Eco-design & Long-Lasting: Biosourced Wear Engines based on PolyHydroxyalkanoates for High-Performance Makeup

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1. Introduction

In the field of make-up, longlasting is one of the strongest expectations expressed by consumers. One of the main attributes sought by consumers is transfer resistance and durability, which means that the coloured deposit should not be transferred to unwanted places (glasses, clothing, etc.), nor should it be removed when water or oils in food or sebum come into contact with the lips or skin [1]. The longlasting performance of a deposit is often obtained via a film-forming polymer conveyed in a volatile solvent. In a typical formulation, film-forming agents are used with rheology modifiers, waxes and pigments in volatile oils. After these oils evaporate, a colored coating forms on the lips or skin. The film-forming polymer will ensure the performance by providing both mechanical resistance (resistance to friction and transfer) and chemical resistance (resistance to water, sebum, olive oil) [2]. For mechanical resistance, the longlasting properties of a cosmetic deposit are achieved by a good balance between cohesion and adhesion. The technologies must have both adhesive and cohesive properties to ensure effective adhesion and solid cohesion of the product to the substrate. The adhesive properties of film-forming polymers allow them to attach to the surface of the skin guaranteeing the non-transfer and resistance of make-up products over a long period of time. The cohesive properties of film-forming polymers are responsible for the resistance and integrity of the formula. These polymers must be able to form a continuous and homogeneous film on the skin that can resist even in the event of friction, perspiration or contact with any chemical element such as water, sebum or edible oil. In long-lasting and transfer-resistant cosmetic applications, trimethylsiloxysilicate silicone resins (also known as MQ) are conventionally used and give lipsticks or foundations exceptional wear and adhesion thanks to their low surface free energy, similar to the surface free energy of human skin. For example, the MQ resin was incorporated into a thermoplastic

elastomer containing a flexible poly(dimethylsiloxane) (PDMS) segment and a polyamide (PA) hard segment that acts as a physical cross-linking through an inter-hydrogen bond between the hard segments for long-lasting, transfer-free lipsticks [3]. The challenge today is to achieve the wear performances of siliconated reference polymers using biobased polymers. Among the different possibilities, we investigated the Polyhydroxyalkanoates (PHA). PHA family consists of a large number of biopolyesters produced by living organisms as a reservoir of carbon and energy in the form of intracellular inclusion bodies [4]. PHAs are grouped into three distinct classes according to the length of the carbon chain. Short-chain PHAs (maximum of 5 carbons in the chain), or *scl*-PHA, medium-chain length PHAs (between 6 and 14 carbons in the chain), *mcl*-PHAs and finally long-chain PHAs (more than 14 carbons in the chain), *lcl*-PHAs. *scl*-PHAs have been studied extensively, particularly for packaging applications. Despite interesting film-forming properties, the use of this class of PHAs is more limited for applications in cosmetic formulas due to their lack of solubility in conventional cosmetic solvents. In this area, their recovery has been targeted mainly to play the role of fillers or in srubbs [5]. Studies on *mcl*-PHAs are much more recent. This class of PHAs is very relevant for valorization in cosmetic formulas because of their compatibility with cosmetic oils [6-7]. *mcl*-PHAs have a lower degree of crystallinity compared to *scl*-PHAs and give them more elastomeric properties. These polymers are film-forming with a very marked cohesive side and the adhesive properties are not at the required level for make-up applications [8]. In this study, a new strategy was implemented to improve the adhesive properties of a *mcl*-PHA. Specific associations have been defined with this biopolymer in order to obtain deposits with longlasting properties at least equivalent to the petrochemical references.

2. Materials and Methods
2.1. Raw materials
mcl-PHA was prepared using the microorganism *Pseudomonas putida* KT2440 ATCC® 47054™, nonanoic acid and acrylic acid. This non-GMO strain is cultivated in a sterile bioreactor using three specific culture media and in particular culture medium 3 containing nonanoic acid and acrylic acid for a duration of 40 hours. The use of acrylic acid allows the inhibition of the β-oxidation pathway during the biosynthesis process and thus to have a *mcl*-PHA structure tending more towards a homopolymeric structure. All culture parameters, such as temperature, stirring, pH, and oxygenation were controlled. Subsequently, cells were collected via centrifugation and subjected to drying by lyophilization. The extraction of *mcl*-PHA involves a specific procedure, resulting in a solid through drying and cutting.

The monomeric composition of the polyhydroxyalkanoate obtained was defined by gas chromatography equipped with a flame ionization detector. Identification is achieved via commercial standard injection and monomeric composition was determined by methanolysis and sylylation treatment. The composition of the biosynthetized *mcl*-PHA is summarized in Table 1. The molecular weight of the *mcl*-PHA was characterized by size exclusion chromatography with a refractive index detection. The number average molecular weight, weight average molecular weight and polydispersity index (Mn, Mw and PDI, respectively) of the *mcl*-PHA are reported in Table 1. This *mcl*-PHA is mainly composed of hydroxynonanoate moieties (86wt-%) and so on, for simplification, will be called PHN for poly(3-HydroxyNonanoate) in the rest of the text.

	Composition (wt-%)			Molecular Weight Distribution		
	HN	HHp	HP	Mn (g/mol)	Mw (g/mol)	PDI
PHN	86	9	5	65 900	143 600	2.2

PHN = mcl-PHA Copolymer of the article – HN = Hydroxynonanoate – HHp = Hydroxyheptanoate – HP = Hydroxypentanoate

Table 1. Composition and molecular mass distribution of the mcl-PHA

For film-forming performance engine dissolution, isododecane from Ineos and absolute etha-nol from Cristalco were used. Waxes were investigated in this study: Helianthus annuus (Sun-flower) wax and Synthetic beeswax from Koster Keunen, Microcrystalline wax from Paramelt, Euphoria Cerifera (Candelilla) wax from Multicerax. Silicon MQ resin, trimethylsiloxysilicate from Momentive in association with Nylon-611/Dimethicone copolymer (PSPA) from Dow were used as a silicone-based film-forming reference material. D&C Red No.7 from Sensient was finally added to color simplified compositions.

2.2. Formulations preparation by Automated formulation platform

The simplified formulations were created using a formulating robot called Formax and pro-posed by Chemspeed, according to the following manufacturing process: isododecane and ethanol are introduced into the reactors at 25°C. After raising the temperature to 80°C, the PHN is introduced into the reactors under rotor-stator agitation at 2500 rpm for 30 minutes. In the case of formulations containing wax, the wax is introduced into the reactors 30 minutes after the PHN, and the agitation speed is increased to 3000 rpm and maintained for 60 minutes. Coloring pigments can also be added at this stage. The manufacturing process concludes by returning to 25°C under agitation at 2500 rpm.

2.3. Thermal analysis

The polymer's thermal properties, namely the glass transition temperature (T_g), the melting temperature (T_m), and the melting enthalpy (ΔH_m) were measured by Differential Scanning Calorimetry (DSC) using a TA Instruments DSC 2500. Initially, the samples were heated and then cooled to erase the material's thermal history before being heated again with a tempera-ture ramp of 20°C/min between - 80°C and 120°C. In some cases, we also added an isotherm step at 32°C during the second heating phase. This isotherm step has been carried out for 2h, 4h, 8h, 16h or 24h.

2.4. Wear and mechanical properties
2.4.1. In-vitro Wear performances

The automated platform FLAME (Flexible Automated Material Evaluations) by Chemspeed is used for longlasting measurements. Unlike conventional rotary tribometers, this platform im-poses linear movements.

- *Friction Resistance Measurements*

The coatings are prepared on a Byko Chart contrast card and let dry for 24 hours at 25°C and 45% relative humidity. The thickness of the deposit is 30µm after drying. A hydrophilic steel ball is used as the friction element. For friction measurements, continuous contact is main-tained during the back-and-forth movement of the ball or friction element. The applied normal

force is 1N, and the sliding speed is 50 mm/s. Multiple passes are performed by the friction element along defined tracks on each film. The number of passes increases for each track. Friction resistance is quantified as the minimum number of passes required to completely wear through the coating. In this study, the number of passes per track are 10, 30, 50, 100, 200, and 300, respectively.

- *Film resistance to Olive oil and Water*

The coatings are prepared following the method previously described for Friction resistance. A 10 µL droplet of olive oil or a 20µL droplet of water is then deposited on the film. The macroscopic appearance of the film is visually observed 1 hour and 24 hours after droplet deposition to assess its integrity. The level of sensitivity is noted according to the scale described in Table 2.

Solubilized	Sticky	Marked	No solubilized
The deposit is solubilized, the support is visible	The deposit is present, but stickiness is noticeable on the finger	The deposit is present, but the imprint of the drop is visible on the deposit	The film has remained intact

Tableau 2. Evaluations of the chemical resistance - Level of sensitivity to chemical aggressors

2.4.2. Mechanical properties under traction

The films are prepared by depositing the formula onto a Teflon mold. The volume of formula needed to obtain a film with a thickness between 250 and 300 µm after drying is poured into the mold. The film is dried for 7 days at 25°C and 45% relative humidity. Its thickness after drying is measured using a caliper. Dumbbell-shaped samples are then cut out using a punch. The effective tensile area is 20 x 4 mm². Tensile measurements are carried out at 25°C using a Zwick tensile testing machine without an extensometer. The distance between the jaws was 30mm. The traction speed was 50mm.s^{-1}. A minimum of five samples were tested for each experiment.

2.5. *In*-vivo performances

In-vivo evaluations were performed on forearms. A thin film from the simplified formula was made on the forearm. After 15 minutes drying, a Kimwipe was pressed on the film for 15 times to see how much color was transferred to the wipe and the damage to the film on the substrate was observed and evaluated. To complete this *in*-vivo evaluation, first evaluations on lips were conducted and the performances observed after application and after a defined lunch. Transfer was also evaluated after pressing a Kimwipe on the lips and taking a photograph of the wipe.

3. Results
3.1. PHN characterizations
3.1.1. Thermal Properties

The thermal properties of pure PHN biopolymer were firstly assessed. A 20wt-% solution of PHN in isododecane/ethanol (78wt%/2wt%) was prepared. The solution was deposited in a 500µm thickness mold in a confined setup at 32°C and 50% relative humidity (RH). This film was analyzed by DSC after 24 hours of drying, when all the volatile solvents are gone. We observe a glass transition around -45°C and a melting point during the first heating phase at 57,6°C. No crystallisation is observed during the cooling phase and neither any melting point during the second heating phase, Figure 1.
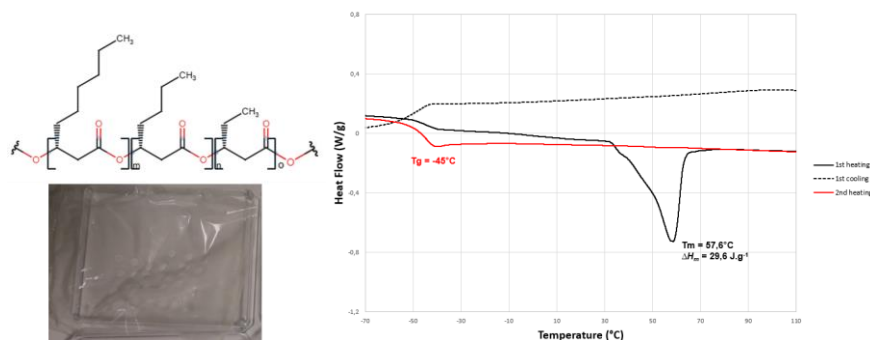
Figure 1. Thermal behaviour of a PHN thin film formed after 24h drying

A second analysis was conducted with an additionnal isotherm step at 32°C during the second heating phase. The isotherm is conducted for 2h, 4h, 8h, 16h and 24h. The thermograms are presented Figure 2. Wheareas no melting peak is observed during the second regular heating phase, we see a melting peak appearing slightly after 2 hours of isotherm at 32°C, and clearly after 4h of isotherm, continuoulsly increasing until reaching an enthalpy of 22.2 J.g^{-1} at 24 hours, Figure 2. These results show a low crystallisation process for the PHN.

Isotherm Time at 32°C	Tm (°C)	ΔH_m (J.g^{-1})
0	-	-
2h	58,0	0,6
4h	57,5	5,1
8h	57,4	20,4
16h	57,4	21,4
24h	57,4	22,2
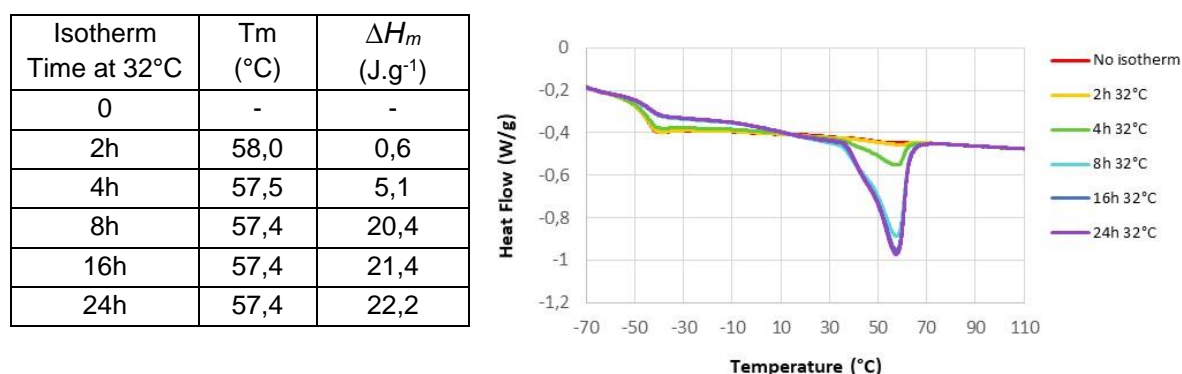
Figure 2. PHN thin deposit thermal behaviour observed during the second heating after various isotherm times at 32°C

3.1.2. PHN wear and mechanical Properties

Figure 1 shows the capacity of the PHN to form a film. The mechanical properties of this film can be characterized thanks to elongation at break experiments. This is conducted on a 300µm thickness PHN film. The results are shown in Figure 3. Films obtained from PHN display a viscoelastic behaviour with high elongation at break. In the experimental conditions, a break was obtained for PHN film when reaching a elongation around 360%.
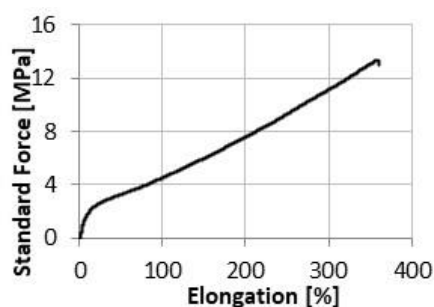
E (MPa)	40
F_{max} (MPa)	14
dL(F_{max}) (%)	360
F_{Rupt} (MPa)	14
dL(rupture) (%)	360

Figure 3. Elongation at break curve for a 300µm thickness PHN film

Thin film (30µm) properties were evaluated thanks to the Flame automated platform, Table 3. PHN material leads to cohesive film-forming deposits, resistant to water, in particular. However, the deposits obtained from PHN showed low dry wear resistance. Indeed, a peeling problem was observed, probably linked to a lack of adhesion of this polymer, Table 3.

3.2. PHN and Waxes associations
3.2.1. PHN and waxes deposits longlasting properties

Associations of PHN with different waxes were made using the automated formulation tool called Formax. The composition of the mixtures is identical regardless of the wax tested with 20wt-% PHN and 5wt-% wax in a solvent mixture containing 72.5wt-% isododecane and 2.5wt-% ethanol. A comparison was made with the MQ/PSPA mixture, two silicone polymers conventionally used in the field of make-up formulated at 25wt-% in isododecan. The mechanical and chemical resistance properties of the deposits were evaluated using the Flame Automated Platform and summarized in the Table 3.
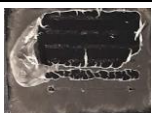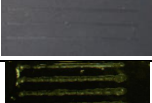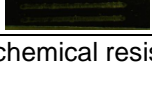
	Mechanical resistance		Chemical resistance
	Resistance to friction		Water resistance
PHN		Resistance inferior to 10 passes of the steel ball	
PHN + Helianthus annuus (Sunflower) wax		Resistance up to 300 passes of the steel ball	
PHN + Synthetic beeswax		Resistance up to 200 passes of the steel ball	
PHN + Microcrystalline wax		Resistance up to 300 passes of the steel ball	
PHN + Euphoria Cerifera (Candelilla) wax		Resistance up to 300 passes of the steel ball	
MQ/PSPA		Resistance up to 300 passes of the steel ball	

Table 3. Mechanical and chemical resistances of PHN, MQ/PSPA and PHN in association with wax

The four waxes associated with the PHN significantly improve the dry wear properties of the deposits. PHN alone is chemically resistant to water. The introduction of wax doesn't degrade this chemical resistance. For the rest of the studies, Sunflower wax was prioritized based on its naturalness, bio-sourcing and environmental impact. Thus, a new green performance engine is designed and constituted by the PHN / Sunflower wax association. This new longlasting engine, in terms of mechanical and chemical resistance, shows similar performances to the MQ/PSPA reference system with dry wear resistance at more than 300 passes of the steel ball and a deposit insensitive to water.

3.2.2. Thermal Properties of a PHn and Sunflower wax association

A solution of PHN at 20% and Sunflower wax at 5% in Isododecane/Ethanol has been prepared. Deposits were formed starting from this solution and let dry for 24h.

A first thin deposit was made on a glass substrate to be microscopically observed under white light and polarized light, Figure 4. Under white light, we observe a continuous deposit with small roughness irregularities. Under polarized light, we observe numerous and well dispersed cristals of Sunflower wax. These observations tend to show that the deposit is made of a continuous film of PHN containing well dispersed inclusion of Sunflower wax crystals.
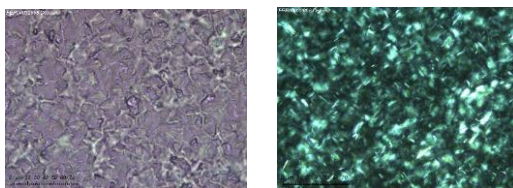
Figure 4. Microscopic observation under white light (on the left) and polarized light (on the right) of a thin dry deposit of a PHN/Sunflower wax (20/5) solution

The second deposit was prepared in the same conditions described earlier for the deposit of PHN. Thermal behaviour of this deposit has been studied by DSC. The thermogram is presented, Figure 5 (b). In parallel, the thermal behaviour of the Sunflower wax as a raw material has been studied by DSC following the same protocol than what has been done for the PHN, Figure 5 (a). We observe a clear melting point around 80°C during both the first and the second heating. The melting enthalpy is very high compared to the one of PHN ($\Delta Hm = 208,5$ J.g^{-1} during the first heating) indicating a strong crystallinity.
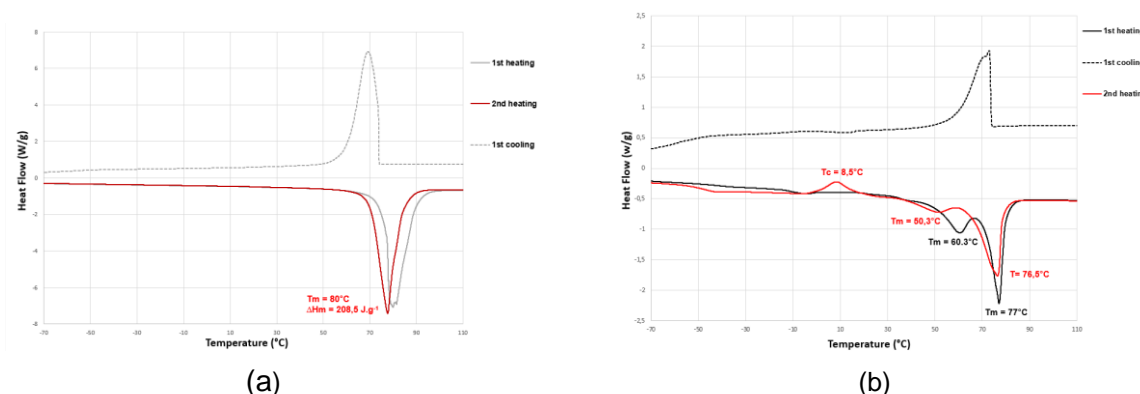

| (a) | (b) |

Figure 5. Thermal behavior of : (a) Sunflower wax – (b) PHN and Sunflower wax (80/20)

For the PHN and Sunflower wax association, during the first heating, we observe two close melting peaks at 60,3°C and 77°C that are very similar to the melting peaks of the PHN alone (Tm = 56,5°C) on one side, and of the Sunflower wax alone (Tm = 80°C) on the other side. This indicates that the two ingredients are still able to crystallize when they are mixed together. During the cooling phase, we observe a crystallisation peak that is similar to the one observed for the Sunflower wax alone (Tc = 72,9°C vs 69,2°C). During the second heating phase, we observe an exothermic peak at 8,5°C, and two endothermic peaks at 50,3°C and 76,5°C

respectively similar to the PHN and Sunflower wax melting peaks. The observation of the PHN melting peak in the PHN/Wax association during the second heating, in comparison to the absence of any melting peak for the PHN alone (Figure 1), suggests that, in the presence of the Sunflower wax, the PHN is forced to crystallize immediately upon heating. The Sunflower wax could be seen as a PHN crystallisation booster.

3.3. Liquid Lipsticks simplified formulations

From the above sections, it is observed that PHN wear engine can meet most of the criteria to be good candidate for longlasting make-up product. To demonstrate it, the *in*-vitro and *in*-vivo performances of the simplified lipstick formulations consisting of PHN +/- Sunflower wax, volatile solvent and pigments were evaluated and compared to a commercial formula containing MQ/PSPA wear engine, Table 4.
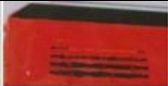
		Formula with PHN	Formula with PHN + Sunflower wax	Commercial Formula containing MQ/PSPA
Composition of liquid lipsticks simplified formula	PHN	20wt-%	20wt-%	-
	Sunflower Wax	-	5wt-%	-
	DC-Red 7	10wt-%	10wt-%	-
	Isododecane	56wt-%	52wt-%	-
	Ethanol	14wt-%	13wt-%	-
In-vitro performances by FLAME	Dry friction resistance			
	Water resistance			
	Olive oil resistance			
In-vivo performances on forearm	Dry friction resistance			
In-vivo performances on lips	Initial	-		
	After lunch	-		
	Transfer	-		

Table 4. In-vitro and in-vivo performances of formula containing PHN alone, PHN in association with Sunflower wax and MQ/PSPA silicone-based performance engines.

An in-vitro evaluation was conducted on the Flame automated platform as well as a first *in*-vivo evaluation on the forearm. The results are summarized in the Table 4. Formulas containing wear engines based on either PHN in combination with Sunflower wax or on the MQ/PSPA silicone-based system have similar *in*-vitro and *in*-vivo mechanical resistance performances. They are very resistant to dry wear by comparison to the PHN alone which does

not resist after a few passes of the steel ball. For chemical resistance, all evaluated formulas are very resistant. The best formula based on PHN in association with Sunflower wax and the commercial formula with MQ/PSPA were applied on the lips. The lastingness of the deposit (Wear and non-transfer) was observed directly after the application and after a lunch, Table 4. The PHN-based formula has a comparable level of longlasting to the commercial formula containing MQ/PSPA and improved transfer resistance.

4. Discussion

The thermal analysis performed on PHN show that it is a semi-crystalline polymer presenting a glass transition around -45°C, a melting temperature around 58°C, and an enthalpy of fusion around 30 J.g^{-1}, Figure 1.The PHN material of the study also presents a slow crystallization process. As shown in Figure 2, we have to wait for few hours at 32°C to see the melting peak appearing. We still see it increasing until at least 24h at 32°C, showing that we need more than 24h to achieve the maximum of crystallization. The low glass transition temperature combined to the crystallinity of the polymer observed until 57°C, gives the PHN the capacity to form solid and highly stretchable films, Figure 3. These observations are in agreement with the litterature around Poly-3-hydroxyoctanoate (PHO), differing from the PHN only by one less carbon atom in its lateral chain. Larranaga et al. [9-10] specifically evaluated the crystallization of a PHO copolymer through thermal and mechanical analysis. Initially amorphous, the copolymer develops crystallinity over time, with the rate dependent on temperature. These confirmed the slow crystallization rate and showed increasing crystallinity and melting temperature over time, further supporting the development of a more ordered crystalline structure. They demonstrated a correlation between crystallinity and mechanical properties. Higher isotherm temperatures resulted in increased Young's modulus and decreased elongation at break, indicating a more rigid and cohesive material. This reinforces the connection between crystal structure and mechanical performance.

The dry PHN deposits show a high chemical resistance at room temperature. This can also be explained by the cristallinity of the PHN. Despite their high level of cohesion, films of PHN show a lack of adhesion, Table 3. In order to improve the global wear performance of deposits made of PHN, we associated it to some waxes like Sunflower wax. Sunflower wax crystals are well dispersed in the PHN film formed upon drying (Figure 4) indicating a good affinity with the PHN. The thermal analysis shows that they act as a booster of the PHN crystallization (Figure 5) thus contributing to mechanical behaviour of the films. Moreover, they highly contribute to the adhesion of the film on a substrate (Tables 3 and 4), thus boosting significantly the friction resistance. Finally, simplex liquid lipsticks formulas were built around the PHN/Sunflower wax wear engine. They show high wear performance both *in*-vitro and *in*-vivo, at the level of the siliconated actual best formulas (Table 4).

5. Conclusion

This study focused on a biopolymer from the mcl-PHA family to provide long-lasting properties to a make-up deposit. This high molecular weight polymer has a semi-crystalline character with slow crystallization kinetics. By controlling this crystallization at the deposition by adding

wax, in particular Sunflower wax, it was possible to obtain deposits with long-lasting properties close to, or even superior to, petrochemical references. The performance of this green performance engine has been demonstrated for application on the lips. This opens up a very important field of possibilities for the entire field of make-up in order to meet the expectations of consumers.

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