Glycerin inclusion levels for skin hydration: a data-driven approach.

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Abstract

Introduction:

Skin hydration claims have been around for many decades and are still one of the most sought after benefit from cosmetic products. There are several strategies to deliver such benefit, with a myriad of formulation available on the market. Glycerin, a by-product of soap manufacturing, is among recurring ingredients with a long history of use for skin hydration. Easiness to formulate and affordability makes it an attractive option , yet it may come at the expense of a tacky feeling in formulation when used in high quantities. We thus aimed at retrospectively analyse the minimum levels of glycerin required in some formulation types that allow to deliver a significant skin hydration effect, measured in clinical settings using the corneometeter.

Methods:

Retrospective corneometer data of 134 products were compiled. Formulation types consisted of emulsions (e.g. creams, lotions, foundations) watery gels or solutions (e.g. toners). These data encompasses skin hydration at baseline and at 2, 8 and 24 hours following a single application of a product. Product were evaluated on the forearms with mixed panels of men and women. The whole dataset was investigated using descriptive statistics and bootstrapped 95% confidence intervals (CI) were calculated.. A linear mixed effect models (LMM) was used to estimate the magnitude of skin hydration increase according to the glycerin content.

Results:

When corrected to an untreated site, similar level of glycerin were found to be sufficient to elicit a significant skin hydration at 2 and 8 hours, while higher quantities were required for a 24 hour sustained effect, as per corneometer measurements. Estimates of expected skin hydration were computed with LMM although the variance explained by the model suggest that explanatory power of the model deserved to be improved, in particular for estimates at 24 hours.

Discussion and Conclusion:

Probing relevant levels of glycerin to achieve skin hydration in clinical settings can help addressing several objectives: gain confidence on expected performance, reduce reliance on prototypes screening but more importantly, inclusion of a sensible amount of glycerin to mitigate detrimental effects on sensorial attributes.

Keywords: Hydration, Clinicals, Statistics, Skin Care

Introduction.

Glycerol, whose name stems from the Greek γλυκός (glykos) meaning “sweet”, is a very common raw material in cosmetics. It is also one of the few that is endogenous to the skin thanks to various multiple sources: either as a catabolic product of hydrolyzed triglycerides, secreted from the sebaceous gland, or being transported from the dermis to the basal layer of the epidermis thanks to Aquaporin-3 (AQP3), proteins located in cells membranes. It has been observed that mice lacking AQP3 had a reduce stratum corneum hydration and elasticity, an impaired stratum corneum biosynthesis and a delayed wound healing. Glycerin can be found further down the skin in adipocytes, as a metabolite of glucose with the enzyme Glycerol 3-P, and transported in the extracellular matrix by AQP7[1].

Its properties are numerous, although hydration is the most sought after when included in formulations. This benefit was thought to come solely from its osmotic and hygroscopic properties, although investigations have challenged this view. In a pig model, diglycerin and triglycerin, albeit having a greater humectant activity, displayed little improvement in skin dryness versus glycerin [2]. It greatly accumulates in the skin and creates a 'reservoir' in the depth of SC without disrupting the lamellar structure of the lipid bilayers. It increases both intracellular and extracellular space among corneocytes, and by doing so improve the water holding capabilities of the SC [3] Previous studies have looked at the corneodesmolytic - degradation of the corneodesmosomes – greatly enhanced by the inclusion of glycerin versus vehicle, thus facilitating desquamation [4]

The hydration boosting effect of glycerin, from a few minutes to several hours after application, has been evidenced by several instrumental methods in vivo relying on distinct electrical principles: conductance (Skicon®), impedance (Nova DPM®) and capacitance (Corneometer®)[5]. It can also mitigate the drying effect of alcohol when included in Cologne at 3%, as measured by capacitance and TEWL, to a greater extent than glycol such as propylene glycol [6]

In this investigation, we aimed at retrospectively revisit the results of 134 products clinically tested with the corneometer, using a data science approach. Objectives were twofold: firstly, to establish the minimum inclusion levels of glycerin for a significant increase in skin hydration at 2, 8 and 24 hours in clinical settings. Secondly, predict percentage increase of skin hydration in vivo according to levels of glycerin.

Materials and Methods.

Data set

The data set contained Corneometer® data of women and men panellists who took part to various studies with the below inclusion criteria & restrictions;

- Healthy

- Panellists aware of the test procedure and having signed an informed consent form
- Aged between 18-65 years
- Absence of hair on the treatment area on the forearms or willingness to have hair removed
- No application of any topical products (including moisturising shower gels) onto the forearms for 7 days before the study commences.
- No shower the morning of the first appointment and throughout the study

The design of experiment was an *in vivo* open study, with randomized and controlled (32mg/cm²) product application performed by a trained investigator on forearms, after baseline Corneometer® measurements. The Corneometer® raw data of 134 products were compiled with formulation types consisting of emulsions (e.g. creams, lotions) watery gels and solutions (e.g. toners).

Software used

All statistical models were established using R (version 4.1.2) and RStudio (2021.09.1+372 "Ghost Orchid" Release) for Windows 10, together with the lme4 package (version 1.1-29) for linear mixed-effect models, ggplot2 package (version 3.3.5) for plots, merTools (version 0.5.2) for predicting hydration values of products outside the dataset based on model's uncertainty, sjPlot (version 2.8.10) for generation of summary table of fixed and random effects and MuMIn package (version 1.46.0) for pseudo-R-squared calculations.

Modelling

In order to determine if different levels of glycerin have a statistically significant impact on hydration, at 2; 8 or 24h, we used a simple nested linear mixed model. ANOVA/linear regression cannot be used here for several reasons linked to the design of the studies and the dataset. First of all, measures are not independent because different products have been tested on the same panelists forearms within a study. We cannot use repeated measures ANOVA

either because the within-subjects factor varies and we have a case of pseudo-replication: products vary from study to study, which would result in a lot of missing data which cannot be handled by an ANOVA test. In fact, repeated measures ANOVA in R and most statistical software uses list wise deletion for missing values, meaning that panelists lacking data for one of the 134 products tested (the within-subjects factor) would be ignored and since no panelists has 134 products tested on, the whole dataset would be disregarded.

In comparison, linear mixed effect models (LMM) are more flexible and will take into consideration the dependence in our dataset and its hierarchical and unbalanced nature by introducing random effects such as the panelists and the studies (see the review article by Magezi for more information [7]). Thus, by explicitly modelling the random effects' structure, we will improve the quality of inferences about our independent variable of interests, which will be "fixed effects" in the formulas.

The nested model we created looks like this:

hydration ~ glycerin + water + time point * baseline + (1|study/panellist)

In this model, the response variable (the effect we try to predict) is hydration (continuous), the fixed effects are glycerin (categorical, 9 levels), water (continuous), time point (categorical, 3 levels) and baseline, which corresponds to hydration at the site before treatment (continuous). The random effects controlling for non-independence of the data are (1|study/panellist) which represents the nested structure of our data such as panellist $\subset$ study. study has 31 levels and panellist, 606. A linear regression/ANOVA would look similar but without the random effect, thus not controlling for the lack of independence in the dataset. There is an interaction term between time point and baseline because we expect hydration values to get closer to baseline as time passes. It is worth mentioning that the glycerin variable was not discrete in the raw data file and was continuous originally, but it was discretized due to its pseudo-discrete distribution. The model has been built using Restricted Maximum Likelihood (REML), an improvement over maximum likelihood estimation for mixed-effects modeling [8]. After filtering of raw data (removal of products with glycerin levels too far from a discrete value, removal of missing values, removal of extre), the model was built on 87 products. All untreated hydration measures at time point 2, 8 and 24h were coded in the

dataset as 0% glycerin and 0% water. For our LMM to be valid, assumptions of linearity of predictors, homoscedasticity of residuals and their normal distribution were checked and deemed to be met.

Results.

Before doing any modeling, we had a look at the relationship between hydration and glycerin levels in our dataset to check if it made sense to even model the relationship further. As shown in **Figure 1**, it looked like an increase in hydration at 2; 8 and even 24h was observed as the % of glycerin increases, meaning that a causal relationship between glycerin levels and hydration was plausible. According to this first analysis, it seemed that 3% glycerin led to a strong increase hydration after 2h and 8h compared to baseline (0h) and corrected to the untreated site. This increase was less clear at 24h, until 6.5% glycerin levels were reached.

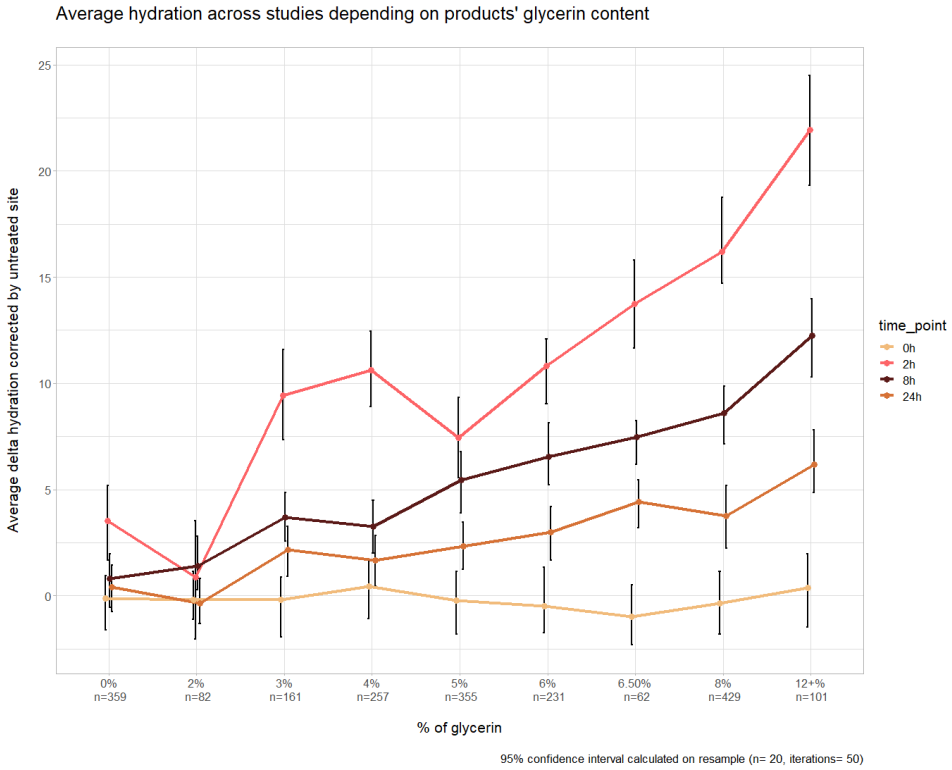


Figure 1: Mean capacitance changes (a.u.) at T0, 2h, 8h and 24h based on % of glycerin in products. The error bars correspond to a pessimistic 95% confidence interval (CI) calculated on 50 resampling of the data with 20 random hydration measures for a given % of glycerin as to simulate a hydration study. "n" represents the number of hydration measures in the study treated with products containing a given % of glycerin.

The explanatory power of the model is substantial with a conditional $R^2 = 0.698$, the part related to the fixed effects alone has a marginal $R^2 = 0.676$. The model is summarised in

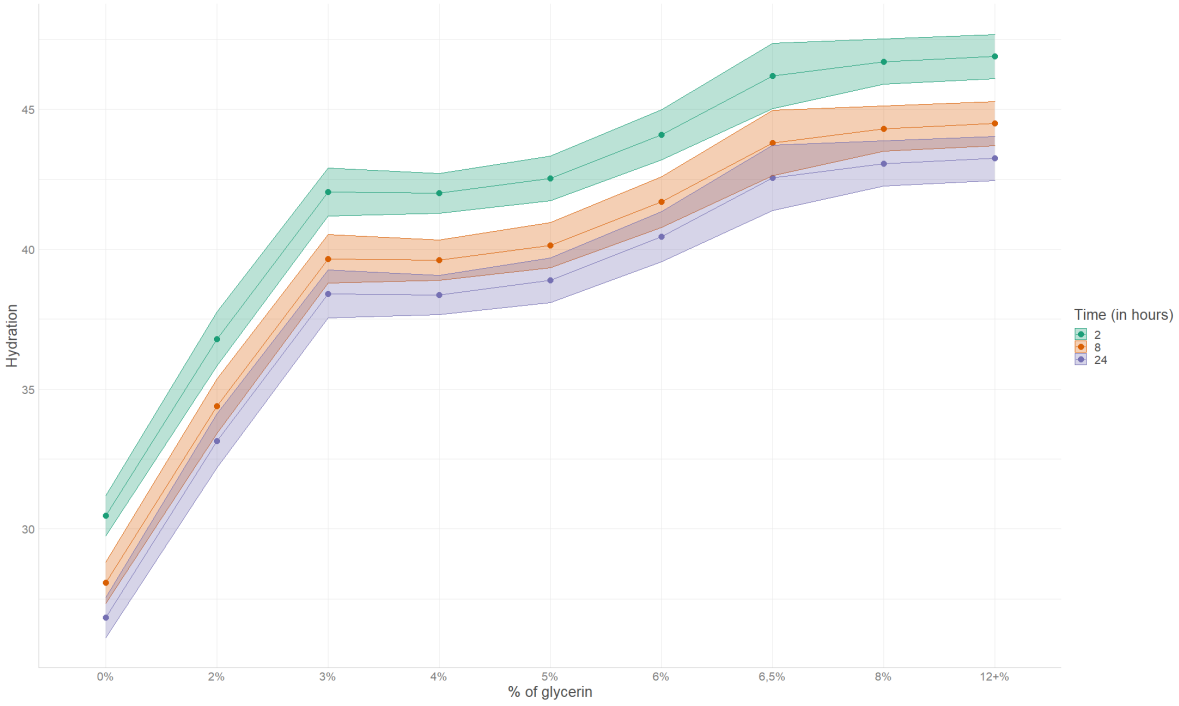
Table 1.

Table 1: Summary table for linear mixed model explaining hydration. Computation of p-values is based on conditional F-tests with Kenward-Roger approximation for the degrees of freedom.

	hydration		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	4.52	3.56 – 5.48	**<0.001**
glycerin cat [2%]	6.31	5.03 – 7.59	**<0.001**
glycerin cat [3%]	11.58	10.32 – 12.84	**<0.001**
glycerin cat [4%]	11.53	10.45 – 12.61	**<0.001**
glycerin cat [5%]	12.06	10.85 – 13.28	**<0.001**
glycerin cat [6%]	13.61	12.29 – 14.93	**<0.001**
glycerin cat [6,5%]	15.72	14.18 – 17.25	**<0.001**
glycerin cat [8%]	16.23	14.99 – 17.48	**<0.001**
glycerin cat [12+%]	16.41	15.48 – 17.35	**<0.001**
water	-0.10	-0.11 – -0.08	**<0.001**
time [8]	0.89	-0.38 – 2.16	0.169
time [24]	2.19	1.03 – 3.35	**<0.001**
baseline	0.93	0.90 – 0.96	**<0.001**
time [8] * baseline	-0.10	-0.14 – -0.06	**<0.001**
time [24] * baseline	-0.18	-0.22 – -0.15	**<0.001**
Random Effects			
σ^2	24.40		
$\tau_{00\ panellist:study}$	0.69		
$\tau_{00\ study}$	1.07		
ICC	0.07		
$N_{\ panellist}$	606		
$N_{\ study}$	31		
Observations	8090		
Marginal R^2 / Conditional R^2	0.676 / 0.698		

As shown in Table 1, the intercept in this model has an average hydration value of 4.52 (95% CI, 3.56 to 5.48) which represents cases where glycerin is 0%, water 0%, at 2 hours after treatment and with a baseline hydration pre-treatment of 0. We can see that all the fixed effects are statistically significant, except time at 8h which has no statistically significant effect on hydration values compared to the reference level for time in this model (2 hours). An important increase of the fixed effect on hydration is observed for 3% of glycerin compared to 2%, jumping from 6.31 (95% CI, 5.03 to 7.59) more hydration on average, to 11.58 (95% CI, 10.32 to 12.84). This increase in hydration is statistically significant compared to basal levels of glycerin (0%). The interaction between baseline (hydration values before treatment) and time is significant both for 8 and 24 hours. This was to be expected as hydration values tend to get closer to baseline as time passes.

Figure 2: Impact of percentage of glycerin and time fixed effects on hydration according to the LMM. The ribbons around each line/dot represent the standard error. Hydration at 24h is in purple, 8h in orange and 2h in green. The uncertainty of the random effects from the full LMM are not taken into consideration here.



In Figure 2, a graphical representation of the impact of two fixed effects (percentage of glycerin and time) on the hydration in our LMM, we can clearly see a sharp increase of

hydration for products with 3% glycerin. Hydration then forms a plateau for glycerin levels above 3 until around 6% of glycerin, where it increases sharply and forms a second plateau from 6.5 to 12+% glycerin. These observations confirm what was previously observed on raw data in Figure 1.

Finally, to validate our hydration LMM, we decided to predict average hydration values of products included in a hydration study external to the dataset used to build the model and compare these predicted values to experimental average hydration values measured by corneometer during the study. Results of the predictions are presented in Figure 3. The generated prediction intervals are satisfactory. For example, the second point from the left on Figure 3 has a predicted average hydration of 28.23, an experimental average hydration value of 29.09, an upper bound of 33.84 and a lower bound of 22.23 (difference of 11.61) when it comes to uncertainty. Only one out of six products had an average experimental hydration value out of bounds with an actual experimental hydration of 25.88 on average and a lower predicted bound of 27.61.

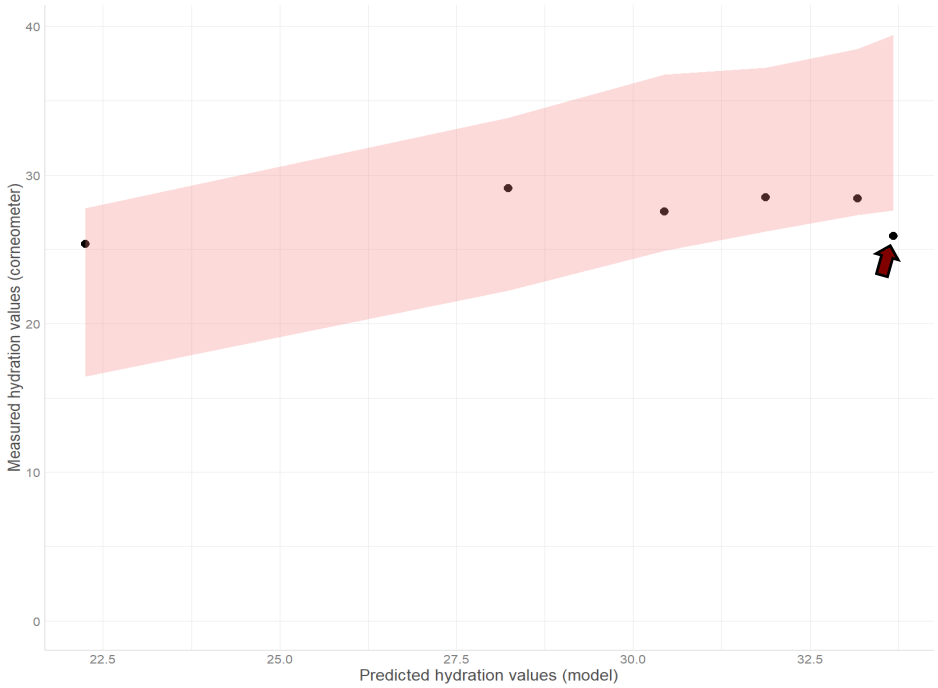


Figure 3: Average hydration values predicted by the LMM compared with average experimental hydration values for a study containing 6 products, acting as a test set. The ribbons around each line/dot represent the prediction intervals (uncertainty) of the LMM calculated by the merTools package in R. The point designated by a brown arrow is a hybrid product close to a foundation.

Discussion.

In the present retrospective study, the clinical data of 134 products tested on human panels using the corneometer were revisited. Inclusion of a minimum of 3% of glycerin in a product guaranteed that a significant boost in hydration could be achieved following a single application, at 2 and 8 hours. For a 24h significant effect, a minimum level of 6.5% was required. These two inclusion levels for glycerin were confirmed to be of interest for hydration by the LMM, as described in the results section for Figure 2 and Table 1. This LMM allows us to predict hydration based mostly on glycerin and water inclusion levels, together with a time component, the baseline of hydration before the start of a study and some blocking parameters (study and panellists). The predictive performances of this model are satisfactory despite its simplicity. Its strength resides in the absence of multiple inclusion levels of various substances, hinting at the preeminence of glycerin inclusion levels to predict hydration.

Water is the main plasticizer of the skin top layers but only application of moisturizer containing key ingredients, glycerin being one of them, can yield a sustained hydrating effects. Early studies reported that skin hydration can be improved with concentration as little as 2% v/v of glycerin, which is ten times higher than endogenous glycerol content in the forearm skin estimated at 0.2 lg/cm². Interestingly, glycerin levels above 3%, matching our established minimum levels for 2h and 8h hydration, was shown to mitigate sodium lauryl sulphate damage as measured with capacitance and TEWL, in a separate investigation**.** Our data also show that level greater than 5% do not induce dehydration of the epidermis through osmotic pressure, as suggested by an investigation based on a sodium lauryl sulphate challenge model [9].

Formulations packed with glycerin grant benefits beyond skin hydration. Interestingly, recent data have reported that moisturizers with high glycerin content may exhibit anti-ageing effects and upregulate proteins involved in desquamation process, glycation/oxidation of proteins, cell-glycan extracellular interaction, tissue mechanical properties and adhesion [10]. Large inclusion of glycerin can lower the water activity of

products and make it less favorable for undesirable microorganisms to growth [11]. As tempting as may be products with substantial glycerin levels, care must be taken to avoid a sticky after-feel [12], and the present data facilitate the choice of relevant glycerin concentrations according to the targeted hydration-based claim.

Despite the inclusion of several galenic in this data set (e.g. O/W emulsion, W/O emulsion, gels etc.), it should be borne in mind that, beyond the level of glycerin in a formula, the vehicle will play a key role. For instance, O/W emulsions are better than W/O emulsion at potentializing the glycerin hydrating effect [13]. Lastly, we did not consider make-up products as their formula backbone(s) differ too drastically from skin or body care products, with more frequent anhydrous format or heavy pigment load. The hybrid product in figure 3, which contains pigments and powders, clearly illustrated that the present model is not suitable for these peculiar products.

Conclusion.

In summary, retrospective analysis of 134 products containing various levels of Glycerin, revealed that in the context of clinical studies that would be performed on 20 volunteers:

▪ A single application of an emulsion, gel or solution containing at least 3 % of glycerin guarantee a significant increase of skin hydration in vivo, 2 and 8 hours after application.

▪ A single application of an emulsion, gel or solution containing at least 6.5 % of glycerin guarantee a significant increase of skin hydration in vivo, 24h after application.

▪ A simple linear mixed model predicting hydration can be established, mostly relying on discrete inclusion levels of glycerin.

Conflict of Interest Statement. None.

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