

Influence of environmental factors in the *in vitro* dehydration of hydrogel and silicone hydrogel contact lenses

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Abstract: Purpose: To analyze *in vitro* the influence of different environmental conditions on the dehydration pattern of seven currently marketed hydrogel (Hy) and silicone hydrogel (Si-Hy) contact lenses (CL). Methods: Three Hy and four Si-Hy CLs were evaluated. CLs were exposed to four different relative humidity (RH) conditions (5%, 30%, 50%, and 70%) and two air flow (AF) rates (0 and 2.75 m/seg) within an environmental chamber. Dehydration was assessed using the gravimetric method. Data were taken at baseline, 5, 10, 15, 20, 30, 45, 60, 90, and 120 minutes of exposure. Dehydration rate (DR), valid dehydration (VD) and stabilization time were calculated. Results: The interaction between RH, AF and the type of the CL material had a significant effect ($p \leq 0.03$) on DR up to 60 minutes. The maximum differences

in VD values among CL occurred around 15 minutes exposure varying from 25.16% to 42.75%. Stabilization time was quicker under the 5%RH with AF condition than under 70% RH without AF one for most CLs. Conclusions: Lower RH seems to increase CL dehydration being further accelerated with the AF presence. The dehydration pattern is material dependent, thus current marketed CLs behave differently under several controlled environmental conditions. Future *in vivo* studies should confirm these outcomes. © 2013 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 00B: 000–000, 2013.

Key Words: contact lens, dehydration, relative humidity, air flow, environmental chamber

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INTRODUCTION

Dehydration of hydrogel (Hy) contact lens (CL) plays a major role in the corneal epithelium integrity¹ and can have a negative impact on CL comfort.^{2–4} Dehydration begins just after CL placement on the eye and it is influenced by several factors such as material features,^{5,6} lens thickness,⁷ or environmental conditions.^{6,8,9} Hydrogel CL wearers are continuously exposed to different environmental conditions such as artificially air-conditioned rooms, vehicles and aircraft cabins; and these indoor conditions may increase CL dehydration provoking the appearance of ocular discomfort and dryness sensation.^{10–14}

Previous studies have evaluated *in vitro* CL dehydration under controlled environmental conditions. Jones et al.¹⁵ have found that adverse environmental conditions [20% relative humidity (RH) and 9ml/min air flow (AF) vs 60%RH and 9ml/min AF] have a negative influence on *in vitro* dehydration, and that increasing AF (60%RH, 30 ml/min AF) have a greater impact than reducing RH on dehydration

rates. Furthermore, González-Méijome et al.^{16–18} have found that HEMA-based lenses dehydrate to a greater extent and faster than silicone hydrogel (Si-Hy) materials, and that initial *in vitro* CL dehydration rate is faster once the lens has been already worn. The importance of CL dehydration is such that, even some authors have created predictive *in vivo* dehydration models based on previous *in vitro* data, reporting that *in vitro* could differ from *in vivo studies* because of variety of external factors like temperature (T), chemical potential of the tear, and dehydration by other mechanisms.¹⁸

Nevertheless, there are some discrepancies in studies performed *in vivo*. Whereas there are some authors who have found correlation between the presence of adverse environmental conditions and the worsening of signs and symptoms of dry eye disease,^{12–14,19} other authors have not found any significant variation in dehydration levels of CL among different environmental conditions.²⁰ Thus, there might be a lack of agreement on the influence that environment might have in CL dehydration between *in vivo* and

Additional Supporting Information may be found in the online version of this article.

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in vitro studies, taking into account that environmental conditions differed between studies.^{12–14,19}

In addition, there are new CL materials in the market, such as second and third generation of Si-Hy, whose chemistry and/or water content (WC) can have a more important role on the dehydration pattern. Consequently, the effect that different environments can produce in their dehydration behavior might also vary. Therefore, the aim of this study was to analyze the influence of different environmental conditions on the *in vitro* dehydration pattern of a broad range of currently marketed CLs.

MATERIAL AND METHODS

Lens materials

Three Hy CLs (HEMA-based): Omaficon A (Proclear; CooperVision, Irvine, CA, USA), Vifilcon A (Focus Visitint; Ciba Vision, Duluth, GA, USA), and Polymacon (Soflens 38; Bausch & Lomb, Contact Lens Division, Rochester, NY, USA); and four Si-Hy CLs: Lotrafilcon B (Air Optix; Ciba Vision, Duluth, GA, USA), Balafilcon A (PureVision; Bausch & Lomb, Contact Lens Division, Rochester, NY, USA), Senofilcon A (Acuvue Oasys; Johnson & Johnson, Jacksonville, FL, USA), and Comfilcon A (Biofinity; CooperVision, Irvine, CA, USA) were analyzed in this study. Technical details of CL studied are summarized in Table I. All the lenses used were -3.00 D back vertex power, to minimize differences in CL thickness that could play a role on the CL dehydration pattern.²¹

Dehydration process

Analysis of the *in vitro* dehydration process was performed using an analytical balance (AS220/C/2, Radwag, Hilden, Germany).⁸ Its scale was capable of measuring up to 0.0001 g. After taking the lens from the blister, the excess water was gently removed by blotting with a Resma filter paper (Filtros Anoa, Barcelona, Spain). The CLs were then placed on a convex plastic in order to simulate the lens on the ocular surface with only the anterior surface directly exposed to air.¹⁷ The maximum time between taking away the CL from the blister and its weighing was 15 seconds in order to minimize dehydration before obtaining the first reading. Measurements were obtained at baseline (0), 5, 10, 15, 20, 30, 45, 60, 90, and 120 minutes of exposure to the selected environment. One measurement was carried on three different samples of each CL brand from the same batch each time for all the environmental conditions.

The parameters derived from the dehydration exhibited by the CLs were expressed as dehydration rate (DR) and valid dehydration (VD).¹⁷

DR: This parameter represents the dehydration per time interval for each lens at a certain time during the dehydration process. It is computed using Eq. (1) where $WT(n)$ is the sample weight at time n and $WT(n-1)$ the sample weight at time $n-1$ (“ n ”: measurement time). Negative values are obtained for this parameter:

$$DR_n(\%) = [(WT(n) - WT(n-1)) / WT(n)] \cdot 100 \quad (1)$$

VD: This parameter represents the loss of weight of each lens at a certain time during the dehydration process

compared to its total loss of weight. It is computed using Eq. (2), where $WT(0)$ is the initial sample weight, $WT(n)$ is the sample weight at time n , and $WT(f)$ the final lens weight. Positive values are obtained because this value is calculated with respect to the final weight of the sample. To better understand the results, we have assumed that CLs reached their full dehydration status after 120 minutes of exposure ($WT(f)$), as previous authors also presupposed.¹⁷ Although this hypothesis might not be totally true in all cases, it can happen for the vast majority of the CLs studied:

$$VD_n(\%) = [(WT(0) - WT(n)) \cdot 100] / (WT(0) - WT(f)) \quad (2)$$

We also assessed the stabilization time, which was defined as the moment when the differences between two consecutive VD values was $\leq 2\%$ and the slope found between two consecutive VD values was < 0.1 . Stabilization time was calculated for the most dissimilar environmental conditions created for this study (5% RH with AF and 70% RH without AF).

Environmental conditions

The environmental exposures were carried out in the Controlled Environment Research Laboratory (CERLab), IOBA, University of Valladolid (Spain).²² Several environmental conditions can be controlled in the CERLab: RH (range 5–80%), T (range 15–30°C), AF, illumination (range 10–1000 lux), and atmospheric pressure (range 1000–450mbar). For this study the samples were exposed to eight different environmental conditions, controlling the RH and the presence of AF. Four different RH conditions (5%, 30%, 50%, and 70%) and two AF rates (0 and 2.75m/s) for each RH were used. We selected these RH conditions because of their real-life relevance: RH values up to 5% have been reported within airplanes,²³ normal indoor conditions are usually close to 30%,²⁴ around 50% is the average of nonarid regions,²⁵ and approximately 70% is the RH magnitude frequently found in coastal areas.²⁶ Besides, we also included the AF to simulate the conditioned air flow that cars and airplanes are equipped with. The pipeline producing AF had a diameter of 4 cm and was positioned at 1 m from the CLs location. The T was maintained always constant (23°C) and the time of exposure was 120 minutes for each lens.

Statistical analysis

Data were analyzed using the SPSS software package (IBM SPSS Statistics 19.0 for Windows; SPSS, Chicago, IL), the SAS software package for Windows (SAS 9.2) and R software.²⁷

For VD analysis, repeated measures ANOVA with two between-subject factors: environmental conditions and CL material, were fitted. In the intersubject variable (time), we included eight levels (the first and the last moment were not included because they are constant). Mauchly’s test was statistically significant and the sphericity could not be assumed, therefore F -ratio was modified by Greenhouse-Geisse correction. The former was fitted to evaluate the effect of time, environmental conditions, and CL material on VD.

TABLE I. Nominal Parameters of CL Used in this Study

USAN BRAND COMPANY BASE CURVE (mm) DIAMETER (mm) CT (mm) EWC (%) Dk FDA GROUP MATERIAL	Hydrogel Contact Lenses			Silicone-Hydrogel Contact Lenses			
	Omafilcon A Proclear CooperVision	Vifilcon A Focus Visitint CIBA-Vision	Polymacon Soflens 38 B&L	Lotrafilcon B Air Optix CIBA-Vision	Balafilcon A PureVision B&L	Senofilcon A Acuvue Oasys Johnson& Johnson	Comfilcon A Biofinity CooperVision
	8.6	8.6	8.7	8.6	8.6	8.4	8.6
	14.2	14.0	14.0	14.2	14.0	14.0	14.0
	0.065	0.100	0.065	0.080	0.090	0.070	0.080
	62	55	38.6	33	36	38	48
	32	20	8.5	110	99	103	128
	II	IV	I	I	III	I	I
	HEMA+PC	HEMA, PVP, MA	HEMA	TRIS+DMA+ DMA	TRIS+NVP+ TPVC+NCVE+ PBVC+	HEMA+PDMS+ DMA+PVP	Aquaform

USAN, United States Adopted Names Council; CT, central thickness; EWC, equilibrium water content; Dk, oxygen permeability. Dk measurement units ($\times 10^{-11}$ cm²/s)(mL O₂/mL x mmHg)); HEMA, 2-hydroxyethyl methacrylate; MA, methacrylic acid; NCVE, N-carboxyvinyl ester; PC, phosphorylcholine; TRIS, 3-methacryloxy-2-hydroxypropyloxy propylbis(trimethylsiloxy)methylsilane; TPVC, tris-(trimethylsiloxy) propylvinyl carbamate; PBVC, poly(vinyl) bis[vinyl] carbamate; PVP, poly(vinyl) pyrrolidone.

In order to evaluate the effect of the CL material and environmental condition (AF and RH) on the dehydration at each time moment (DR), linear mixed effects models (LMM) were used. At each time moment, DR outcome was fitted as the sum of a fixed effect, CL material, and two random effects, AF and RH. The LMM have allowed us to account for the correlation between samples and environmental conditions. In addition, we have been able to consider the AF and RH levels as a random sample for an environmental condition population. Residuals were used to assess the adequacy of the fitted models. In all cases, models were meaningful to fit data (residuals were independent and normally distributed).

p -values ≤ 0.05 were considered statistically significant.

RESULTS

Dehydration rate (DR)

Each environmental condition caused different dehydration profiles. Figure 1 (A–H) shows DR results for 5%, 30%, 50%, and 70% RH with and without AF conditions during the 120 minutes exposure for each CL analyzed. Figure 1 shows how the different environmental conditions had different impacts on DR patterns. Low RH accelerates dehydration, which graphically seems to be, further increased with the presence of AF. High values of DR are observed at earlier measuring times when RH is low, while these peaks tend to move towards later measuring times as RH increases. The same effect is observed for AF, finding higher DR values in the presence of AF at earlier measuring times than those obtained without AF.

Analyzing DR curves over time for the different CLs studied (Figure 1), Comfilcon A showed the highest DR value at 5 minutes ($p \leq 0.0001$) for 50%, 30%, and 5% RH with AF and 5% RH without AF environments. This material showed also the maximum DR value at 5 minutes for 30% RH without AF and 70% RH with AF conditions compared with all the CLs except for Senofilcon A. Omafilcon A and Vifilcon A yielded similar DR profiles, showing a similar dehydration peak during the 10–15 minute interval under the following environmental conditions: 5% RH without AF and 5%, 30%, and 50%RH with AF. This dehydration peak was observed at later times (20–30 minute interval) when these CL materials were exposed to less desiccating conditions (50% and 70% RH without AF and 70% RH with AF). Balafilcon A showed a different behavior compared with the other CLs; whereas most of the CLs showed one peak on the DR curve, Balafilcon A presented a double peak for the majority of the environmental conditions. The rest of CL had a more homogeneous DR profile (Figure 1).

The significant outcomes obtained for the DR analysis are showed in Table II. Air flow solely had a significant effect over CL dehydration at minutes 5 ($p = 0.0115$), 10 ($p = 0.0003$), 45 ($p = 0.0459$), and 90 ($p = 0.0392$). CL material also showed a significant effect on CL dehydration, but in this case, the effect was constant until 90 minutes ($p < 0.04$); however, the effect of the interaction between AF and CL material on water loss was only significant up to 5 minutes ($p = 0.0288$). The interaction between RH and CL material showed a significant effect only at minute 10

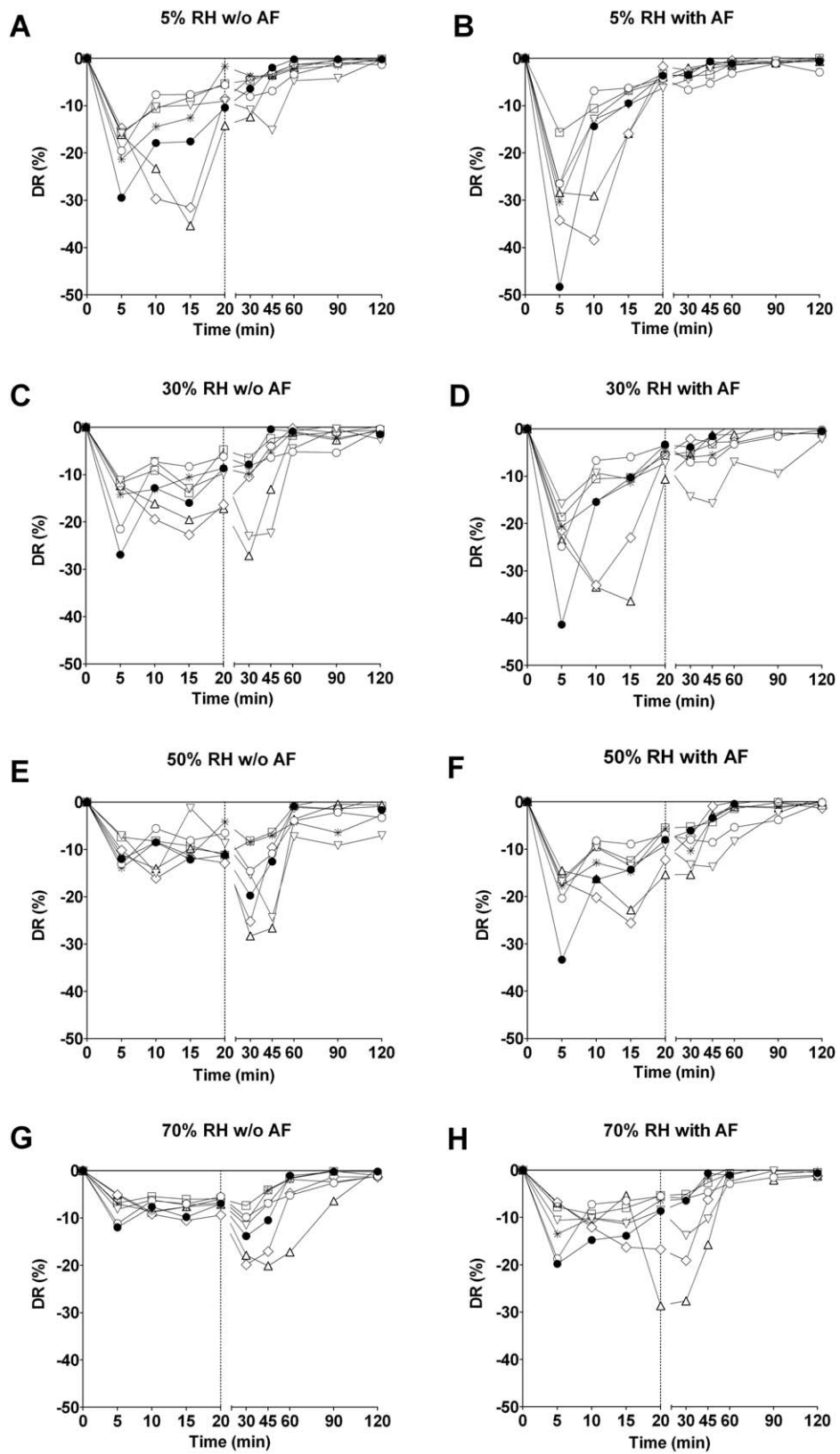


FIGURE 1. (A–H) Dehydration rate curves obtained for all the environmental conditions recreated. DR, dehydration rate; RH, relative humidity; AF, air flow; w/o, without; Δ , Omafilcon A; \diamond , Vifilcon A; $*$, Polymacon; \square , Lotrafilcon B; ∇ , Balafilcon A; \circ , Senofilcon A; \bullet , Comfilcon A.

TABLE II. Effect of CL Material, RH, and AF on CL Dehydration Rate

Time (min)	AF (<i>p</i> -value)	CL Material (<i>p</i> -value)	RH (<i>p</i> -value)	AF*CL Material (<i>p</i> -value)	AF*RH (<i>p</i> -value)	CL Material*RH (<i>p</i> -value)	AF*RH*CL Material (<i>p</i> -value)
5	0.0115	<0.0001	NS	0.0288	NS	NS	0.0095
10	0.0003	0.0001	NS	NS	NS	0.0158	0.0261
15	NS	<0.0001	NS	NS	NS	NS	<0.0001
20	NS	<0.0001	NS	NS	NS	NS	0.0002
30	NS	0.0026	NS	NS	NS	NS	0.0062
45	0.0459	0.0026	NS	NS	NS	NS	0.03
60	NS	0.0107	NS	NS	NS	NS	0.0019
90	0.0392	0.0392	NS	NS	NS	NS	NS
120	NS	NS	NS	NS	NS	NS	NS

NS, non-significant. ■, Random effects. □, Fixed effects. CL, Contact lens. RH, Relative Humidity. AF, Air Flow.

($p = 0.0158$); however, the triple interaction among RH, AF and CL material had a significant effect ($p \leq 0.03$) on CL dehydration up to 60 minutes.

Valid dehydration

VD results are shown in Figure 2(A–H). From a general point of view, it can be observed that the decrease in RH and the presence of AF increased the loss of WC in all the CLs evaluated. However, significant different dehydration behaviors have been observed among the several CL analyzed for all the environmental conditions (Figure 2) (see Supporting Information Tables S1–S4, which shows the VD *p*-values among different lenses during all environmental conditions for each time measured).

The maximum differences in VD values among CL in the majority of the environmental conditions occurred at 15 minutes exposure and the range of these differences was from 25.16% to 42.75% VD. The greatest VD difference among CLs was found between Balafilcon A and Vifilcon A (27.35%, $p < 0.0001$) after 15 minutes exposure in the 5% RH without AF condition; for the same RH magnitude adding AF, the maximum VD difference was found between Senofilcon A and Vifilcon A (26.2%, $p < 0.0001$) after 15 minutes exposure. Under 30% RH without AF condition, we observed the greatest difference among CL at 15 minutes, which was 35.33% ($p < 0.0001$) between Balafilcon A and Comfilcon A. After incorporating AF to the previous condition, the maximum VD difference was again seen between both CL materials at 10 minutes (42.75%, $p < 0.0001$). The widest dehydration difference during the 50% RH without AF condition was found between Balafilcon A and Lotrafilcon B at 15 minutes (29.01%, $p < 0.0001$), whereas Balafilcon A and Comfilcon A obtained the maximum VD difference at 15 minutes among CL evaluated (28.06%, $p < 0.0001$) when AF was also incorporated. The 70% RH without AF condition elicited a maximum VD difference of 25.16% ($p < 0.0001$) between Omaficon A and Lotrafilcon B at minute 20, this maximum VD difference was even greater (32.52%, $p < 0.0001$) between Comfilcon A and Omaficon A at minute 15 when AF was also used, keeping the same RH magnitude.

Table III shows the stabilization time for each CL under the less (70% RH without AF) and the most desiccating

(5% RH with AF) condition. Stabilization time was earliest for the 5% RH with AF condition than the 70% RH without AF one for all the lenses, except for Balafilcon A and Lotrafilcon B, which had the same stabilization time for both environments (60 minutes).

DISCUSSION

We demonstrated that a wide range of environmental conditions provoked dissimilar dehydration profiles in several CLs studied. We selected several RH conditions because of their real-life relevance. CL wearers can be daily exposed to variable RH values within indoor conditions like airplanes (5% RH)²³ and conventional offices (30% RH),²⁴ and in outdoor conditions like non-arid regions (50% RH)²⁵ and coastal areas (70% RH).²⁶ Our outcomes indicated that low RH and especially the presence of AF, elicit the acceleration of CL water loss because adverse conditions caused a more rapid dehydration than milder conditions did. This claim has previously caused controversy among *in vivo* studies. On the one hand, Andrasko⁷ and Andrasko and Schoessler⁸ reported higher levels of dehydration for both 55% and 71% WC CLs under low RH conditions compared with higher RH ones. However, the former study was conducted only in one subject,⁷ and the latter did not describe the number of subjects included.⁸ On the other hand, Morgan and Efron²⁰ showed that Etafilcon A and Balafilcon A dehydration levels were not significantly different among three dissimilar environmental conditions. They used three completely different conditions in terms of RH and *T* (5% RH and 30°C; 70% RH and 22°C; 90% RH and 5°C). Therefore, their outcomes might be difficult to be interpreted because first, they did not keep constant any environmental variable and second, it must be taken into account that RH values depend on *T*, such that, the same amount of water vapor gives different measures of RH at different *T*.²⁸

In our study, DR evaluation indicates graphically that the presence of AF led to a major CL water loss producing further dehydrating impact than low RH. This finding is similar to that reported by Jones et al.¹⁵ who evaluated the effect of two RH values (20% and 60%) combined with two different AF rates (9 and 30 mL/min). DR parameter might be helpful to easily identify some dehydration profiles over time,

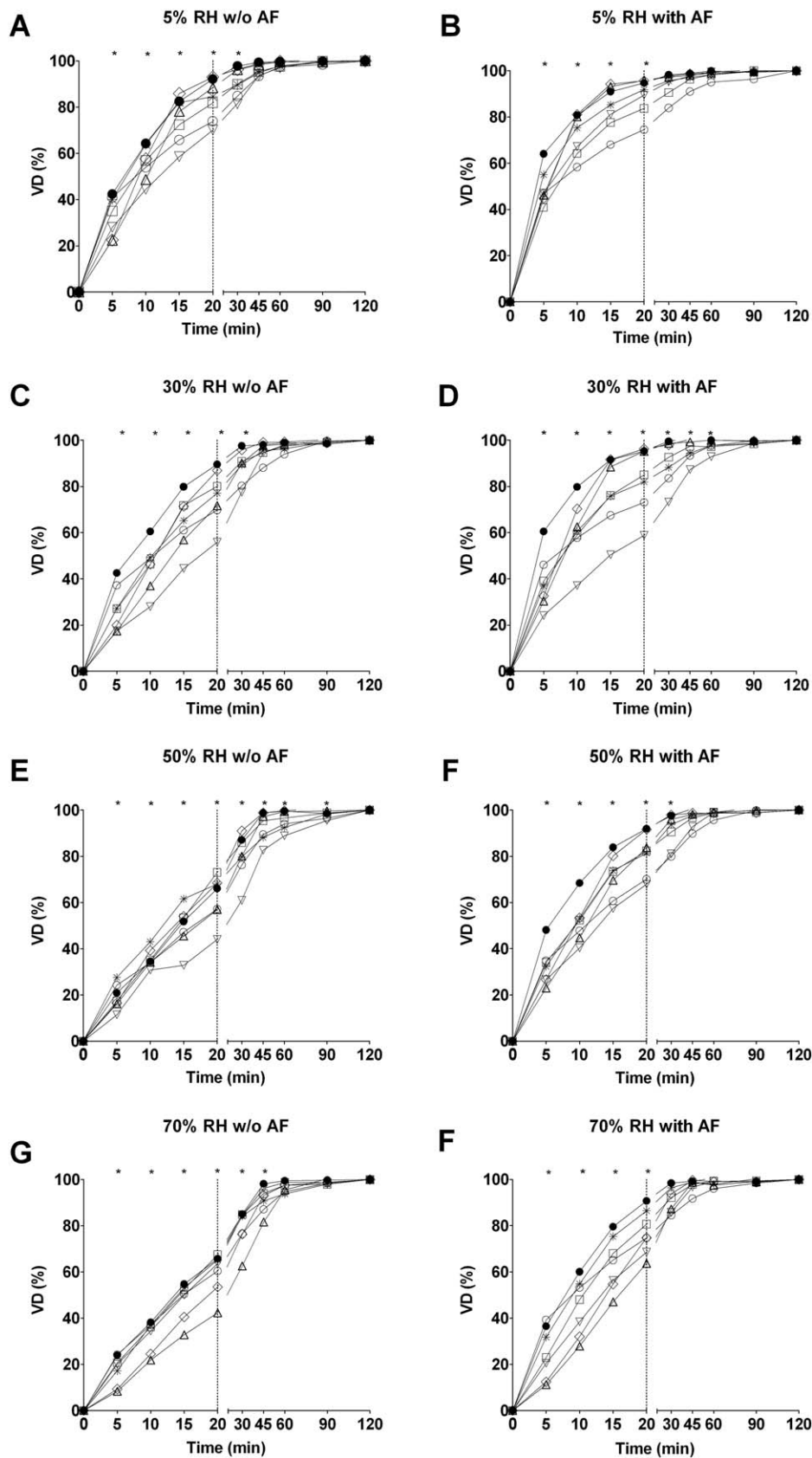


FIGURE 2. (A–H) Valid dehydration curves obtained for all the environmental conditions recreated. *Statistically significant differences ($p < 0.05$) between at least two CLs materials at that exposure time. VD, Valid dehydration; RH, Relative Humidity; AF, Air Flow; w/o, without; Δ , Omafilcon A; \diamond , Vifilcon A; \ast , Polymacon; \square , Lotrafilcon B; ∇ , Balafilcon A; \circ , Senofilcon A; \bullet , Comfilcon A.

TABLE III. Stabilization Time for each Contact Lens Material Under the Less (70% RH without AF) and the Most Desiccating (5% RH with AF) Condition

CL Material	5% RH with AF		70% RH w/o AF	
	Stabilization Time (minutes)	VD %(at the stabilization time)	Stabilization Time (minutes)	VD %(at the stabilization time)
Omafilcon A	30	97.27	90	100
Vifilcon A	30	97.67	60	97.58
Polymacon	30	95.65	90	98.02
Lotrafilcon B	60	98.33	60	97.61
Balafilcon A	60	99.57	60	98.74
Senofilcon A	Not stabilized	Not stabilized	90	98.41
Comfilcon A	30	98.25	45	98.15

CL, Contact lens; VD, Dehydration rate; RH, Relative humidity; AF, Air Flow; w/o, without

which are more difficult to be noticed using VD. DR curves showed similar dehydration profiles for Vifilcon A and Omafilcon A during the vast majority of the environmental conditions. These similar behaviors have not been previously reported and it could indicate that polyvinyl pyrrolidone and phosphorylcholine, both wetting agents of Vifilcon A and Omafilcon A, respectively, could lead similar resistance to *in vitro* dehydration in conventional hydrogels CLs. Another major item regarding DR curves is Comfilcon A dehydration during the first moments of exposure. This material seems to be the least resistant to dehydration in most environments; however, further *in vivo* studies are needed to understand the actual impact of this fact on clinical practice.

We performed a multivariate analysis to fit DR value based on three different factors (RH, AF, and CL material) because we wanted to assess how each factor could affect CL dehydration by themselves and grouped (Table II). Our outcomes showed that AF itself was significant during the first minutes (up to 10) of the exposure, which shows that water loss is more dependent on this variable at the beginning as compared with the last moments. Besides, CL material alone was a factor that affected dehydration continuously until minute 90, which means that dehydration pattern is material dependent. Surprisingly, we could not find any effect of RH alone on water loss. Nonetheless, when the interaction among the three factors on dehydration was studied, data showed that water loss will be different depending on each CL material under diverse RH values and whether there is presence of AF.

We could notice that the *in vitro* dehydration profile over time of the CLs studied showed a different dehydration curve depending on the environmental conditions. The VD curves acquired an ogival shape (Figure 2) when CLs were exposed to the more desiccating conditions (5% and 30% with and without AF), whereas they looked almost like a straight line when the CLs were subjected to less desiccating conditions (50% and 70% RH with and without AF). During the first minutes of exposure, water loss was always quickest such as other authors have already described;¹⁷ besides, the lower the RH was, the higher the VD values were. This dehydration behavior was also noticed when AF was present [Figure 2(B,D,F,H)]. During the mid time of the

exposure, water loss was not so remarkable compared to the first minutes, especially under higher RH conditions. At this stage, we obtained the highest amount of VD differences among CLs, showing that at this point, the influence of the CL material could be an important variable for CL water loss. VD curves at the final stage of the exposure showed almost no water loss because within this period the stabilization time was produced, leading to an almost totally dehydrated CL at the end of the exposure.

Based on the VD curves (Figure 2) it is possible to compare the different CL behaviours under the environmental conditions recreated. Comfilcon A showed a characteristic dehydration profile that was highly differentiated from the other CLs. This material was the most affected one by either AF or low RH, showing the greatest dehydration values during the early stages of exposure in almost all the environmental conditions studied (Figure 2). Even, its dehydration behavior is different from the other Si-Hy materials studied. Our results regarding Comfilcon A were similar to those previously reported by González-Méjome et al.¹⁶ who have found that this CL had the highest dehydration rate among several CL materials (Galyfilcon A, Lotrafilcon B, Balafilcon A and Lotrafilcon A) during the first 5 minutes. On the other side, Balafilcon A showed the lowest VD values at the middle stages of exposure for most of the environments recreated.

The stabilization time is a useful measure that shows when CLs have almost lost all the WC, which usually occurs when the middle stage of the VD curve has finished. Our results (Table III) indicated that the stabilization time occurred sooner when CL were subjected to the most desiccating conditions (5% RH with AF) as expected; showing that Balafilcon A, Lotrafilcon B, and Senofilcon A had the longer stabilization time, which indicates that these materials might have higher ability to avoid dehydration. For the less adverse condition (70% RH without AF), Comfilcon A reached its stabilization time the sooner, whereas Omafilcon A, Senofilcon A, and Polymacon achieved this point much later.

The major limitation of the present study was the CL weighing procedure performed when assessing CL dehydration, because the methodology used for removing the excess water of the CLs prior to be weighed might have introduced moderate variability on the results. Nonetheless, this procedure was always performed by the same trained researcher.

Another limitation in our study is not using always the same CLs area, because the marketed CLs included in the study had different diameters. A CL with either a larger diameter or a broader thickness (or both) might spend more time to reach the stabilization time because there is more water volume to be lost. Nonetheless, our aim was assessing the intrinsic characteristics of each CL material regarding dehydration; thus, we did not provide absolute dehydration values, and instead we evaluated the relative dehydration changes observed for each CL in terms of VD and DR magnitude. Finally, the sample size was small and a larger one could have increased the reliability of the results.

In conclusion, our results provide information about the influence that several environmental conditions have on the *in vitro* dehydration of current marketed CLs. Differences in water loss among CL materials were more evident during the middle stage of exposure, especially between 10 and 20 minutes. Differences found in the CL dehydration behavior can be mainly explained by differences in chemical composition of the different materials studied taking also into account the design of the CL, such as thickness profile and diameter. Therefore, the present study shows how current marketed CLs behave *in vitro* under different environmental conditions, despite this *in vitro* dehydration can differ from the *in vivo* one.

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