IMPROVEMENT OF THE BIO-AVALAIBILITY OF AN HYDROPHILIC ACTIVE IN DEEPER SKIN LAYERS:GALENIC DEVELOPMENT AROUND A COMPLEX EQUILIBRIUM OF GLYCOLS AND OILS

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1 INTRODUCTION

The case of hydrophilic actives vectorization is quite special as they display a great affinity for most of the galenic that are used and hence, in most cases, a poor skin delivery at average concentrations. In this paper we discuss the way a new galenic was designed to perform at the same time from a biological performance standpoint and delivering sensory cues that strengthen the performance from a consumer standpoint.

In order to maximize the targeting and delivery of hydrophilic anti-ageing actives, it is important to select the right referent molecule but also the right galenic to propel it. The active that was selected is the C-xyloside (Figure 1) that displays both a strong hydrophilicity (logP = -1.7) and biological effects. In vitro cultured keratinocytes and fibroblasts data show that C-Xyloside can induce different biomarkers related to skin regeneration and firmness [1].
The selected galenic is a surfactant free micro-emulsion [2]. It is designed to be a ternary between water, octanol, and ethanol. In this work, the system was complexified to an unprecedented extent to monophasic systems based on aliphatic oils, triglycerides, fatty alcohol, glycols and ethanol considering Hansen's Parameters. This system presents two major advantages. First of all, the polarity of the medium is unfavorable to C-xyloside which increases its chemical potential and thus fosters a partition of the active towards the skin once the formula gets in contact with it [3;4]. The second advantage is that this formulation increases the skin permeability of actives via the transitory disturbance of the lipidic structure of the stratum corneum.

2 MATERIALS & METHODS

Design of clinical test :
This 6 months study was conducted as a double blind, randomized and comparative clinical test. Subjects served as their own reference for the comparisons in time. Ages: 45 to 65 years old. All skin type (except very oily & very dry skin). The product was applied twice a day. Two cells were established, as following: Cell 1: The specific surfactant-free microemulsion containing 3% of C-xyloside (Formula A) was applied prior to a classical cream that did not contain any C-xyloside (Formula B). Cell 2: The same classical cream than in cell 1 was applied. This time, this cream did contain 3% of C-xyloside (Formula B'). The study took place at the contract research organization (CRO) Spincontrol in Tours.

High resolution ultrasound imaging of the dermis :
A high frequency transducer was used (50 MHz ultrasound scanner; Dub SkinScanner). The depth examined for such an emission frequency is 3 - 4 mm for an intrinsic image resolution of 31 µm.

Self-evaluation questionnaire :
The subjects had to fill in a questionnaire in order to evaluate their overall opinion and their attitude towards the effectiveness of the products being tested.
The questionnaires were filled at Timmediate, T+28 days, T+56 days, T+112 days and T+140 days and T+168 days.

Proposed formulations

Table 1. Composition of a new Surfactant-free Microemulsion (Formula A)

CAS Number	Raw Materials	%
107-41-5	HEXYLENE GLYCOL	9.5
25265-71-8	DIPROPYLENE GLYCOL	8.5
439685-79-7	HYDROXYPROPYL TETRAHYDROPYRANTRIOL (C-xyloside)	3.0
/	OILS MIX	65.1
68002-94-8	OLEYL ALCOHOL	4.9
64-17-5	ETHANOL	7.5
7732-18-5	WATER	Q.S. 100

Table 2. Compositions of classical emulsions with (Formula B') and without (Formula B) C-xylose

CAS Number	Raw Materials	Classical emulsion w/ C-xyloside Formula B' (%)	Classical emulsion w/o C-xyloside Formula B (%)
/	SURFACTANT MIX	5.1	5.1
/	CHELANT	0.1	0.1
/	FATTY COMPOUNDS	16.6	16.6
/	THICKENERS	0.7	0.7
439685-79-7	HYDROXYPROPYL TETRAHYDROPYRANTRIOL	3.2	/
/	PRESERVATIVES	0.5	0.5
504-63-2	PROPANEDIOL	2.3	2.3
5343-92-0	PENTYLENE GLYCOL	3.0	3.0
56-81-5	GLYCERIN	12.0	12.0
7732-18-5	WATER	Q.S. 100	Q.S. 100

Bioavailability assessment method
The bioavailability study was conducted according to the SCCS guidelines on excised full thickness human skin from abdominal plastic surgery obtained from at least 8 different donors. The study was performed using a static Franz-type diffusion cell having 2 cm^2 exposure area. C-xyloside was assessed in the stratum corneum, epidermis, superficial dermis and receptor fluid by validated LC/MS/MS analytical method. Mean concentration in epidermis was calculated assuming an epidermis default thickness value at 100 µm.

Differential Scanning Calorimetry
Samples of SC (30 x 10 mm) are placed in a glove box at RT and 75% RH for 1 night. This phase allows hydration of the materials which improves the lipid organization.

3 RESULTS & DISCUSSION

Hansen Solubility Parameters (HSP) & LogP:
The formulation was designed to deliver a monophasic system using the Hansen solubility parameters to have both a high level of fatty compounds and a stability throughout time at room temperature.
The first step of the strategy has been to fix a desirable concentration of oil and then to adapt the percentages of glycols and ethanol. Considering an association of three glycols is equivalent as researching the crossing point of the surface passing through three glycols, and the segment between C-xyloside and oil mixture (Figure 1).

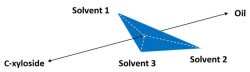


Figure 1. Scheme of a 3 solvents mix with oil and C-xyloside

This technology is a thermodynamic dispersion of nanodroplets of water, most probably containing C-xyloside. The way to process was design to obtain formulas where the C-xyloside is in solubility limit enabling us to have an active with a high degree of saturation.

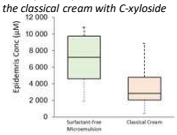
Skin permeability modification & C-xyloside skin delivery :
The skin permeability modification has been studied indirectly following the modification of lipids melting point of the stratum corneum (SC) via Differential Scanning Calorimetry (DSC). The thermogram of the untreated stratum corneum is characterized by differents phase transitions. Among them : T2 (~72 °C : melting of alkyl chains) and T3 (~85 °C : disturbance of the lamellar arrangement of lipids and loss of ordering at the level of polar heads – attributed to corneocyte bound lipids) [5]. These 2 transition temperatures following treatment are interpreted as evidence of product penetration into the SC (transitory modification of its barriers properties), interaction with intercellular lipids and disordering effect on their crystal organization. A decrease of -2 °C is considered significant. The analysis is based on the 2 main transitions of the SC. Results are reported in Table 3.

Table 3. DSC results for T2 and T3 variations for both the surfactant-free microemulsion and the classical cream both containing 3% C-xyloside

Formula	DSC	
	°C ΔT2	°C ΔT3
Surfactant-free microemulsion (Formula A)	-7.9 ± 1.3	-7.2 ± 1.4
Classical cream (Formula B')	-1.2 ± 0.1	-2.5 ± 0.02

Results indicated that there is a notable difference between the surfactant-free microemulsion (Formula A) and the classical cream (Formula B'). Formula A strongly reduces both melting point of SC lipidic structure (both inter-corneocytes lipids and lipids bound to corneocytes) whereas Formula B' has a significantly lesser effect comparatively.
The skin absorption of the C-xyloside was assessed. According to each assay, the concentration in epidermis using surfactant-free microemulsion (Formula A) was 1.4 to 3.3 times (mean 2.5 times) higher than the concentration in epidermis measured for the classical cream also containing 3% C-xyloside (Formula B'). Our hypothesis and formula design has hence been validated.

Figure 2. Bioavailability chart of the C-xyloside within the epidermis for both the surfactant-free microemulsion and the classical cream with C-xyloside



Clinical and Perceived efficacy - Impact of delivery :
Via high resolution ultrasound imaging 50 mHz, a favorable tendency of increase (limit to significance) at D28 for cell 1 (A+B) can be observed compared to baseline (Table 5), whereas no improvement was noticed for cell 2 (B'). From D28 to D168 of application, we obtained a plateau of efficacy. The results demonstrated a statistical increase of the dermis thickness at D84 compared to baseline for cell 1 (A+B) with a large effect size and cell 2 (B') with a moderate effect size. After 168 days of application, the results showed a statistical increase compared to baseline for cell 1 only, that leaded to +8.5% of dermis thickness, with a moderate effect size. In parallel, the statistical increase of dermis thickness at D168 for Cell 2 was not demonstrated (+2.8%).

Table 5. Change from baseline – descriptive & statistical analysis of dermis thickness
Cell 1 (surfactant-free microemulsion containing 3% of C-xyloside, Formula A, was applied prior to a classical cream that did not contain any C-xyloside, Formula B) and Cell 2 (classical cream containing 3% of C-xyloside, Formula B') P-value significance; p ≤ 0.05

Cell	Time	mean	med	sd	min	max	Relative change mean %	p value	Effect Size (ES)
1 (A+B)	D28	79	47.0	169	-266	516	8.0	0.0585	0.421 (moderate)
	D84	94	86.0	165	-266	524	9.4	0.018	0.504 (large)
	D168	83	51.0	169	-235	461	8.5	0.042	0.428 (moderate)
2 (B')	D28	20	47.0	151	-446	211	2.5	0.2682	0.248 (small)
	D84	57	70.5	134	-321	383	5.3	0.018	0.482 (moderate)
	D168	26	32.0	126	-289	211	2.8	0.2186	0.270 (small)

Efficacy perception results were assessed at T 1 month (D28) and T 6 months (D168). The results at D28 show a higher number of items perceived by at least 60% of the panelists in Cell 1 (1.5-2 times more items of benefits very well evaluated) than Cell 2. This is especially true for the perceived mechanical attributes (in Table 6) such as deep firmness feel and tightened skin look. At D168, the difference between the cells is much lower. Nonetheless cell 1 still outperforms cell 2 on perceived mechanical items of benefits.

Table 6. Perceived efficacy of the routines for both Cell 1 (A+B) and Cell 2 (B')
The '+' marks a high level of positive responses (at least 60% of the panel that declared agree or somewhat agree)

Items of questionnaire (Mechanical attributes only)	T 1 month (D28)		T 6 months (D168)	
	Cell 1	Cell 2	Cell 1	Cell 2
Cheeks appear less hollowed				
Face contours look more defined				
Product(s) seems to deeply firm the skin	+		+	
Skin appears less saggy, as retightened	+		+	+
Skin features look relaxed	+	+	+	+
Skin feels bouncy	+		+	
Skin looks more elastic	+		+	
Skin looks tightened, as lifted	+		+	
Skin seems firmer	+		+	+
Skin seems suppler	+	+	+	+
Fine lines appear reduced	+		+	+
Wrinkles appear reduced	+			

4 CONCLUSIONS

Hydrophilic anti-ageing actives that target deep layers of the skin need an effective delivery system to be delivered. The designed formulation to achieve this goal is a complex monophasic equilibrium of oils and glycols. This new galenic showed a strong delivery of the candidate hydrophilic anti-ageing active, C-xyloside. This delivery could be correlated to a significative growth of the derm compartment over one month compared to when the active was vectorized within a classical emulsion. This functionalized galenic also triggered very strong perceived efficacy. This is especially true for perceived skin mechanical properties with many items of benefits reaching high level of positive responses once again versus the classical emulsion containing C-xyloside.

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