer surface-gel layers with water content >80% [24–28]. As daily disposables, DAILIES TOTAL1[®] lenses are designed for use without care solutions. Here, solute partitioning is used to ascertain the hydrogel structure. Because the reported surface-gel layers are of high water content, their mesh sizes are expected to be larger than those of the core region. Accordingly, larger aqueous solutes likely partition more favorably into the surface-gel region than into the core region [13,16,17]. Further, by comparing uptake of aqueous ionogenic solutes in their charged and neutral states, information can be obtained on the charge of the polymer strands in the surface and core gels. By comparing partitioning into a SiHy-core prototype lens (e.g. O₂OPTIX[™] [29]), the core SiHy structure of DAILIES TOTAL1[®] can be validated. Finally, partitioning of oleophilic solutes from an oil solvent can be

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pursued to establish whether or not an aqueous-saturated surface gel can forestall low aqueous-soluble lipid penetration into the core SiHy region.

We measure uptake of six prototypical fluorescent solutes in DAILIES TOTAL1[®] SCLs. Two-photon fluorescence confocal laser-scanning microscopy (FCLSM) obtained profiles and partition coefficients of both hydrophilic (i.e. fluorescently labeled avidin and dextrans) and oleophilic (i.e. Nile Red and fluorescently labeled cholesterol) solutes. For the hydrophilic solutes in DAILIES TOTAL1[®], FCLSM measurements confirmed consistently greater solute partitioning in the surface-gel layers and, therefore, a higher water content compared with that in the SiHy core. FCLSM confirmed both the layered structure and established the surface-gel-layer thickness. Higher surface-layer water content was validated using attenuated total-reflectance Fourier-transform infrared spectroscopy (ATR-FTIR). Penetration of two oleophilic solutes from silicone oil into the SiHy core was not prevented by the surface-gel layers. To establish a baseline, solute-uptake and water-content measurements were also performed on a single-water-content prototype SiHy SCL: O₂OPTIX[™].

2. Materials and methods

2.1. Soft contact lenses

Two commercially available Alcon (Fort Worth, TX) SiHy SCLs were used in this study: DAILIES TOTAL1[®] (delefilcon A; composition unavailable) and O₂OPTIX[™] (lotrafilcon B; containing *N,N*-dimethylacrylamide and methacryloxypropyl tris(trimethylsiloxy)silane [29]). The diameter, base curve, and were 14.1 and 14.2 mm, 8.5 and 8.6 mm, and –0.75 and –2.0, for DAILIES TOTAL1[®] and O₂OPTIX[™], respectively. Prior to each measurement, lenses were extracted in excess pH 7.4 phosphate buffer saline solution (PBS) for at least 48 h to remove preservatives and surfactants from the packaging solutions. PBS was prepared as described previously [13,16,17]. For measurements at pH 4, extracted lenses were subsequently equilibrated for 24 h in excess pH-4 citrate buffer (1.2 × 10⁻² M citric acid anhydrous, Cat. No. A940-500, Fisher Scientific; 8.2 × 10⁻³ M sodium citrate dihydrate, SX0445-1, EMD Chemicals; 0.15 M NaCl, S271-3, Fisher Scientific). All experiments were performed at ambient temperature.

2.2. ATR-FTIR

SCL surface water content was determined using ATR-FTIR [30–32]. IR spectra were obtained using a Nicolet 6700 FTIR spectrometer (Thermo Scientific, Madison, WI) equipped with a DTGS-KBr detector and a single-reflection Smart OMNI-Sampler ATR cell (No. 0028-899, Thermo Scientific). In all cases, ambient air was used as the reference spectrum. Prior to each water-content measurement, SCLs were removed from the extraction solution, lightly blotted with Fisherbrand[®] weighing paper (Fisher Scientific, Pittsburgh, PA), and forced onto the ATR crystal using the instrument supplied fixture or light weights to ensure intimate crystal contact. The applied force was low enough to ensure no gel collapse [33]. Subsequently, FTIR spectra were obtained in the range of wavenumber from 4000 to 650 cm⁻¹ during 64 scans, with 2 cm⁻¹ resolution. Each measurement was performed in triplicate.

Total internal reflectance probes surface properties through an exponentially decaying evanescent wave of penetration depth d_p , given by

$$d_p = \frac{\lambda}{2\pi n_1 [\sin^2 \theta - (n_2/n_1)^2]^{1/2}} \quad (1)$$

where λ is the wavelength of light, θ is the angle of incidence (45°), and n_1 and n_2 are the refractive indices of the ATR crystal

(Germanium: $n_1 = 4$) and either the surface-gel layer (approximated as water: $n_2 \approx 1.33$) or O₂OPTIX[™] ($n_2 = 1.42$ [29]), respectively. Over the wavenumbers investigated, penetration depths into the lenses ranged from 0.1 to 1 μm, which are considerably smaller than the observed DAILIES TOTAL1[®] surface-layer thicknesses [27]. For O₂OPTIX[™], however, penetration depths are considerably greater than the 25-nm-thick plasma coating [29]. In this case, water contents measured by ATR-FTIR are averaged over the plasma-surface-coated region and the untreated bulk SiHy material.

Fig. 1 displays ATR-FTIR spectra for PBS solution, DAILIES TOTAL1[®], and O₂OPTIX[™] over the range of wavenumbers between 4000 and 2500 cm⁻¹, where water absorbs strongly. For clarity, the baseline was subtracted. In all cases, the O–H stretching band (3500–3000 cm⁻¹ [34]) is clearly observed. Following Wilson et al. [30], the surface water content was calculated from the peak area between 3600 and 3000 cm⁻¹. To account for the contribution of lens polymer to the IR spectra (e.g. at 2950 cm⁻¹), overlapping peaks were deconvoluted using dry-lens spectra; thus, only peak areas corresponding to O–H stretching were used in the calculation. We neglected the O–H bending band, because the matrix polymer contributes significantly at these wavenumbers. ATR-FTIR-measured mass water contents were validated from in-house synthesized 2-hydroxyethyl methacrylate (HEMA)/methacrylic acid (MAA) copolymer hydrogels [13] of known bulk gravimetric water content. Fig. 2 plots the ATR-FTIR-measured water content against known gravimetric water content for four HEMA/MAA hydrogels. Water content obtained by ATR-FTIR is in good agreement with that determined gravimetrically over the range of water contents studied.

2.3. Fluorescent solutes

PBS (pH 7.4) and citrate buffer (pH 4) solutions, prepared as described above, were solvents for the hydrophilic fluorescent solutes. Fluorescein isothiocyanate dextrans (FITC-dextran4, MW = 4000 g mol⁻¹; FITC-dextran20, MW = 20,000 g mol⁻¹; FITC-dextran70, MW = 70,000 g mol⁻¹) were obtained from TdBCons (Uppsala, Sweden). To remove free label (i.e. FITC), FITC-dextran solutions were extensively dialyzed in Slide-A-Lyzer Dialysis Cassettes (No. 66212, Thermo Scientific, cutoff MW = 2000 g mol⁻¹) for 1 week at 25 °C, with the surrounding dialyzing solution changed daily. Cationic FITC-conjugated avidin (FITC-avidin, MW = 68,000 g mol⁻¹) was acquired from Invitrogen (Eugene,

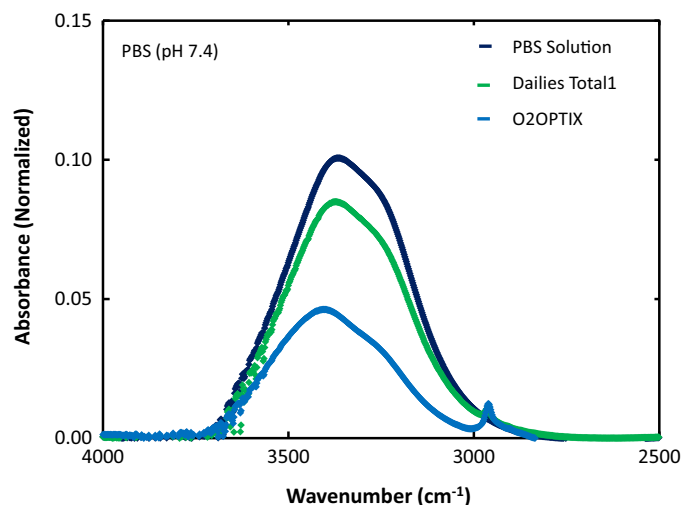


Fig. 1. Truncated ATR-FTIR spectra for PBS solution, DAILIES TOTAL1[®], and O₂OPTIX[™] over the range of wavenumbers between 4000 and 2500 cm⁻¹. The measured surface mass water content of DAILIES TOTAL1[®] and O₂OPTIX[™] was 82 ± 3% and 43 ± 2%, respectively.

OR). In this case, free FITC label was removed by filter centrifugation at 25 °C using an Amicon Ultra-4 membrane (10,000 g mol⁻¹, UFC801008, EMD Millipore Corp., Billerica, MA) for 3 cycles at 4000 rpm.

Silicone oil was purchased from Fisher Scientific (500 cSt, S159-500, Pittsburgh, PA) and used as the solvent for oleophilic-fluorescent solutes. Nile Red (N1142) and 25-[N-[(7-nitro-2-1, 3-benzoxadiazol-4-yl)methyl]amino]-27-norcholesterol (NBD-cholesterol, 810250P) solutes were purchased from Invitrogen and Avanti Polar Lipids (Alabaster, AL), respectively, and used without further purification. Table 1 reports the molecular weight and hydrodynamic radius a_s of all solutes.

2.4. Solute partition coefficients

Solute partition coefficients in the SCLs were obtained using two-photon FCLSM [35,36] excited at 780 nm, as described previously [13,16,17]. With the exception of Nile Red, emission for all solutes was detected through a 500–550-nm emission filter. For Nile Red, a 685-nm short-pass emission filter was employed. Prior to the uptake measurement, extracted SCLs were soaked in the pertinent solute-containing solution under magnetic stirring for at least 2 d at 400 rpm. After equilibration, a 1-mm-thick layer of the bulk-solute solution in a small Petri dish was placed on the microscope platform and scanned in the vertical (z) direction at 2- μ m intervals to a depth of at least 250 μ m. Thereafter, a solute-loaded SCL was placed on a microscope slide (48300-047, VWR International, West Chester, PA, USA) and placed on the microscope for scanning in the z-direction at the same laser power and detector setting as those during scanning of the bulk-solute solution. Background fluorescence intensity was recorded and subtracted from solution and SCL signals. In the concentration range studied (i.e. 10⁻⁵ to 5 \times 10⁻⁴ M), detected solute intensities inside the SCL and in the surrounding bulk solution were proportional to dye concentration [13,16,17]. The partition coefficient k , which is the concentration of solute in the gel phase divided by the concentration in the bulk surrounding phase, is thus given by the ratio of solute intensity in the SCL to that in the loading solution. Equilibrium was confirmed for all solutes excluding the protein FITC-avidin [17]. With the exception of FITC-avidin, loading concentration was varied over a factor of 10, with no change in the measured partition coefficient.

Prior to FCLSM with the oleophilic solutes, SCLs were first saturated with aqueous PBS for 48 h and then immersed in silicone oil containing dissolved Nile Red or NBD-cholesterol. FCLSM was performed as described above. The measured intensity of Nile Red depends strongly on the polarity of its environment [37]. As a result, partition coefficients were confirmed by back extraction [13] both into silicone oil and into decane as an alternate solvent. Agreement was excellent in all cases. Gravimetric oil-uptake measurements reveal little to no swelling of dry SiHy lenses when immersed in 500-cSt silicone oil, likely due to size exclusion from silicone microdomains as a result of the large molecular weight of the oil. Additionally, water-saturated lens thicknesses obtained from FCLSM of oleophilic and hydrophilic solutes verify minimal

imbibition of silicone oil into water-saturated DAILIES TOTAL1[®] and O₂OPTIX[™] lenses.

3. Results

Table 2 reports mass water contents w_1 obtained by ATR-FTIR for the DAILIES TOTAL1[®] surface layer and O₂OPTIX[™] in aqueous pH 7.4 and 4. Also reported are gravimetric water contents of the DAILIES TOTAL1[®] SiHy core and O₂OPTIX[™]. Core water content was obtained from the measured gravimetric water content of the entire lens, corrected for the measured water content of the surface layers of known thickness (see below) using a typical core dry-polymer density of 1067 kg m⁻³ [16,17]. Several features are salient. For both the SiHy core of DAILIES TOTAL1[®] and O₂OPTIX[™], water content does not vary over the range of pH studied. Conversely, for the DAILIES TOTAL1[®] surface layers, water content rises significantly with increased aqueous pH (i.e. from 63 to 82%) indicating a polyelectrolyte gel [13]. ATR-FTIR water-content measurements at pH 6.5 demonstrate no significant de-swelling compared with that at pH 7.4 (data not shown). Therefore, the surface-gel-layer water content does not vary over the reported range of tear-film physiological pH (i.e. 6.5–7.8) [39]. In all cases, the DAILIES TOTAL1[®] surface-layer water content is significantly higher than that of the SiHy core, with core gravimetric water content identical to that of O₂OPTIX[™]. ATR-FTIR surface-water content for O₂OPTIX[™] is slightly higher than bulk water content obtained gravimetrically, but is not significant, owing to limitations in deconvoluting the water signature in high-polymer-content SCLs.

As discussed previously [13,16,17], higher water content reflects larger water-filled meshes and leads to greater solute partitioning into hydrogels, all else being equal. Fig. 3 displays typical fluorescence-confocal-microscopy images of aqueous FITC-dextran4 in (a) DAILIES TOTAL1[®] and (b) O₂OPTIX[™] equilibrated at pH 7.4. Scale bars represent 20 μ m in the vertical direction. Fig. 3a reveals the layered structure of DAILIES TOTAL1[®]. Uptake of FITC-dextran4 in the surface-gel layers is clearly greater than that in the SiHy core. The resulting fluorescence intensity profile (not shown) establishes a surface-layer thickness of 11 \pm 4 μ m. Conversely, in Fig. 3b, FITC-dextran4 uptake in O₂OPTIX[™] is spatially uniform. Comparison of intensities in Fig. 3a and b demonstrates that FITC-dextran4 partitioning in the SiHy core of DAILIES TOTAL1[®] is similar to that in O₂OPTIX[™].

Fig. 4 plots partition coefficients at pH 7.4 as a function of hydrodynamic radius for several fluorescently labeled aqueous solutes in DAILIES TOTAL1[®] and O₂OPTIX[™]. At this pH, the aqueous solutes are anionic [13,38]. In all cases, the DAILIES TOTAL1[®]-core partition coefficients are similar to those of O₂OPTIX[™]. This result indicates that the chemical and physical structures of the core of DAILIES TOTAL1[®] and O₂OPTIX[™] are similar, confirming the reported SiHy structure of the DAILIES TOTAL1[®] core [9,27,28]. Hydrophilic solutes partition primarily into the hydrophilic domains of SiHys. Since the water contents of the DAILIES TOTAL1[®] core and O₂OPTIX[™] are identical,

Table 1
Solute properties in aqueous pH 7.4.

Solute	M_w (g mol ⁻¹)	a_s (nm)
Nile Red	320	0.8 [47]
NBD-cholesterol	560	0.9 ^a [48]
FITC-dextran4	4000	1.6 [16,17]
FITC-dextran20	20,000	3.4 [16,17]
FITC-avidin	68,000	3.5 [17]
FITC-dextran70	70,000	5.8 [16,17]

^a Approximated as cholesterol in water-saturated chloroform.

Table 2
Water content of DAILIES TOTAL1[®] and O₂OPTIX[™] at aqueous pH 7.44.

SCL	w_1 (%) pH 7.4/pH 4	w_1 (%) lit.
DAILIES TOTAL1 [®] (surface layer)	82 \pm 3 ^a /63 \pm 2 ^a	85 [27,28]
DAILIES TOTAL1 [®] (core)	29 \pm 5 ^b /34 \pm 6 ^b	33 [24–28]
O ₂ OPTIX [™]	43 \pm 2 ^a /44 \pm 2 ^a	33 [29]
	33 \pm 6 ^b /38 \pm 3 ^b	

^a Obtained from ATR-IR.

^b Obtained gravimetrically [13].

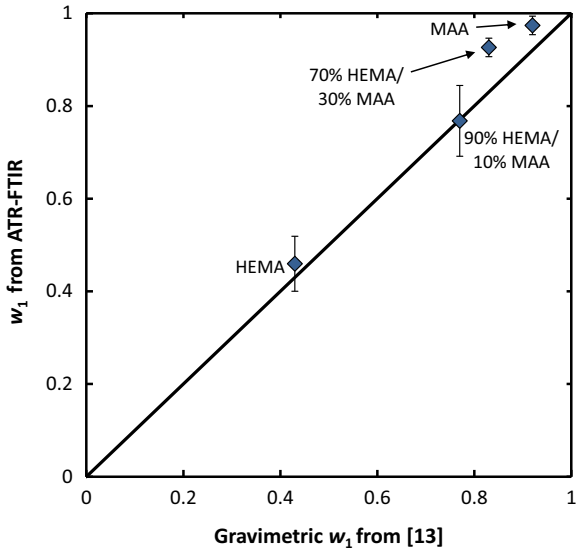


Fig. 2. ATR-FTIR-measured mass water content as a function of known bulk gravimetric water content for HEMA/MAA synthesized hydrogels.

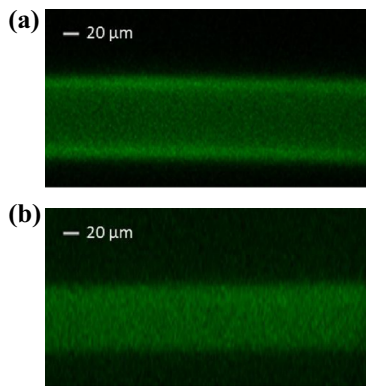


Fig. 3. Fluorescence-confocal-microscopy images of FITC-dextran4 at equilibrium in (a) DAILIES TOTAL1® and (b) O2OPTIX™. Scale bars represent 20 μm in the vertical direction.

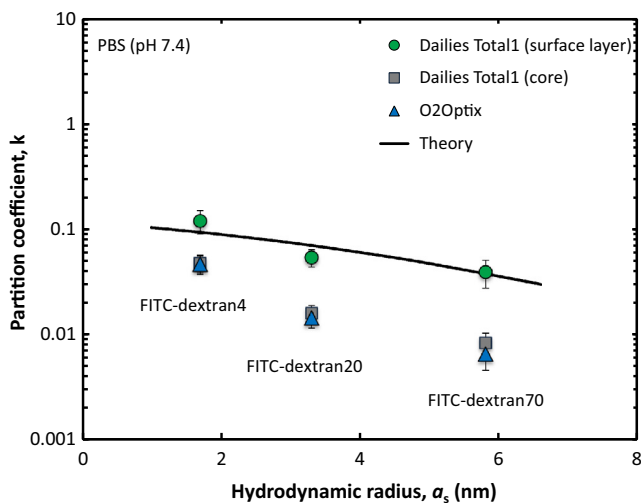


Fig. 4. Hydrophilic solute partition coefficients k as a function of hydrodynamic radius a_s at pH 7.4 for FITC-dextran4, FITC-dextran20 and FITC-dextran70, in DAILIES TOTAL1® and O2OPTIX™. The line is drawn according to Eq. (2), with $\phi_1 = 0.83$, $a_r = 6.6$ nm, $E_i^{cl} = 0.14$ and $E_i^{ad} = 1$.

corresponding partition coefficients are essentially identical. Larger solutes exhibit progressively smaller partition coefficients, indicative of size exclusion from the water domains of the SCL [13,16,17]. Importantly, solute partition coefficients in the DAILIES TOTAL1® surface-gel layers are greater than those in the underlying SiHy core, confirming higher water content in the surface layers. The solid line in Fig. 4 corresponds to predicted FITC-dextran partition coefficients in the surface-gel layer according to theory below (i.e. Eq. (2)).

Fig. 5 also plots aqueous solute partition coefficients as a function of hydrodynamic radius in DAILIES TOTAL1® and O2OPTIX™, but now for pH 4. At this lower pH, the hydrophilic solutes are partially anionic [13,38]. Trends are identical to those observed at pH 7.4: partition coefficients are identical in O2OPTIX™ and the DAILIES TOTAL1® SiHy core, and higher in the surface layers of DAILIES TOTAL1®. Partition coefficients again decline with increasing solute size. Here too, a solid line is drawn for the predicted FITC-dextran partition coefficients in the surface-gel layer according to Eq. (2) below.

Comparison of Figs. 4 and 5 reveals that, at pH 4, measured partition coefficients are large (i.e. greater than or equal to the water content), whereas at pH 7.4, measured partition coefficients are small (i.e. less than or equal to the lens water content) for the same solutes. This result is characteristic of aqueous ionized-solute partitioning into SCL materials due to repulsion between solutes and hydrogel strands of like-charge, and/or due to diminished specific interactions between ionized solutes and polymer chains [13]. At pH 7.4, FITC-dextran ($pK_a = 6.7, 4.4$ [38]) are dianionic. They are repelled from coionic SCLs and also exhibit weaker specific adsorption to uncharged SCLs compared with that of the counterpart neutral solutes [13]. Conversely at pH 4, the aqueous solutes are predominately neutral, and are taken up more strongly by the SCLs. Measured FITC-dextran partition coefficients in the surface layer of DAILIES TOTAL1® increase more substantially with decreasing pH compared with those of the SiHy core and O2OPTIX™, despite the larger surface-layer water content. Again, this result suggests that the DAILIES TOTAL1® surface gels are anionic. For O2OPTIX™ and the SiHy core of DAILIES TOTAL1®, however, increased uptake at pH 4 compared with that at pH 7.4 is most likely explained by greater specific adsorption on a neutral polymer matrix due to decreased solute ionization.

Table 3 displays measured partition coefficients for the cationic protein FITC-avidin at pH 7.4. Partitioning of similarly sized

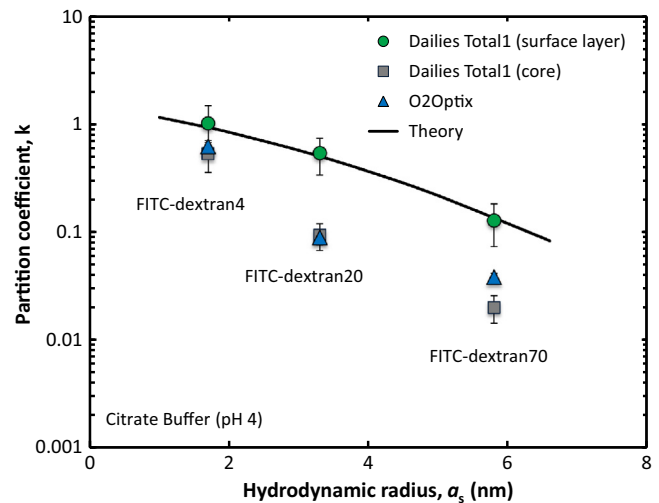


Fig. 5. Hydrophilic solute partition coefficients k as a function of hydrodynamic radius a_s at pH 4 for FITC-dextran4, FITC-dextran20 and FITC-dextran70 in DAILIES TOTAL1® and O2OPTIX™. The line is drawn according to Eq. (2), with $\phi_1 = 0.64$, $a_r = 6.6$ nm, $E_i^{cl} = 1$ and $E_i^{ad} = 2.3$.

Table 3

Partition coefficients of FITC-avidin and FITC-dextran20 in DAILIES TOTAL1® and O₂OPTIX™ at pH 7.4.

SCL	FITC-avidin	FITC-dextran20
DAILIES TOTAL1® (surface layer)	1.50 ± 0.75	0.054 ± 0.023
DAILIES TOTAL1® (core)	0.062 ± 0.023	0.016 ± 0.001
O ₂ OPTIX™	0.12 ± 0.05	0.014 ± 0.003

FITC-dextran20 is shown for comparison. In the DAILIES TOTAL1® core and O₂OPTIX™, partition coefficients for the positively charged protein FITC-avidin are larger than those of similar-sized FITC-dextran20, again owing to specific solute interaction with the polymer matrix. In the DAILIES TOTAL1® surface layer, however, FITC-avidin partition coefficients are nearly 30 times larger than those of FITC-dextran20. Strong specific adsorption of FITC-avidin to the negatively charged surface gel is indicated. We conclude that the high-water-content surface-gel layers of DAILIES TOTAL1® are anionic, whereas both the SiHy core of DAILIES TOTAL1® and O₂OPTIX™ are nonionic at physiological pH.

In contrast to the hydrophilic solutes in Fig. 4 that exhibit small partition coefficients in the SiHy core (and in O₂OPTIX™), oleophilic solutes (e.g. wax esters and sterols) have a high affinity for silicone microdomains [40]. Fig. 6 shows typical fluorescence-confocal-microscopy images of oleophilic Nile Red absorbed from silicone oil into water-saturated (a) DAILIES TOTAL1® and (b) O₂OPTIX™. Scale bars represent 20 μm in the vertical direction. Clearly, the oleophilic dye penetrates both SCLs after 2 d of loading. Table 4 summarizes partition coefficients for Nile Red and NBD-cholesterol in the DAILIES TOTAL1® core and O₂OPTIX™. Partition coefficients in the thin surface gel are not reported because the high intensity of the neighboring SiHy core interferes with measured intensities in the surface layer. Nevertheless, opposing fringes of low intensity (i.e. black) at the anterior and posterior of the lens are barely observed, confirming the presence of surface-gel layers.

Both Nile Red and NBD-cholesterol exhibit greater-than-unity partition coefficients in the SiHy core of DAILIES TOTAL1® and in O₂OPTIX™, apparently because of strong specific adsorption to silicone moieties [13]. Again, partition coefficients in the SiHy core of DAILIES TOTAL1® and in O₂OPTIX™ are similar (compare Fig. 6a and b). Delivery of oleophilic solutes from silicone oil into a water-saturated SiHy lens does not mimic that on eye. That process involves lipid-solute delivery from lens-deposited patches or spots of tear-film lipids and proteins, not from continuous oil [11,41,42]. However, since oily deposits on worn SCLs directly contact the lens surface, local oil-soluble solute delivery to a water-saturated bulk lens is not disparate to that from continuous oil.

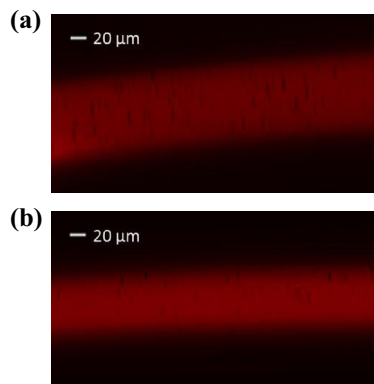


Fig. 6. Fluorescence-confocal-microscopy images of Nile Red at equilibrium in (a) DAILIES TOTAL1® and (b) O₂OPTIX™. Scale bars represent 20 μm in the vertical direction. The dye is dissolved in silicone oil and delivered to water-saturated lenses.

Table 4

Oleophilic solute partition coefficients in DAILIES TOTAL1® and O₂OPTIX™.

SCL	Nile Red	NBD-cholesterol
DAILIES TOTAL1® (core)	7.5 ± 0.5	5.8 ± 0.4
O ₂ OPTIX™	10.9 ± 0.9	7.4 ± 1.3

4. Discussion

We successfully implement fluorescent-solute partitioning experiments to characterize the layered structure of DAILIES TOTAL1® water-gradient lenses. Fluorescent-solute loading established a surface-layer thickness of ~10 μm, in good agreement with those reported earlier from atomic force microscopy [27,28]. Additionally, FCLSM measurements confirm consistently greater solute partitioning in the surface-gel layer, reflecting large water-filled liquid spaces and, therefore, higher water content compared with that in the SiHy core. Surface-layer mass water content was established as 82 ± 3% using ATR-FTIR compared with a core water content of 34 ± 6%.

Fluorescent-solute partitioning experiments revealed the similarity of the DAILIES TOTAL1® SiHy core and O₂OPTIX™. Both hydrophilic and oleophilic-solute partition coefficients in the SiHy core and in O₂OPTIX™ were nearly identical, regardless of the solute and aqueous pH studied. This is likely due to their similar structure and chemistry giving equal water contents of 33%, reflective of similar-sized water-filled meshes. Likewise, similar SiHy compositions and structure lead to similar solute-gel interactions [13,16,17]. Additionally, we find that silicone-microdomain size scales as submicron in both O₂OPTIX™ and DAILIES TOTAL1®, since they are smaller than the resolution of the confocal microscope (~1 μm). This is clearly shown by the locally uniform intensities in Figs. 3 and 6 and also by lens transparency.

Solute partitioning may also be used to determine the charge valence of SCLs. This chemical property is important for understanding care-solution and tear-film component uptake into lenses, since an ionic lens attracts counterion solutes and repels coion solutes [9,13,16]. Comparison of aqueous-solute partitioning at pH 4 compared with that at pH 7.4 reveals that the surface-gel layers of DAILIES TOTAL1® are anionic at physiological pH 7.4. The large partition coefficient of the positively charged protein, FITC-avidin, further supports this finding and also indicates specific ion binding of the protein to the negatively charged SCL-gel matrix. The large increase in surface-gel water content with increasing pH provides yet more confirmation of charged surface-gel layers.

Theory for hydrophilic-solute partition coefficients further documents our measured water content and charge valence of the DAILIES TOTAL1® surface gels. The partition coefficient k_i of an aqueous solute i in a homogeneous gel is the product of the water volume fraction ϕ_1 and individual enhancement factors [13]

$$k_i = \phi_1 E_i^{ex} E_i^{el} E_i^{ad} \quad (2)$$

where E_i^{ex} , E_i^{el} and E_i^{ad} denote size exclusion, electrostatic interaction and specific solute adsorption to the SCL polymer network strands, respectively. For an ideal point solute, k_i equals the hydrogel water volume fraction (i.e. $E_i^{ex} = E_i^{el} = E_i^{ad} = 1$). Actual solutes, however, are excluded from a gel matrix owing to finite size ($E_i^{ex} < 1$), are attracted ($E_i^{el} > 1$) or repelled ($E_i^{el} < 1$) by electrostatic interaction with the polymer chains, and may specifically adsorb to the SCL polymer strands ($E_i^{ad} > 1$) [13]. In Eq. (2), ϕ_1 is calculated from measured mass water contents of the surface layers (Table 2) and a dry polymer density characteristic of the SCLs (1067 kg m⁻³ [16]). Following Kotsmar et al. [16], E_i^{ex} is given by a mesh-size distribution (e.g. see Eq. (8) in Ref. [16]) with a fiber radius of 6.6 nm calculated

from rubber elastic theory and ϕ_1 (specifically, the fiber radius was calculated using Eqs. (6) and (8) in Ref. [16] with the measured surface-layer water volume fraction at pH 7.4 (Table 2), the length of a carbon–carbon bond (0.154 nm), the DAILIES TOTAL1[®] surface-gel elastic-modulus from nanoindentation tribology (0.025 MPa) [25], the Flory characteristic ratio typical of polymers with similar moduli ($C_n = 4$) [43], the dry polymer density characteristic of SCLs (1067 kg m⁻³) [16,17], and a typical molecular weight of a repeat unit (300 g mol⁻¹). At pH 4, the surface gels are essentially unchanged (i.e. the overall matrix monomer fractional degree of ionization, f_{i-} , equals zero). Therefore, we take $E_i^{el} = 1$ at pH 4 and fit an average $E_i^{ad} = 2.3$ to the measured solute partition coefficients following Dursch et al. [13]. Conversely, $E_i^{ad} = 1$ at pH 7.4, since FITC-dextran is highly water soluble and not likely to interact specifically with the SCL polymer strands [16]. In this case, the anionic degree of ionization is taken as an adjustable constant, $f_{i-} = 0.17$, and $E_i^{el} = 0.14$ is calculated from Eqs. (8) and (9) of [13]. Importantly, all parameters are physically based [13,16,19].

Lines in Figs. 4 and 5 for the surface-gel partition coefficients are predicted from Eq. (2) according to the chosen parameter set. Excellent agreement is found for both pH values studied. Although the calculation is impacted by parameter choice, hydrophilic-solute-partitioning theory confirms the high water content and negative charge of the surface-gel layers at physiologic pH 7.4. Theory is not available for the heterogeneous SiHy gels in Figs. 4 and 5.

Despite the high-water-content coating of DAILIES TOTAL1[®], oleophilic Nile Red and NBD-cholesterol partition into the underlying SiHy core. In fact, equilibrium partition coefficients in the SiHy core of DAILIES TOTAL1[®] are similar to those in O₂OPTIX[™] (Table 3). Oleophilic solutes penetrate the surface-gel layers by three possible mechanisms: (1) dissolution and bulk diffusion through the water fraction; (2) surface diffusion along polymer chains [17,44]; and (3) partial collapse of the surface gel when immersed in the oil, allowing more direct access of the Nile Red to the core lens. Significant collapse, however, is unlikely because of the extremely low solubility of water in silicone oil [45]. We saturated DAILIES TOTAL1[®] lenses with aqueous FITC-dextran4, equilibrated with FITC-dextran4-saturated silicone oil, and scanned with FCLSM. To within the precision of the experiment, no decrease in surface-gel thickness was observed, demonstrating diffusion through the surface gel as the most likely access mechanism.

Although the aqueous solubilities of Nile Red and NBD-cholesterol are small [46], they are apparently non-negligible. Bulk diffusion coefficients of similar sized solutes in water are of the order 10⁻⁶ cm² s⁻¹ [13]. Thus, dissolution and diffusion through the water fraction of the surface gel is a likely pathway for oleophilic-solute initial penetration into the supporting SiHy core of the lens [44]. Later saturation of the SiHy core by oleophilic dyes likely involves diffusion in both the hydrophobic silicone and hydrophilic phase-separated microdomains. In our experiments, the presence of a hydrophilic high-water-content surface gel did not prevent lipid penetration into a SiHy-lens core. We further conclude that transport of oil-soluble dyes through SiHy SCLs does not validate a percolated (e.g. gyroid [40]) microstructure for the silicone domains unless the aqueous-solute pathway can be completely eliminated.

5. Conclusions

Solute partitioning in SCLs provides valuable information on gel structure and composition in addition to quantifying uptake and release amounts. Using FCLSM and ATR-FTIR, we confirm the layered structure of DAILIES TOTAL1[®] with hydrophilic surface-gel layers of water content near 82% compared with 33% for the SiHy core. Consequent high aqueous-solute uptake was much higher in the surface gels compared with that in the SiHy-like core.

Changes in the ionicity of the aqueous solutes evaluated the charge valence on the surface-gel layers as anionic at physiologic pH 7.4. Despite the high-water-content surface-gel layers, oleophilic Nile Red and NBD-cholesterol partitioned significantly from continuous oil into the SiHy core of DAILIES TOTAL1[®] with greater-than-unity partition coefficients because of strong specific adsorption in the silicone domains. Fluorescent-solute partitioning is a useful tool for characterizing SCLs, especially those with surface coatings within the micron resolution of FCLSM.

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Appendix A. Figures with essential color discrimination

Certain figures in this article, particularly Figs. 1–6 are difficult to interpret in black and white. The full color images can be found in the on-line version, at <http://dx.doi.org/10.1016/j.actbio.2014.11.046>.

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