Supplementary Material

The effects of polar excipients transcutol and dexpanthenol on molecular mobility, permeability, and electrical impedance of the skin barrier

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Supplementary Tables

Table S1. Metronidazole solubility (mg g^{-1}), adjusted formulation concentration (mg g^{-1}), and water activity (a_w) in the formulations. Deviations are given by the standard error of the mean (±SEM).

Formulation[†]	[Solubility]	[Adjusted]	a_w
PBS	12.5 ± 0.19 (*n*=6)	7.50	0.992 ± 0.002 (*n*=2)
TC5	14.3 ± 0.22 (*n*=6)	8.62	0.984 ± 0.002 (*n*=2)
TC10	15.8 ± 0.27 (*n*=6)	9.55	0.975 ± 0.001 (*n*=2)
TC15	17.4 ± 0.29 (*n*=6)	10.5	0.965 ± 0.001 (*n*=2)
TC30	22.1 ± 0.52 (*n*=3)	13.3	0.936 ± 0.000 (*n*=2)
DexP5	14.1 ± 0.18 (*n*=6)	8.51	0.986 ± 0.001 (*n*=2)
DexP10	15.5± 0.08 (*n*=6)	9.35	0.981 ± 0.001 (*n*=2)
DexP15	16.8 ± 0.18 (*n*=5)	10.1	0.976 ± 0.001 (*n*=2)
DexP30	20.4 ± 0.14 (*n*=5)	12.3	0.955 ± 0.001 (*n*=2)

[†]Abbreviations: TC - transcutol, DexP – dexpanthenol. Numbers give concentration in wt%.

Table S2. Impedance spectroscopy data of initial (*i*) and final (*f*) values of R (Ohm cm^2) and CPE parameters α, Q_{eff} (Ohm^{-1} cm^{-2} s$^{\alpha}$), and C_{eff} (nF cm^{-2}).

Formulation†	R		α		Q_{eff}		C_{eff}	
	i	*f*	*i*	*f*	*i*	*f*	*i*	*f*
PBS A	21691	1818	0.830	0.599	1.29E-07	2.55E-06	39	70
PBS B	7844	752	0.769	0.375	3.22E-07	3.89E-05	53	107
PBS C	14685	1268	0.816	0.490	1.53E-07	1.06E-05	39	119
PBS D	13069	988	0.790	0.469	2.65E-07	1.59E-05	59	145
PBS E	6915	1369	0.757	0.565	3.68E-07	4.93E-06	54	105
PBS F	11544	801	0.754	0.508	3.31E-07	1.03E-05	54	98
PBS G	11239	2227	0.686	0.663	4.72E-07	1.37E-06	43	72
PBS H	3899	548	0.704	0.412	7.30E-07	3.64E-05	62	137
PBS I	11625	1073	0.780	0.557	2.58E-07	5.59E-06	50	96
PBS J	4196	1270	0.713	0.592	5.13E-07	3.67E-06	43	91
TC5 A	7142	1072	0.747	0.583	5.46E-07	4.87E-06	83	114
TC5 B	4487	971	0.747	0.476	4.06E-07	1.29E-05	48	104
TC5 C	7744	834	0.767	0.509	3.56E-07	1.10E-05	59	119
TC5 D	10736	1398	0.678	0.580	7.67E-07	4.20E-06	78	101
TC5 E	3988	1629	0.684	0.589	7.46E-07	2.74E-06	51	62
TC30 A	7136	831	0.751	0.556	4.80E-07	7.16E-06	73	120
TC30 B	8009	2033	0.753	0.674	3.63E-07	1.19E-06	53	65
TC30 C	3015	995	0.494	0.581	5.44E-06	4.46E-06	80	89
TC30 D	4232	1232	0.526	0.610	3.32E-06	2.74E-06	71	72
TC30 E	5870	687	0.719	0.495	6.21E-07	1.40E-05	69	122
DexP5 A	9398	1682	0.790	0.584	2.66E-07	3.89E-06	54	108
DexP5 B	4257	1092	0.738	0.556	4.20E-07	4.59E-06	44	67
DexP5 C	5191	664	0.738	0.419	6.02E-07	3.04E-05	78	137
DexP5 D	8794	1118	0.757	0.540	4.18E-07	7.08E-06	69	115
DexP5 E	9343	633	0.7667	0.423	4.27E-07	3.29E-05	79	167
DexP30 A	8919	8828	0.757	0.767	3.14E-07	3.50E-07	48	60
DexP30 B	9373	7952	0.789	0.761	2.42E-07	4.01E-07	48	66
DexP30 C	5183	1427	0.724	0.640	6.15E-07	2.45E-06	69	101
DexP30 D	2733	1888	0.699	0.669	6.49E-07	1.34E-06	43	69
DexP30 E*	861	-	0.38	-	2.3E-05	-	41	-

†Abbreviations: TC - transcutol, DexP - dexpanthenol. Numbers give concentration in wt%.
*Membrane defect at final measurement

Table S3. Relative change of R (Ohm cm^2), CPE parameters α and Q_{eff} (Ohm^{-1} cm^{-2} s$^{\alpha}$), and C_{eff} (nF cm^{-2}) given by the ratio of the final (f) and initial (i) values.

Formulation†	R_f / R_i	α_f / α_i	$Q_{eff,f}$ / $Q_{eff,i}$	$C_{eff,f}$ / $C_{eff,i}$
PBS A	0.08	0.72	19.8	1.8
PBS B	0.10	0.49	120.6	2.0
PBS C	0.09	0.60	69.5	3.1
PBS D	0.08	0.59	60.0	2.5
PBS E	0.20	0.75	13.4	1.9
PBS F	0.07	0.67	31.1	1.8
PBS G	0.20	0.97	2.9	1.7
PBS H	0.14	0.59	50.0	2.2
PBS I	0.09	0.71	21.7	1.9
PBS J	0.30	0.83	7.2	2.1
TC5 A	0.15	0.78	8.9	1.4
TC5 B	0.22	0.64	31.7	2.2
TC5 C	0.11	0.66	30.9	2.0
TC5 D	0.13	0.86	5.5	1.3
TC5 E	0.41	0.86	3.7	1.2
TC30 A	0.12	0.74	14.9	1.6
TC30 B	0.25	0.90	3.3	1.2
TC30 C	0.33	1.18	0.8	1.1
TC30 D	0.29	1.16	0.8	1.0
TC30 E	0.12	0.69	22.5	1.8
DexP5 A	0.18	0.74	14.6	2.0
DexP5 B	0.26	0.75	10.9	1.5
DexP5 C	0.13	0.57	50.5	1.8
DexP5 D	0.13	0.71	16.9	1.7
DexP5 E	0.07	0.55	77.1	2.1
DexP30 A	0.99	1.01	1.1	1.3
DexP30 B	0.85	0.96	1.7	1.4
DexP30 C	0.28	0.88	4.0	1.5
DexP30 D	0.69	0.96	2.1	1.6
DexP30 E*	-	-	-	-

†Abbreviations: TC - transcutol, DexP - dexpanthenol. Numbers give concentration in wt%.
*Membrane defect at final measurement

Supplementary Figures

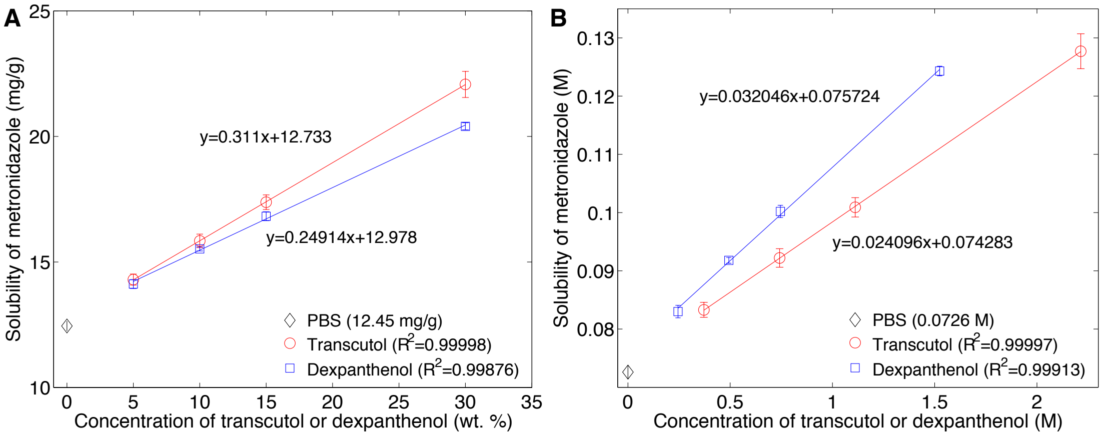

Figure S1. (A) Metronidazole solubility (mg/g) as a function of transcutol or dexpanthenol concentration (wt%). (B) Metronidazole solubility (M) as a function of transcutol or dexpanthenol concentration (M), showing that dexpanthenol is more efficient in solubilizing metronidazole in a mole-per-mole basis, as compared to transcutol.

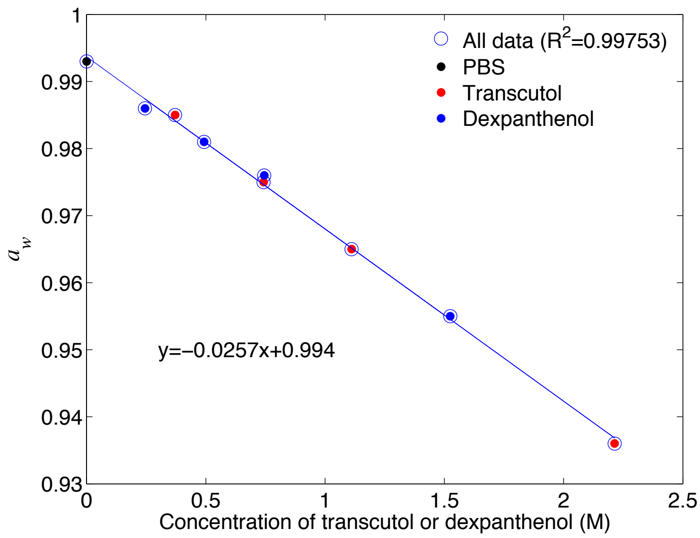

Figure S2. Water activity in the formulations as a function of transcutol or dexpanthenol concentration (M). The regression line shows good correlation, which suggests that the water activity is mainly determined by the number of transcutol or dexpanthenol molecules, irrespective of type.

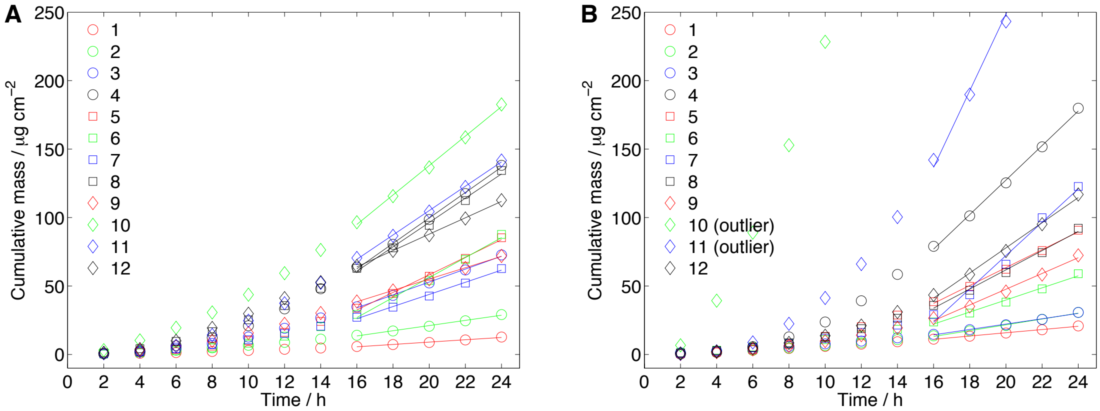

Figure S3. Determination of steady-state flux of metronidazole across skin membranes from (A) neat PBS formulation (*n*=12) and (B) formulation containing 5 wt% TC (*n*=10, 2 outliers). Cumulative mass of permeated metronidazole is plotted as a function of time and the steady-state flux is given by the slope of each individual curve between 16 and 24 h. Statistical outliers were excluded based on a two sided Grubbs' test at p=0.05.

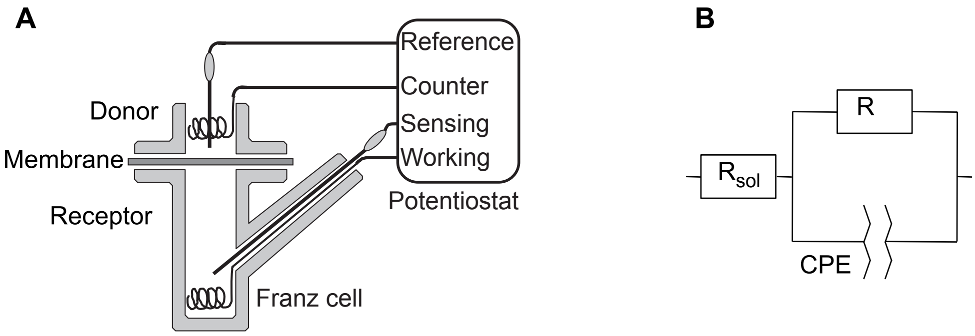

Figure S4. (A) Schematic representation of the Franz cell equipped with four electrodes for electrical impedance measurements. Two platinum wires served as working and counter electrodes and two Ag/AgCl/3M KCl electrodes were used as sensing and reference electrodes. The water jacket of the Franz cell was kept at 32 °C by a circulating water bath. (B) Equivalent electrical circuit used to model the experimental impedance data. R_{sol} is the resistance of the donor and receptor solution, R is the membrane resistance, and CPE is a constant-phase element.

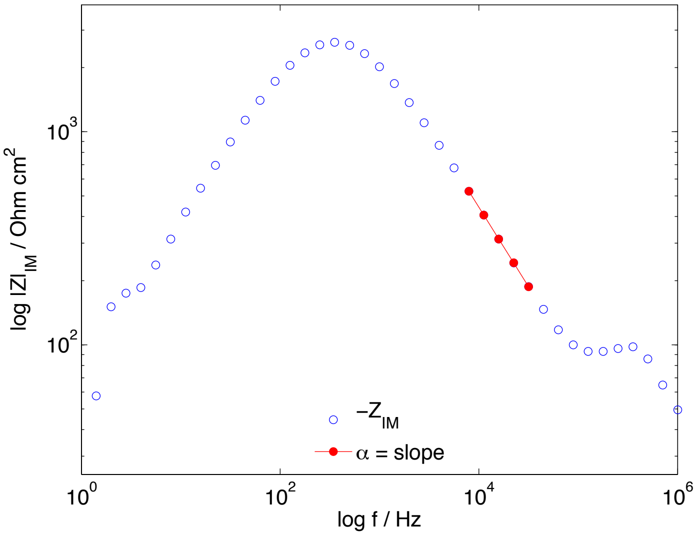

Figure S5. The imaginary part of the impedance as a function of frequency in logarithmic coordinates. Parameter α was determined from the high frequency region where the imaginary part contributes greatest to the total impedance, relative to the real part of the impedance (in this region, the phase angle is approaching its maximum value; cf. Fig. S8). Parameter α was graphically determined from the slope of the decaying region, which for the presented data corresponds to the slope of the solid circles in red.

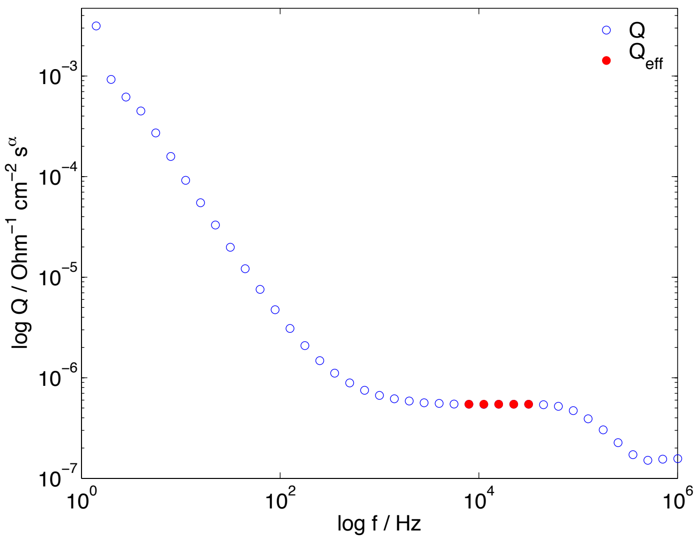

Figure S6. CPE parameter Q as a function of frequency in logarithmic coordinates. Solid red circles represent data points used to determine Q_{eff}, which corresponds to the mean value of these data points.

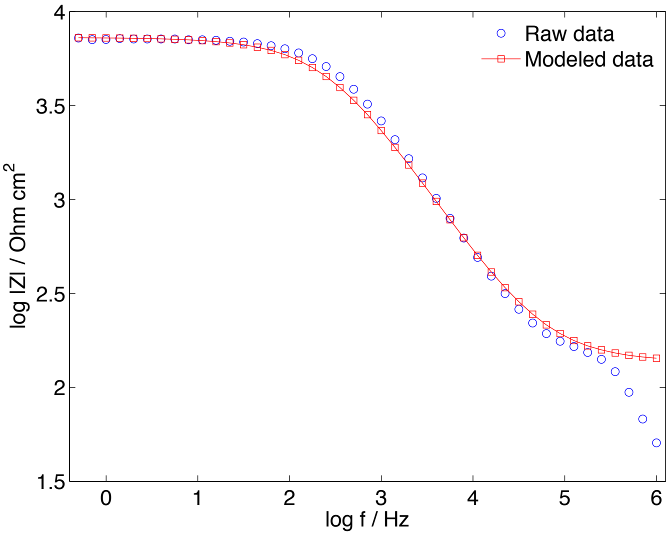

Figure S7. Total impedance as a function of frequency in logarithmic coordinates. Blue circles represent raw data, while red squares represent modeled data according to (cf. model circuit in Fig. S4 B). Input data: R = 7142 Ohm cm^2, α = 0.7465, Q_{eff} = 5.458*10^{-7} Ohm^{-1} cm^{-2} s$^{\alpha}$. The values of these parameters were determined following the graphical method described in the supplementary text below.

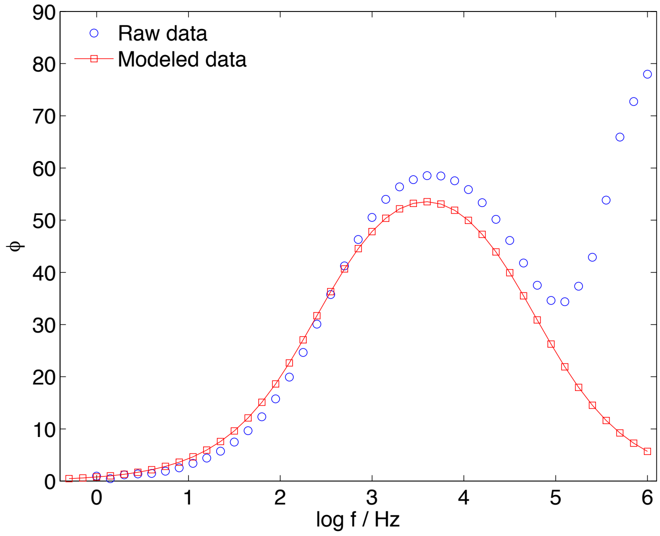

Figure S8. Phase angle ϕ as a function of frequency in logarithmic coordinates. Blue circles represent raw data, while red squares represent calculated data according to the model described by eq. S1. Input data: R= 7142 Ohm cm^2, α = 0.7465, Q_{eff} = 5.458*10^{-7} Ohm^{-1} cm^{-2} s$^{\alpha}$. The values of these parameters were determined following the graphical method described in the supplementary text below.

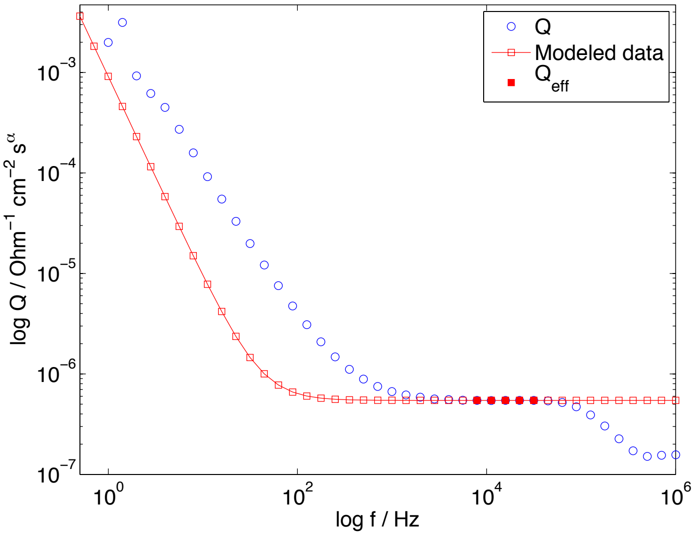

Figure S9. Effective CPE parameter Q as a function of frequency in logarithmic coordinates. Red squares represent data points used to determine Q_{eff}, while the modeled data were calculated from eq. S1 and S2 with $\alpha = 1$, $R = 7142$ Ohm cm^2, C_{ideal} (= Q) = 546 nF cm^{-2}. The good agreement between the modeled data under ideal conditions and the value of Q_{eff} in the high frequency region (from which Q_{eff} was obtained) confirms the validity of the method used to determine Q_{eff}.

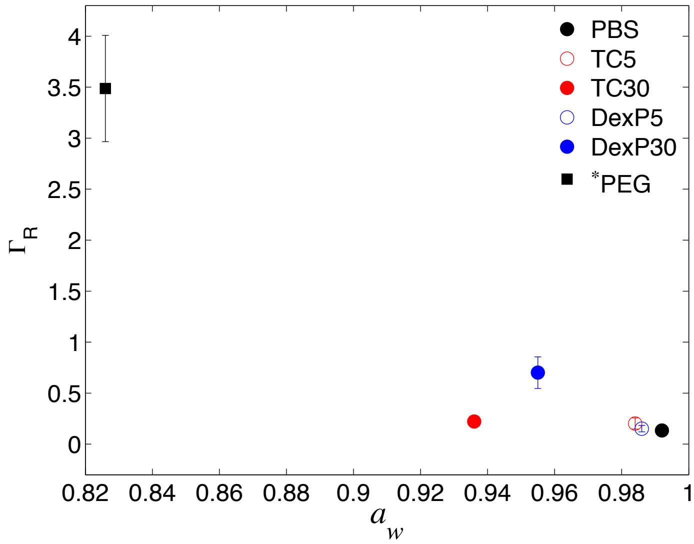

Figure S10. Relative change of skin membrane resistance R as a function of water activity (a_w) in the donor solution. The relative change is given by the ratio of the final value, after 24 h exposure of the formulation, and the initial value before the steady-state diffusion experiment. Abbreviations: TC - transcutol, DexP - dexpanthenol. Numbers give concentration in wt%. *PEG shows data from [1].

Supplementary Text

Impedance spectroscopy

The total impedance Z of the equivalent circuit in Fig. S4 is given by eq. S1.

$$Z = R_{sol} + \frac{R}{1 + (i2\pi f)^{\alpha} QR} \tag{1}$$

In eq. S1, $2\pi f$ is the angular frequency, while Q and α are CPE parameters. The resistance values were obtained from the real part of the impedance in the frequency regions where the imaginary part gives minimum contribution to the total impedance. For R_{sol}, this region corresponds to high frequencies in the range of ~ 0.2-0.1 MHz. In average, R_{sol}=134 ± 4 Ohm (±SEM) including all measurements (n=60). The corresponding frequency region for the membrane resistance R occurs at low frequencies close to direct current (DC) where $R = Z_{Re} - R_{sol}$. In this analysis, all data were normalized with the skin membrane area (0.64 cm^2) to get units in Ohm cm^2. It should be noted that the measured value of R_{sol} depends on the distance between the reference and sensing electrodes, which in the present experiments was subject to minor variations. Therefore small deviations from the separately determined values of R_{sol} were expected and accepted. R_{sol} was in all cases significantly lower than R, which assures that the effect of the uncertainty of R_{sol} on the determined value of R is negligible.

Referring to eq. S1, when α equals unity, Q represents an ideal capacitor with units of F m^{-2}. However, in the present work α was in the order of ~ 0.50-0.85, which is consistent with several impedance studies on skin [2-4]. The deviation of the heterogeneous skin membrane from ideal properties is accounted for by the empirical CPE element, which can be used to derive an effective capacitance of the SC [5]. For this, we followed a procedure in which the SC layers are considered to have a distribution of varying time-constants in the vertical direction across the skin membrane [2]. In this case, the effective CPE coefficient Q_{eff} is derived from the high frequency region where Q_{eff} in theory provides a correct value of Q in the case when α equals unity (see Fig. S9) [5]. The CPE parameters, α and Q_{eff} were obtained from the imaginary impedance data following a graphical representation method, which does not involve any fitting [5]. In brief, α was determined by plotting the imaginary part of the impedance as a function of frequency in logarithmic coordinates, where the slope of the decaying region corresponds to α (Fig. S5). The parameter α was consistently calculated in the high frequency region where the imaginary part contributes greatest to the total impedance, relative to the real part. Once parameter α is obtained, Q_{eff} is determined from the imaginary part of the impedance, Z_{im}, according to eq. S2.

$$Q_{eff} = sin\left(\frac{\alpha\pi}{2}\right) \frac{-1}{Z_{im}(f)(2\pi f)^{\alpha}} \tag{2}$$

The average value of the high frequency asymptote data, corresponding to the frequencies used to calculate α, was used to determine Q_{eff} (Fig. S6). Finally, the effective capacitance, C_{eff}, of the skin membrane was calculated using eq. S3 with the determined values of Q_{eff}, α, and R.

$$C_{eff} = Q_{eff}^{1/\alpha} R^{(1-\alpha)/\alpha} \tag{3}$$

The impedance data were evaluated in Matlab (www.mathworks.com) to derive R, Q_{eff}, α, and C_{eff} and the validity of this procedure to extract the skin impedance parameters is further commented in relation to Fig. S7-S9. The values of these parameters from the initial and final measurements are compiled in Table S2. From these data, the general observation is that R and α decreases, while Q_{eff} and C_{eff} increases when comparing the initial and final values. In addition, the results illustrate the natural variability of impedance data, generally observed for different skin membranes [1].

Supplementary References

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[2] B. Hirschorn, M.E. Orazem, B. Tribollet, V. Vivier, I. Frateur, M. Musiani, Determination of effective capacitance and film thickness from constant-phase-element parameters, Electrochimica Acta, 55 (2010) 6218-6227.

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