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Determination of Oxygen Permeability/Transmissibility and Storage of Contact Lenses Using HPLC with Reductive Electrochemical Detection in Combination with a Specifically Designed Sampling Unit

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A new analytical technique for the determination of oxygen permeability/transmissibility of contact lenses is presented in this paper. The method is based on high performance liquid chromatography (HPLC) with reductive electrochemical detection at -750 mV (vs Ag/AgCl) in combination with a patented sampling chamber that was designed especially for the purpose to determine oxygen at nanomolar levels. Compared to conventional method, the new technique exhibits higher sensitivity, selectivity, and versatility. The method permits the selective determination of oxygen permeability (Dk/L)/transmissibility (Dk) of soft as well as rigid contact lenses with good agreement with the Dk/L (Dk) values reported in the literature. Precision was determined by repeated measurements and yielded relative standard deviations of 3–8% for hydrophilic lenses and 5–13% for rigid contact lenses. Because of the extraordinarily high sensitivity of the chromatographic oxygen sensor for the first time, the capability of hydrogel lenses to store oxygen could also be directly monitored.

Contact lenses must transmit sufficient oxygen toward the cornea to ensure a minimum oxygen tension of at least 12–19 mmHg beneath the lens (corresponding to a closed eye lid) in order to allow the cornea to maintain its normal oxygen uptake rate. Otherwise, discomfort, swelling of the cornea, and other abnormal changes in the corneal tissue are very likely to occur.^{1–4} Though there are also other parameters to consider for successful contact lens wear, the adequate supply of the cornea with oxygen is of utmost importance. In recent years, considerable attention has been paid to the development of new contact lens materials with very high oxygen permeabilities that should be especially suitable for extended wear.⁵ As a consequence of these developments, not only theoretical and practical aspects of oxygen

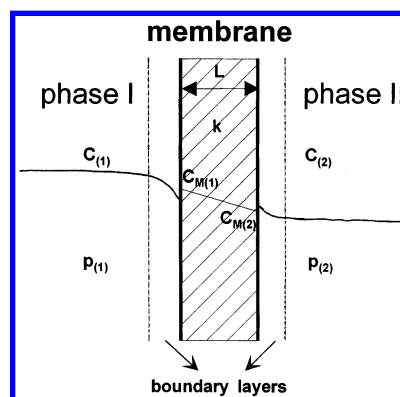


Figure 1. Schematic representation of the concentration profiles when a gas (oxygen) passes through a membrane.

transport characteristics across contact lenses have received increased attention but also analytical methodologies to measure this intrinsic property of lens materials^{6–9} have been studied. In the following section, a short review concerning the basic theoretical concepts and principles of measurement of oxygen permeability of contact lenses shall be given.

Fick's law of diffusion is the basis for the definition of both permeability and transmissibility of a membrane. Figure 1 shows the concentration profiles arising when a gas, for example, oxygen, passes through a membrane that separates two phases with different partial pressures of oxygen. The driving force for the passage of oxygen through a membrane of thickness L and area A is the concentration difference ($C_{M(1)} - C_{M(2)}$) across the membrane. According to Henry's law, the concentration of a dissolved gas is proportional to its partial pressure above the solution. Thus, a direct correlation between the flux j , defined as flow rate per unit area, and the partial pressure difference in phase I and phase II can be given according to the following equation:

$$j = \frac{1}{A} \frac{dn}{dt} = \frac{D}{L} (C_{M(1)} - C_{M(2)}) = \frac{Dk}{L} (p_{(1)} - p_{(2)}) \quad (1)$$

wherein j = oxygen flux; D = diffusion coefficient of oxygen [cm^2

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s^{-1} ; L = thickness of the membrane [cm]; A = diffusion area [cm^2]; $C_{M(1)}$ and $C_{M(2)}$ = oxygen concentrations [mol L^{-1}] within the membrane at the surfaces in contact with phase I and phase II, respectively; k = solubility coefficient of oxygen in the membrane material [$\mu\text{L (STP)}/\text{cm}^3 \text{ mmHg}$]; and $p_{(1)}$ and $p_{(2)}$ = the partial pressures of oxygen in phase I and phase II.

Oxygen flux is expressed in milliliters of oxygen (STP) per second per square centimeter. The abbreviation "STP" means that the gas volume is measured at standard temperature and pressure of 0 °C and one atmosphere. In contact lens literature, the product of the diffusion coefficient D and the solubility coefficient k , Dk , is used to denote the *permeability* of lens materials. Dk characterizes the rate of oxygen flow under specified conditions through a membrane of unit thickness when subjected to unit pressure difference. The value of oxygen permeability divided by the lens thickness L [cm], Dk/L , is termed *transmissibility*. Although the Dk value is an intensive physical property of the lens material independent of shape or thickness of the sample, the Dk/L value depends on lens design and lens thickness. The traditional unit to express the oxygen permeability, Dk , of a contact lens is $10^{-11} \text{ mL O}_2 \text{ cm}/(\text{cm}^2 \cdot \text{s} \cdot \text{mmHg})$, which has historically been referred to as "barrer".⁵ The traditional unit for oxygen transmissibility, Dk/L , is $10^{-9} \text{ mL O}_2/(\text{cm}^2 \cdot \text{s} \cdot \text{mmHg})$.

Many different methodologies have been developed to measure the oxygen transport characteristics of polymer membranes. In contact lens research, most of the Dk measurements are made with one form or other of electrochemical device,^{7,8} the most popular methods being the single-chamber "polarographic" (SCP) technique developed by Fatt et al.^{9–11} and the coulometric carrier gas (CCG) method.^{12–14} The Fatt method, which is based on the amperometric oxygen sensor (Clark electrode),¹⁵ has become standard for the determination of oxygen permeability of soft hydrogel lenses. Oxygen permeability of rigid contact lenses is usually determined as gas-to-gas permeability using the dual-chamber coulometric method, which is also more suited than the polarographic method for assessing lenses with oxygen permeability of over 100 barrer/cm. Although, at first sight, both "ISO" measurement systems seem to complement each other, it is well-known that both methods suffer from some problems and are still subject to experimental errors.

One of the main disadvantages of the coulometric method is that it can be used only for rigid lens materials and that the measurement takes very long. At the beginning, oxygen has to be removed completely from the chambers, and the samples have to be dried for ~48 h before the measurement. Failure to remove all residual oxygen from the chambers initially as well as uncontrolled diffusion of oxygen due to leakage of the test

chamber, absorbance, or release of oxygen by the material constituting the test chamber will lead to considerable errors in permeability determinations.

A further disadvantage arising from the measurement principle of amperometric sensors is oxygen consumption of the cathode during the measurement at rates that depend on the applied polarizing voltage, the size of the cathode, and the partial pressure of oxygen. Because measurements take several hours, a substantial amount of oxygen that has entered the chamber may be consumed in this time, leading to changes in the diffusion rate of oxygen to the cathode and to irreproducible and erroneous analytical readings.

Without the application of mathematical correction factors, the standard methods do not necessarily lead to the same results. In certain cases, differences between the dissolved oxygen permeability, determined with an aqueous-membrane-aqueous system, such as the Fatt method, and the gaseous oxygen permeability obtained with a gas-membrane-gas system (e.g., by the coulometric method) have been found. These discrepancies can be explained by experimental errors, known as edge effect^{11,16,17} and boundary effect,^{6,7,14,18,19} which unless corrected, will lead to an overestimation and an underestimation, respectively, of oxygen permeability of the lens material.

The edge-effect arises as a result of a mismatch between the size of the cathode and the sample during measurements with amperometric sensors according to the Fatt method. As an additional consequence, a lateral component of oxygen diffusion in addition to the perpendicular component is collected by the cathode from a sample area somewhat greater than the cathode diameter, leading to an overestimation of the oxygen permeability. This lateral component of oxygen diffusion has to be taken into account by complicated mathematical correction factors in order to obtain the "intrinsic" permeability value of the lens material.

The formation of stagnant liquid layers at the surfaces of the sample being measured will also produce a significant resistance to the flow of oxygen. This effect is known as the boundary effect and will result in underestimation of the oxygen permeability of the sample. The resistance of boundary layers is always of concern when the sample is measured with one or both surfaces in contact with fluid, especially when the sample is very thin or of high oxygen permeability. The correction for this effect is achieved by measuring several samples of the same material but of different thicknesses. Plotting the reciprocal of the measured oxygen transmissibility (L/Dk) versus the lens thickness L results in a straight line and from the inverse of the slope of the line the intrinsic, or true, Dk of the material can be derived. The intercept of the linear regression line on the L/Dk axis is the sum of any diffusional resistances of all boundary layers, including also the resistances due to the sensor membrane and layers between sample and membrane. The same corrections have to be made to account for the various layers of resistance between lens and cathode in an amperometric oxygen sensor (Fatt method). Changes in sensor-membrane permeability have to be taken into

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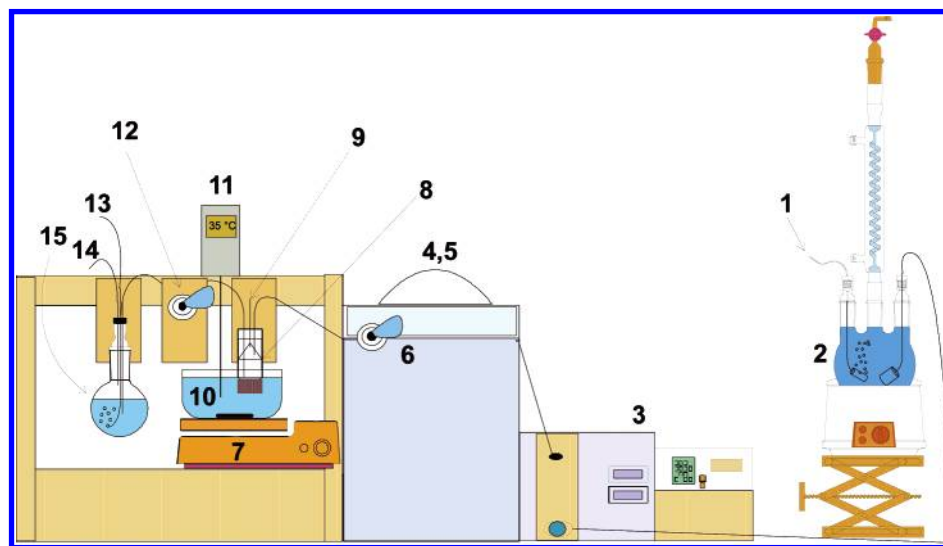


Figure 2. Scheme of the chromatographic oxygen sensor, sampling chamber, and the sample application system for the determination of oxygen permeability: (1) inlet for helium, (2) solvent (mobile phase) reservoir and heating mantle, (3) HPLC pump, (4, 5) amperometric detector (column inserted), (6) injection valve II for HPLC, (7) magnetic stirrer, (8) sample chamber, (9) overflow of the chamber, (10) thermostated water-bath, (11) contact thermometer, (12) injection valve I for calibration by standard additions, (13) overflow of the reservoir, (14) inlet for argon, (15) filling solution (0.95% NaCl (w/v)).

account during calibration; otherwise, they will lead to erroneous analytical results.

Since manufactureres very often do not publish the apparatus and methodology that have been used to obtain the permeability data of the lenses, direct comparison of Dk values from one manufacturer's lens to another's may be very difficult for the optician and, finally, for the customer. Therefore, it still seems to be important to develop new methods for permeability determinations that lead to unequivocal results for all kinds of lens materials without complicated correction factors being necessary. In this paper, we report about the use of the chromatographic oxygen sensor as a new measurement system for transmissibility/permeability determinations, which exhibits several advantages as compared to the conventional methods.

EXPERIMENTAL SECTION

Apparatus. Apart from the chromatographic system that has already been described elsewhere in detail,^{20,21} a second chromatographic system was applied in this work, which consisted of a low-pressure gradient pump (Rheos 4000, Flux Instruments, Basel, Switzerland) in combination with an injection valve (model 7010, Rheodyne, Supelco, Vienna) with a 20- μ L sample loop. The column was 60 \times 4 mm i.d. packed with 5- μ m LiChrospher RP-8 particles (including precolumn). An amperometric detector (DECADE, Antec Leyden, Netherlands) with an electrochemical flow-cell (model VT-03, Antec Leyden, Netherlands) consisting of a three-electrode configuration with a glassy carbon working electrode, a silver/silver chloride (3 M KCl) reference electrode, and a glassy carbon auxiliary electrode was used for electrochemical detection. Recording of the chromatograms as well as quantitative data analysis was accomplished on a PC-based data system (Chromeleon, Version 6.0, Dionex, Vienna). The barometric air

pressure was determined at least once a day using a barometer of high precision (Lufft, Fellbach, Germany).

Reagents. Buffer solutions were prepared by dissolving the appropriate amount of phosphate salts, $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ and Na_2HPO_4 (puriss. p.a., Fluka, Vienna) in high-purity water (Epure, Barnstead Co., Newton, MA). The pH value of the final solution (ionic strength 0.01 mol L⁻¹) was controlled with a glass electrode and a calibrated pH meter. Acetonitrile was obtained from Fluka (puriss. p.a.). The mobile phase consisted of phosphate buffer (pH 7.0) and acetonitrile (9:1, v/v) and was filtered through 0.45- μ m cellulose acetate filters (Millipore Corp.) before use. NaCl, 0.95% (w/v), (puriss. p.a., Fluka, Vienna) was dissolved in high-purity water and used as filling solution. Helium 5.0 (AGA, Schwechat, Austria) and argon 5.0 (AGA, Schwechat, Austria) in combination with an oxsorb scrubber (AGA, Schwechat, Austria) were used for deoxygenation of the mobile phase and the filling solution.

Oxygen Removal from Mobile Phase and Filling Solution. The mobile phase was refluxed at 60–80 °C, and helium gas was gently bubbled through the liquid. Deoxygenation of the mobile phase was performed overnight (at a minimum eluent flow rate of 50 μ L min⁻¹) and continued during the measurement. All plastic (Teflon) tubings were replaced by stainless steel, glass or titanium capillaries. The filling solution in the solvent reservoir was deoxygenated by bubbling argon through the solution for at least 20 min before filling the sampling chamber.

Two-Chamber-Siphon Technique. This technique has been described elsewhere in detail.^{20,21} The oxygen measurement system consisted of a sample chamber, an additional injection valve (10-port, Valco Instruments Co. Inc.), and a solvent reservoir. The chamber and the application system together with the chromatographic oxygen sensor based on HPLC-EC are depicted in Figure 2. To measure the oxygen permeability/transmissibility of contact lenses, the sample chamber had to be modified so that a contact lens together with a sealing could be tightly fixed by a

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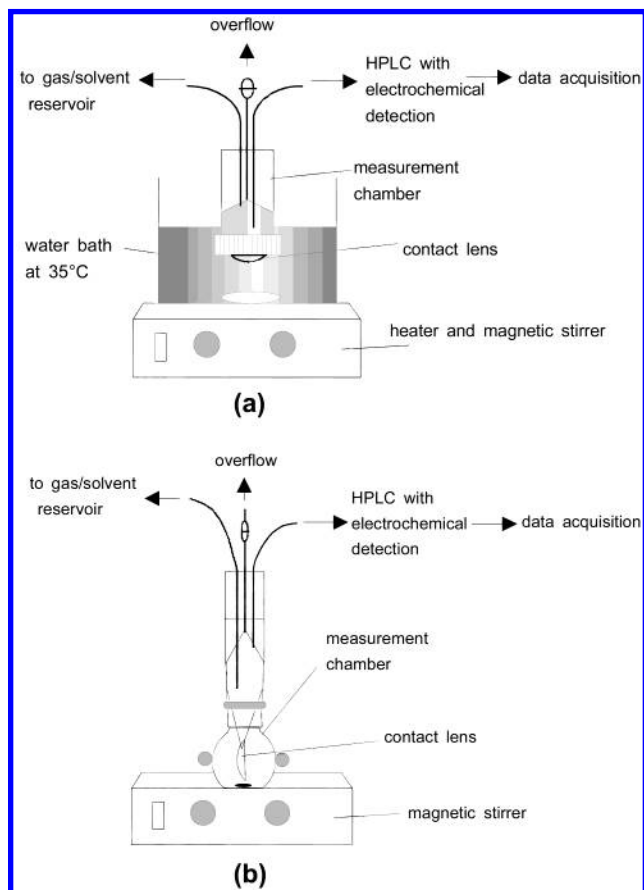


Figure 3. Different design of the sampling chambers used for measuring (a) the oxygen permeability/transmissibility of contact lenses and (b) the storage of oxygen by a lens.

screw cap onto the lower part of the sampling chamber (Figure 3a).

Measurement of Oxygen Permeability/Transmissibility.

The contact lens was fixed to the sampling chamber, and the chamber with the lens was immersed into a tempered water bath (35 °C), which was saturated with air. All air bubbles at the bottom of the contact lens were removed by a syringe adapted especially for this purpose with cannula and plastic tip. For ~10 minutes, argon (pressure 0.5 bar) was passed into the chamber to generate an oxygen-free atmosphere within. Then the chamber was filled with oxygen-free electrolyte (0.95% NaCl solution (w/v)). The changes in the oxygen concentration in the chamber with time were monitored at short time intervals (every 2 min) for 25 min. For this purpose, small aliquots of the solution (150–200 μ L) were removed from the chamber and transferred into the sample loop by pulling at the injection syringe of injector II (suction loading instead of direct injection, with the injector being in load position) and then injected into the solvent stream. The volume removed from the chamber was replaced simultaneously and automatically by deoxygenated solvent from the reservoir. In the detection unit, oxygen was reduced at a constant potential of –750 mV (vs Ag/AgCl/(3M KCl)), and the corresponding reduction current was measured. The increase in the reduction current with time reflects the oxygen flux across a given lens into the chamber.

Calibration. For calibration of the measurement system, an air-saturated aqueous solution was used as the oxygen standard. The oxygen concentration of air-saturated water depends on temperature and pressure and can be found in reference tables

or calculated from a semiempirical equation.^{15,20,21} A stainless steel capillary immersing into a water bath (at 35 °C) was connected to injector II (6), and the 20- μ L sample loop was filled with air-saturated aqueous solution (“oxygen standard”) by pulling at the injection syringe. The solution was injected into the solvent stream, and the current corresponding to the reduction of oxygen was recorded. This procedure was carried out at least three times.

$$O_2 [\text{mol}] = V_{\text{inj}} C_{\text{sat aq soln}} \quad (2)$$

$$i_{\text{meas}} \frac{V_{\text{inj}}}{V_{\text{chamber}}} = i_{\text{dil soln}}$$

The current corresponding to the reduction of a defined amount of oxygen (eq 2) can be calculated [nA/mol O_2] from the calibration measurements after having taken into account the dilution effect that would occur if the same solution were injected directly into the sampling chamber.

Calculation of the Oxygen Permeability (Dk/L)/Transmissibility (Dk). The amount of oxygen [nmol] diffusing into the chamber was calculated by dividing the peak currents [nA] by the ratio of current to amount of oxygen [nA/nmol O_2] obtained during the calibration procedure. Transformation of moles into milliliters of oxygen [cm^3] was achieved by multiplication with the value of the STP molar volume (22.4 L/mole of an ideal gas at a standard temperature of 0 °C and a standard pressure of one atmosphere). The oxygen volumes were plotted versus time, and the slope (b) of the resulting straight line reflected the amount of oxygen passing the lens and diffusing into the chamber within a given time. From the slope b , the Dk value [$\text{cm}^3 \cdot \text{cm} / \text{s} \cdot \text{cm}^2 \cdot \text{mmHg}$] was calculated according to eq 3,

$$Dk = \frac{bL}{ApO_2} \quad (3)$$

where L = the thickness of lens [cm], A = the diffusion area of the exposed lens [cm^2], and pO_2 = the partial pressure of oxygen [mmHg].

The partial pressure of oxygen is given by the formula

$$pO_2 = 0.2094(p_0 - p_{H_2O}) \quad (4)$$

where p_0 is the barometric air pressure [mbar] corrected by the partial pressure of water vapor p_{H_2O} . The dependence of vapor pressure on temperature can be expressed by eq 5.¹⁵

$$p_{H_2O} = 0.01 \times 10^{(10.09 - 1668/(228 + T(^{\circ}\text{C})))} \quad (5)$$

Test of Tightness of the Sampling Chamber (Edge Effect).

Instead of a contact lens, a piece of Parafilm or aluminum foil, which had been cut into the same size as the lens, was fastened to the sampling chamber, and the measurements were performed as described above.

Elimination of the Resistance of Boundary Layers (Boundary effect). The influence of the resistance of boundary layers was canceled by performing repeated measurements of the same sample as described above, varying the thickness of the lens material by stacking two to a maximum of five lenses, carefully

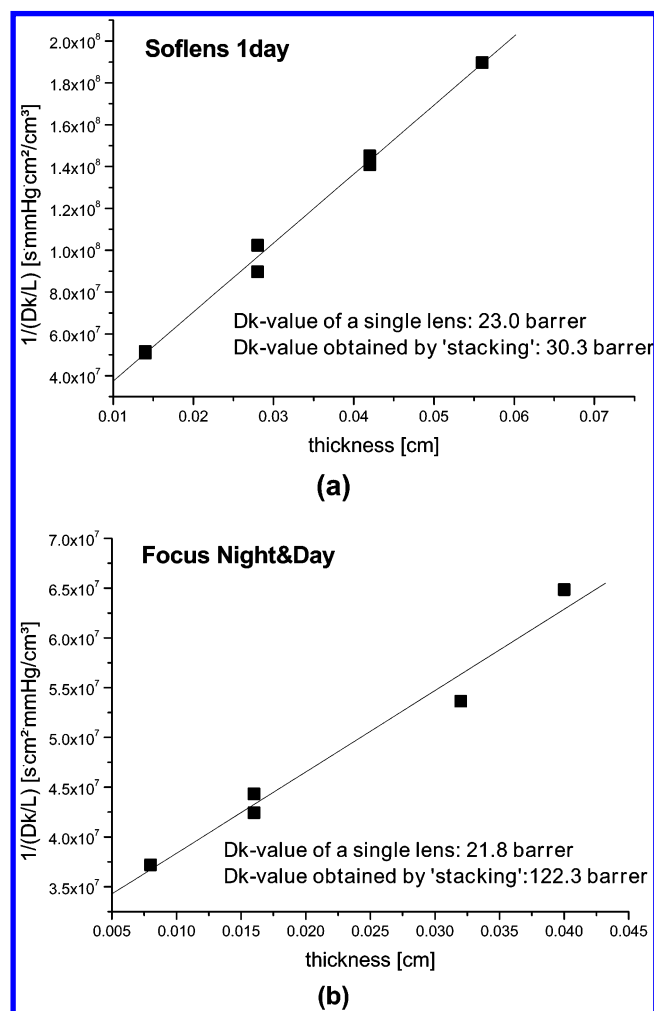


Figure 4. Results of stacking experiments performed with two different types of soft lenses: (a) Soflens 1day, (b) Focus Night&Day.

avoiding trapped air in between. In a diagram, the reciprocal of the measured transmissibility, L/Dk , was plotted against the lens thickness (Figure 4) and from the reciprocal of the slope of the resulting straight line the intrinsic Dk value of the lens material was obtained.

Measurement of Oxygen Storage by a Lens. The lens was fixed on a glass capillary in the closed chamber depicted in Figure 3b. Chamber and lens were deoxygenated by passing argon through the chamber for a defined period of time (15–20 min). After having filled the chamber with oxygen-free electrolyte solution, a defined small amount of oxygen was added to the solution. Concentration changes within the chamber were monitored by removing aliquots of the solution and measuring the oxygen content in the detection unit as described above.

RESULTS AND DISCUSSION

The apparatus used for the transmissibility/permeability determinations described in this paper is schematically depicted in Figure 2. It is based on conventional high-performance liquid chromatography with reductive electrochemical detection in combination with a patented sample application system,²² which was developed at the University of Innsbruck for the determination

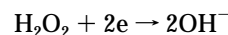
of oxygen at nanomolar levels.^{20,21} The sample application system permits the injection of a sample solution containing very low amounts of oxygen into the solvent stream without the interference of atmospheric oxygen. This is achieved by coupling the sampling chamber with a solvent reservoir containing a deoxygenated electrolyte solution. Whenever a small aliquot of the sample solution is removed by suction with a syringe, the removed amount of liquid is replaced automatically and simultaneously by deoxygenated solution from the reservoir. Thus, uncontrolled influx of oxygen during the injection process can be avoided.

The chromatographic conditions were further optimized with respect to a relatively short retention time ($t = 1.5$ min) and sufficient separation of the oxygen reduction peak from the injection signal. The best results were obtained with a LiChrospher 100 RP-8 ec column (60×4 mm, including precolumn) with a mixture of phosphate buffer (pH 7, ionic strength 0.01 mol L^{-1}) and acetonitrile in a volume ratio of 9:1 (v/v) as mobile phase at a flow rate of 1 mL min^{-1} . Figure 3 shows the sampling chambers that have been developed and optimized for the determination of oxygen transmissibility/permeability as well as oxygen storage of contact lenses.

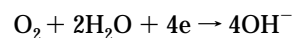
At the beginning of the transmissibility measurement, the lens is fixed tightly to the sampling chamber, then chamber and lens are immersed into a tempered water bath at 35°C that is saturated with air. After deoxygenation with argon, the chamber is filled with oxygen-free electrolyte. In this way, both sides of the lens are in contact with liquid phase, which corresponds largely to the physiological situation. Because of the difference in partial pressures in front and in back of the lens (inside and outside of the chamber), oxygen is driven across the lens into the sampling chamber, and the oxygen content within the chamber increases steadily. The changes in oxygen concentration within the chamber with time are measured by removing small aliquots of the electrolyte at selected time intervals and injecting them into the solvent stream. In the detection unit, oxygen contained in the injected solution is reduced at a constant potential of -750 mV (vs Ag/AgCl reference electrode), and the resulting reduction current is recorded as a function of time. The mechanism of the electrochemical reduction of oxygen is rather complex, because it involves multiple electron and proton-transfer reactions.^{23,24} In aqueous solution, the mechanism depends on electrode material, pH, and electrode potential, the most common reaction pathway being the two-electron reduction to hydrogen peroxide as an intermediate and the four-electron reduction to water (or in neutral and basic media to OH^-) (equation 6). At mercury and glassy carbon (GC) electrodes, oxygen is reduced in two well-defined steps according to eq 6, separated by $\sim 0.3 \text{ V}$ (GC)²³ up to 1 V (mercury).^{20,24}



and



overall reaction:



(22) Stubauer, G.; Seppi, T.; Obendorf, D.; Lukas, P. Österreichisches Patent 406712, 1999.

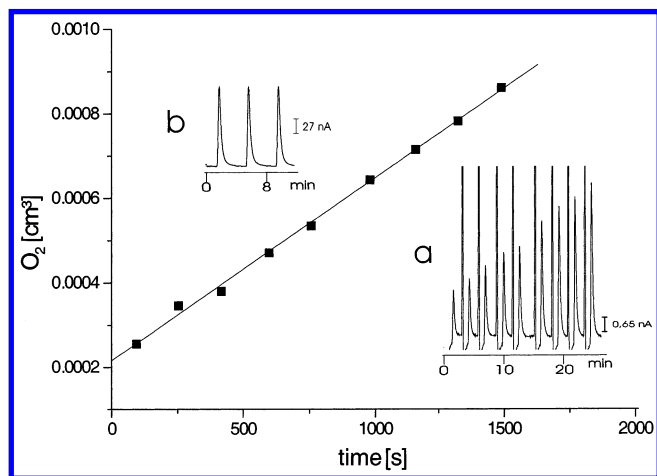


Figure 5. Measurement of the oxygen permeability of a lens: (a) corresponding chromatogram monitoring the increase of the oxygen content in the sampling chamber with time, (b) chromatogram obtained during the calibration procedure.

Although the highest and, thus, the most sensitive analytical signal would be expected at more negative potentials (< -1.0 V), in the potential region of a four-electron transfer, the best results concerning both sensitivity and signal-to-noise ratio were obtained at potentials between -600 and -800 mV. At the selected potential of -750 mV, sensitivity is lower because only the two-electron reduction of oxygen is likely to occur, leading to the formation of hydrogen peroxide as an intermediate. On the other hand, at this potential, the background current was lower than 5 nA with a baseline noise lower than 5 pA. Therefore, all measurements were performed at a working electrode potential of -750 mV.

In contrast to the ISO–Fatt method or coulometric method, in which a constant flux of oxygen to the cathode leads to the measurement of a steady state current, the chromatographic oxygen sensor monitors directly the diffusion of oxygen through the lens into the chamber. A typical chromatogram and the corresponding i_p/t diagram in Figure 5 show the increase of the reduction peak current i_p with time corresponding to the gradual increasing oxygen concentration within the sampling chamber. Calibration is most easily achieved by injection of a defined amount of air-saturated water (Figure 5b), the oxygen concentration of which can be easily derived from tables.¹⁵ Since there is a direct correlation between the amount of oxygen and the reduction peak current, the Dk value of the lens can be directly calculated from the increase of the peak currents with time according to eqs 2 and 3 (see Experimental Section).

Compared to the standard methods, the new measurement system exhibits several advantages. First of all, the new technique is by far more sensitive and accurate. Oxygen concentrations down to 4.5×10^{-9} mol L⁻¹ can be detected with the chromatographic oxygen sensor. This concentration is ~ 2 – 3 orders of magnitude lower than the concentration that can be measured with any other conventional method. Thus, very small changes in oxygen concentration within the chamber can be registered reliably with HPLC/EC-detection.

Table 1. Statistical Data of Oxygen Transmissibility (Dk/L) Determinations of Some Soft Contact Lenses with the Chromatographic Oxygen Sensor (HPLC/EC)

contact lens	dioptr	Dk/L^a cm ³ /s cm ² mmHg	SD	RSD %
Optima 38	3	16.0×10^{-9}	1.10×10^{-9}	6.84
Soflens 1 day	-3.25	27.2×10^{-9}	8.49×10^{-10}	3.12
Conso	4.25 u. 4.5	13.9×10^{-9}	9.90×10^{-10}	7.12
One Soflens	-2	16.4×10^{-9}	1.47×10^{-9}	8.97
GoldMedalist	-4.75	18.0×10^{-9}	1.04×10^{-9}	5.80
Unilens	4.5	10.8×10^{-9}	6.86×10^{-10}	6.32
Pure Vision ^b	-3.25	37.5×10^{-9}	1.63×10^{-9}	4.34

^a Mean of four measurements. ^b Dk value obtained after measurement of one single contact lens (instead of performing stacking-experiments).

The tightness of the sampling chamber and screw cap were thoroughly tested in order to avoid all errors that might arise due to uncontrolled diffusion of oxygen into the sampling chamber (edge effect). If an oxygen-impermeable membrane, such as Parafilm or aluminum foil was fastened to the chamber instead of the lens, the influx of oxygen was negligibly small. Five times out of six, the current values increased by < 0.2 nA within 30 min, whereas in the presence of a lens, the current increased between ~ 5 and 20 nA within 30 min (between 0.17 and 0.67 nA min⁻¹), depending on the lens type used. This means that the maximum level of leakage of 6.7 pA/min is considerably lower than the values obtained with lenses and can therefore be neglected. Thus, in contrast to the standard methods where zero oxygen currents or voltage outputs have to be compensated for, no correction of the measured peak currents is necessary.

Further advantages arise from the spatial separation of sampling chamber and electrochemical detection unit. As a consequence, there is no effect of temperature on the measurement, and there are no erroneous results caused by oxygen consumption of the cathode. Any conventional electrochemical detector can be used because size and shape of the working electrode do not influence the diffusion of oxygen through the lens into the chamber, and thus, in contrast to the ISO–Fatt method, there is no need for complicated correction factors to compensate for the effect of different electrode geometries on the results of the measurement. In this paper, the same permeability data were obtained with two different electrochemical detectors equipped either with a glassy carbon electrode or a hanging mercury drop electrode as the working electrode (see Experimental Section).

Compared to other methods, determination of permeability/transmissibility values of contact lenses is rapidly achieved with the chromatographic oxygen sensor. Immediately after having filled the sampling chamber with oxygen-free electrolyte, the increase of the oxygen concentration with time within the chamber can be registered. There is no need to wait for steady state-flux conditions, as is the case in both ISO-methods, which can take hours if lenses with low oxygen permeability are analyzed.

The new system is extremely versatile. Dk/L values of both soft and rigid contact lenses can be determined with very good reproducibility and with good agreement with the values published in the literature. In Tables 1–4, the results of the Dk determinations of soft and rigid lenses are summarized. Hard lenses are

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Table 2. Comparison of the Dk Values Obtained with HPLC-EC for Some Soft Contact Lenses to Values Reported in Literature or Given by the Manufacturer

code	lens material	contact lens (manufacturer)	Dk value		
			(HPLC-EC) single lens [barrer]	(HPLC-EC) stacking [barrer]	(ISO-Fatt) stacking [barrer]
A	Ocufilcon D	Fit 58 UV (Hecht)	19.6	20.6	21.6
B	Alphafilcon A	Soflens 66 (B&L)	32.5	34.1	32
C	Balafilcon A	Pure Vision (B&L)	25.2	75.7 u. 117.4 ^a	110
D	Etafilcon A	Acuvue 1day (J&J)	22.3	20.9	21.4
E	Hilafilcon A	Soflens 1day (B&L)	23.0	30.3 ^b bzw. 36.8 ^c	35
F	Lotrafilcon A	Focus Night&Day (Ciba Vision)	21.8	122.3 u. 125.2	> 140 ^d
G	Nelfilcon A	Dailies (Ciba Vision)	20.2	26.3	26
H	Hioxifilcon A	Extreme H ₂ O (Galifa)	19.9	22.8	20
V	GMA-HEMA Cop	Visell (Hecht)	15.4	15.2	15

^d Determined with a modified coulometric method.⁵ ^a Two different charges. ^b Two different values of L were provided by the manufacturer for the same lens type: (b) calculated with a lens thickness L of 0.014 cm and (c) calculated with a lens thickness L of 0.017 cm.

Table 3. Results of Oxygen Transmissibility (Dk/L) Determinations of Some Hard Contact Lenses with HPLC/EC

contact lens	dioptr	Dk/L ^a [cm ³ /s cm ² mmHg]	SD	RSD [%]
Persecon	-3.25	3.80×10^{-9}	4.24×10^{-10}	11.2
Ascon-XL	-10.5	5.90×10^{-9}	4.24×10^{-10}	7.19
Ascon_BO7	-11.25	8.65×10^{-9}	7.07×10^{-11}	0.82
Ascon_ES	-23.75	3.45×10^{-9}	2.12×10^{-10}	6.15

^a Mean of three measurements.

usually not ready to use but are especially fabricated to fit the eye geometry of a patient and, therefore, rather expensive. This is the reason that only a few hard lens types could be investigated in this study (Table 3). Instead, RGP (rigid gas permeable) test buttons with a constant thickness of 0.025 cm were analyzed, to be able to compare Dk values of rigid contact lens materials obtained with the new method to literature values. The results are presented in Table 4. Reproducibility (given as relative standard deviations, $n = 4$) of the Dk measurements lay between 3 and 8% for hydrogels and between 5 and 13% for rigid contact lens buttons.

Usually, there is a very good agreement between the Dk values obtained with the new HPLC/EC technique and the values reported in the literature. It has to be noted, however, that with any method measuring oxygen permeability in solution, correction for boundary layers has to be made if the Dk value of the lens is higher than, let us say, 50 barrer, which is best achieved by so-called stacking experiments,^{6,7,14,18,19} as described in the Experimental Section. In Figure 4, the results of stacking experiments are demonstrated for two lens types differing extremely in oxygen permeability. In the case of the lens with high oxygen permeability, there is an enormous difference between the Dk value obtained by measuring one single lens (21.8 barrer) and the value obtained by stacking experiments (122.3 barrer) (Figure 4b), whereas almost the same values are obtained for a lens with low oxygen permeability (23.0 for a single lens and 30.3 barrer obtained by stacking) (Figure 4a). Thus, whenever the oxygen permeability of an unknown lens material is to be investigated in solution, stacking experiments are highly recommended.

When we did measurements with various hydrogels, we made some striking observations. We noticed that the results of the Dk

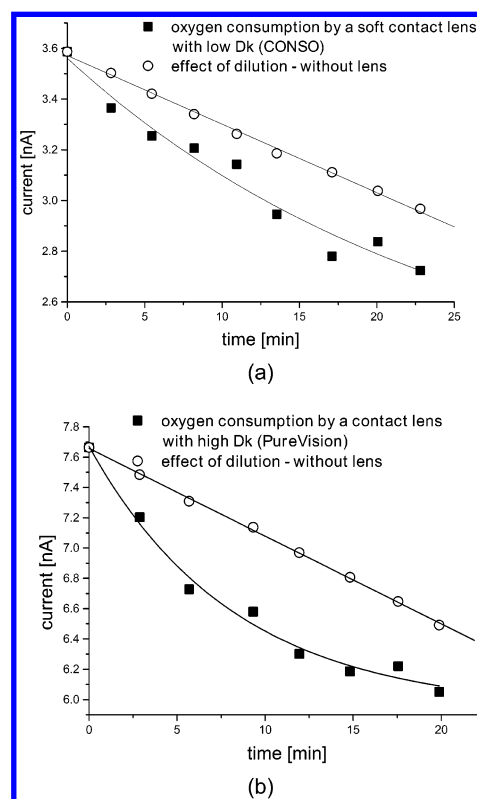


Figure 6. Monitoring of the storage of oxygen by (a) a soft contact lens (Conso) with low Dk value and (b) a soft contact lens (Pure Vision) with high Dk value.

determinations depended strongly and reproducibly on the pretreatment of the lens before the measurement. If the lens was taken directly out of the wrapping, a lower Dk value was obtained than in cases where the lens had been stored for 24 h in air-saturated Renu solution. The difference was usually significantly higher than the standard deviation obtained for repeated measurements of the same lens type under defined experimental conditions. For some lens materials (Table 5), a difference as large as 20% was found. This behavior can only be explained if an interaction between oxygen and the lens occurs, e.g., dissolution or storage of oxygen within the material. To prove this assumption, we did the following experiments using the type of chamber depicted in Figure 3b. A lens was fixed in the closed chamber, which was then degassed with argon and filled with deoxygenated

Table 4. Comparison of Dk Values Obtained with HPLC/EC for Some Hard Contact Lens Buttons^a to the Values Reported by the Manufacturer

contact lens material	Dk^b [cm ³ cm/s cm ² mmHg]	SD	RSD [%]	Dk^c [cm ³ cm/s cm ² mmHg]
Boston7	46.4×10^{-11}	5.05×10^{-11}	10.9	49.0×10^{-11}
Boston4	21.3×10^{-11}	2.05×10^{-11}	9.65	19.0×10^{-11}
BES	19.3×10^{-11}	2.55×10^{-11}	13.2	18.0×10^{-11}
BEO	31.1×10^{-11}	3.85×10^{-11}	12.4	58.0×10^{-11}
BXO	65.6×10^{-11}	3.61×10^{-11}	5.50	100×10^{-11}

^a $L = 0.025$ cm. ^b Mean of four measurements. ^c Obtained from literature/manufacturer.

Table 5. Influence of Storage Conditions on the Results of Dk/L Measurements of Some Soft Contact Lenses

contact lens	Dk/L^a [cm ³ /s mmHg cm ²]	Dk/L^b [cm ³ /s mmHg cm ²]	difference [%]
Unilens	10.9×10^{-9}	11.8×10^{-9}	7.63
Hilafilcon	16.8×10^{-9}	21.2×10^{-9}	20.8
Focus Night&Day ^c	27.1×10^{-9}	30.1×10^{-9}	9.97

^a Lens taken directly out of wrapping. ^b After storage of the lens for 24 h in air-saturated lens care solution (Renu, B&L). ^c Dk/L value obtained after measurement of one single contact lens (instead of performing stacking-experiments).

saline solution. After having added a defined amount of oxygen, the changes in oxygen concentration within the chamber were monitored for ~20 min. The same experiment was performed without a lens. The results are demonstrated in Figure 6.

If no lens is present, the oxygen concentration decreases linearly in the chamber as a result of the gradual but constant dilution occurring during the measurement, because defined small amounts of the solution are removed and replaced simultaneously by deoxygenated solution from the reservoir. In the presence of a soft lens, the oxygen concentration decreases much faster and exponentially. The half-life period, that is, the time it takes until the oxygen concentration reaches half the value of the initial concentration, can be used to express the capability of a soft lens to store oxygen. The extent of oxygen consumption by the lens seems to depend primarily on the polymer type of the lens, its water content, or both. There might even be a correlation between the half-life period and the term k (solubility coefficient of oxygen within the material) in the Dk value of a lens, a shorter half-life period indicating a higher k value. To our knowledge, this is the first time that this feature can be directly monitored by an analytical method, and this might be a new way to characterize the capability of soft lens materials to supply the cornea with oxygen. However, these results can be considered as only preliminary, and further studies will have to be performed to quantify reliably the ability of hydrogels to store oxygen. In the case of RGP (rigid gas permeable) materials, no storage effects could be noticed.

CONCLUSION

The results presented in this paper demonstrate that the combination of liquid chromatography with reductive electro-

chemical detection together with a specially designed sampling chamber provides a very powerful alternative to already existing methods for the determination of oxygen transmissibility and permeability of contact lens materials. The new method is not only superior in sensitivity and accuracy, but also much faster and more versatile. Dk values of rigid and soft contact lens materials can be determined in the same manner with good reproducibility and good agreement with published data. Moreover, for the first time, the capability of hydrogels to store oxygen could be directly monitored, which until now was not possible with any other analytical method. In the future, after having performed further studies, this feature might prove to be an additional quality criterion for wearing comfort of soft contact lenses.

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