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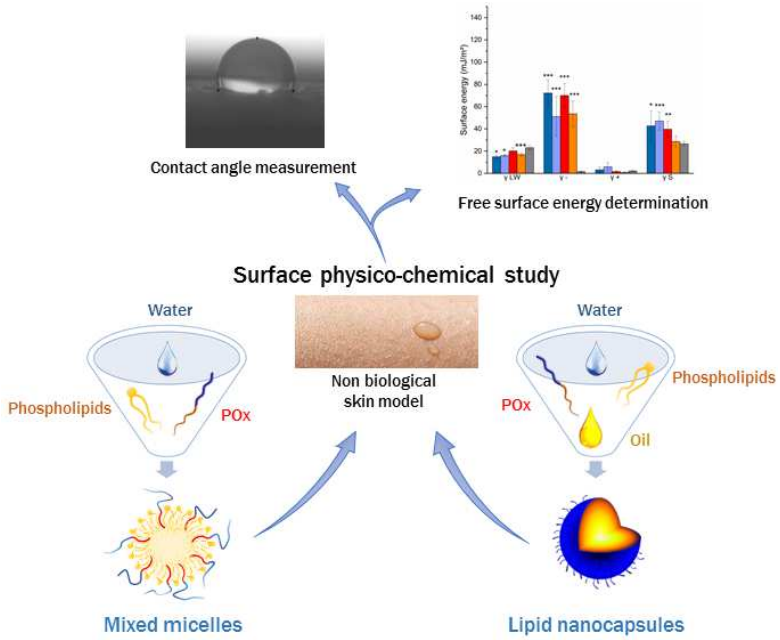
Abstract

The behavior of polyoxazolines based mixed micelles and lipid nanocapsules on skin surface was studied on nonbiological human skin surface model to assess the formulations potential for topical delivery. Two amphiphilic polyoxazolines, saturated ($C_{16}POx_{15}$) and unsaturated ($C_{18:2}POx_{15}$), were used to evaluate the polymer architecture impact on formulations interaction with skin surface. To do so, the formulations spontaneous spreading and their residual film left on surface after manual application was investigated using contact angle measurements and free surface energy determination. The Van Oss model was employed to identify the physicochemical interactions in order to understand how the formulations can change the skin surface properties. In brief, both formulations showed a good affinity with the surface but depending on the polyoxazoline used, the spontaneous formulation spreading was modulated. Overall, the residual film left on the model surface modified the skin model physicochemical properties leading to a better interaction with hydrophilic and polar compounds. Regarding in detail each excipient impact on the surface physicochemical properties further explained the resulting formulation behavior on the skin surface model and highlighted the crucial role of the main component.

Key words: Skin surface model, physicochemical properties, polyoxazoline, lipid nanocapsule, mixed micelles, free surface energy, contact angle

Graphical abstract



I.1 Introduction

To protect the human body from external aggressions, the skin acts as a shield. More especially, the *stratum corneum* with its unique composition and structure of corneocytes embedded in lipid matrix such as "brick and mortar" ensures stiffness and protection [1]. The external aggressions defined as "skin exposome" [2] (pollution, cigarette smoke, UV) directly impact the skin by inducing increased production of radical oxygen species (ROS). Overall, the excess of ROS accelerated skin aging and higher risks of skin cancer [3]. To help the skin maintains its essential integrity for its barrier function, topical products are proposed to deliver active ingredients and to prevent skin damages from happening. Once applied on the skin, topical product spreads onto the surface and then interacts with the skin surface components mainly the sebum. Therefore, it is of interest to predict and understand how the product interacts and modifies the skin surface properties to enhance penetration. To do so, a method was developed based on physicochemical interactions and free surface energy of the skin surface determination. Van Oss et al. studied the interactions of Lifshitz-Van der Waals gathering the forces of Keesom (orientation), Debye (induction) and London (dispersion) and the interactions acide-base (electron acceptor and electron donor, respectively) to characterize the interfacial energy [4]. Mavon et al. applied this equation model to study the effect of sebum on human skin and proved that skin behaves like a basic monopolar surface and sebum increases its hydrophilic affinity (strong electron donor component) [5],[6].

Therefore, Van Oss model and method can predict the spreading and affinity of the topical product on the skin. However, to avoid toxic hazard during *in vivo* testing or wasting expensive *ex vivo* skin, nonbiological skin models (NBSM) were fine tuned to mimic the skin surface properties. Eudier et al. previously deeply examined some nonbiological model regarding their physicochemistry [7] and patented a model reproducing the skin topography and sebum composition. A correlation between the tested solution on their model and *in vivo* results was established [8].

Thus, this NBSM was selected to investigate the surface interactions with new lipid nanoformulations based on amphiphilic polyoxazolines. Polyoxazoline (POx) is a bioinspired nonionic polymer with a risen interest for therapeutic use due to its excellent biomedical properties [9]. The hydrophilic POx was associated with lipid alkyl chain to form amphiphilic POx also called LipoPOx [10]. The LipoPOx will be called POx in this article to ease the comprehension of the study. Various POx were investigated for their interesting features in development of nanodrug delivery systems and many were developed for intravenous injection delivery. However, POx was also formulated for skin delivery in lipid based nanoformulations such as liposomes, mixed micelles (MM) [11] and lipid nanocapsules (LNC) [12] as stabilizing agent and potential penetration enhancer. MM and LNC were proved to encapsulate, protect and deliver while maintaining the molecule bioactivity in our case a strong antioxidant: quercetin. These two formulations have different composition and morphology suggesting divergent behavior. MM is composed of phospholipids and POx with a hydrophobic core made of the entanglement of hydrophobic chains. On the contrary, LNC is highly ordered with an oily core surrounded by a phospholipid rigid capsule and POx corona. The resulting nanoscale mechanical properties might influence the interaction with the NBSM. Now, as POx have never been used for topical delivery, our work evaluates the MM and LNC interactions with the NBSM and to characterize the free surface energy. Two different POx, one with a linear saturated and one with a linear unsaturated alkyl chain, were explored to see if one of them leads to better skin affinity and penetration efficiency. To go further into understanding the surface physicochemistry, each component of the two formulations will be investigated such as POx.

I.2 Materials and Methods

-For POx synthesis:

2-Methyl-2-oxazoline (MOx, Sigma Aldrich, 99.0%) was dried over CaH_2, distilled at reduced pressure and stored under nitrogen atmosphere. Iodo-hexadecane (95.0 %), potassium hydroxide (KOH), lithium aluminum hydride ($LiAlH_4$, 95 %), trimethylamine (98 %), methanesulfonyl chloride (99.7 %) acetonitrile, diethyl ether, anhydrous tetrahydrofuran (99 %), methanol and acetone were bought from Sigma Aldrich (Germany). Linseed oil was brought from Bioplanète (France).

-For POx based nanoformulations:

Phosphatidylcholine (L-α-Phosphatidylcholine from egg yolk ≈ 60%) and Brij®58 were purchased from Sigma Aldrich (Sigma Aldrich, Germany). Lipophilic Labrafac® WL 1349 (caprylic/capric triglycerides) was brought from Gattefossé (France) and Lipoid S75 (soybean phospholipids with 70% phosphatidylcholine) was kindly provided by Lipoid (Germany). Sodium chloride (NaCl) was bought from VWR. MilliQ water was produced from Milli-Q Gradient A10 (Merck Millipore, Germany) apparatus.

-For contact angle measurements:
Three reference liquids were used to perform surface free energy calculations: ultrapure water (resistivity of 18 $M\Omega.cm^{-1}$ at 25 °C) from Merck Millipore (Germany), diiodomethane (analytical grade, 99% purity) and formamide (analytical grade, 99% purity) from Sigma Aldrich (Germany).

I.2.1 Amphiphilic polyoxazolines (POx) synthesis: $C_{16}POx_n$ and $C_{18:2}POx_n$

Both polyoxazolines (POx) syntheses were based on cationic ring-opening polymerization of MOx performed under nitrogen atmosphere.

The polymerization of $C_{16}POx_n$ was initiated with the commercialized iodohexadecane. Regarding $C_{18:2}POx_n$, the initiator of the polymerization was produced from the linseed oil in two steps. First the linseed oil (4 g, 4.55 mmol) was reduced by lithium aluminum hydride (9 eq, 41 mmol) under nitrogen atmosphere in dry tetrahydrofuran for 8 h. The organic phase was then solubilized in dichloromethane and washed with a solution of sodium chloride saturated. The corresponding fatty alcohol (NMR spectra Figure SI 1) was obtained with a yield of 98 %. Then, methanesulfonyl chloride (1.5 eq, 22.5 mmol) and trimethylamine (1.5 eq, 22.5 mmol) were added to the fatty alcohol in dry diethyl ether for 8 h. After washing with water and dried on $MgSO_4$, the initiator of the polymerization ($C_{18:2}OMs$) was synthesized (NMR spectra Figure SI 2) with a yield of 93 %.

$C_{16}POx_n$ was synthesized after dissolving iodohexadecane (3.564 g, 10.1 mmol) and MOx (15 eq, 151.7 mmol) in dry acetonitrile (0.5 M). After strong agitation at 80 °C for 8 h, the solution was quenched with potassium hydroxide dissolved in methanol (5 eq, 50.5 mmol, 5M). The mixture was left under magnetic stirring at 40 °C for 8 h. The $C_{16}POx_{15}$ was obtained after precipitation dropwised in cold diethyl ether and filtration [11]. $C_{16}POx_{15}$ had a molar mass of 1500 g/mol and a critical aggregation concentration of respectively 150 mg/L. $C_{18:2}POx_{15}$ synthesis occurred in the same conditions with the initiator ($C_{18:2}OMs$) (2.5 g, 7.8 mmol) left to react with MOx (15 eq, 117 mmol) in dry acetonitrile (0.5 M). The polymerization and purification conditions remained the same as $C_{16}POx_{15}$. $C_{18:2}POx_{15}$ (NMR spectra Figure SI 3) had a molar mass of 1500 g/mol and a critical aggregation concentration of respectively 100 mg/L.

The HLB of both polyoxazoline was calculated based on the Griffin method adapted for nonionic surfactant as the equation:

$$\frac{molar\ mass\ of\ hydrophilic\ part}{total\ molar\ mass}\ x\ 20$$

I.2.2 Preparation of mixed micelles (MM)

The mixed micelles (MM) were produced using the thin film method with POx and phosphatidylcholine (PC) at the molar ratio 1:40. The components were dissolved in chloroform: acetone in 1:1 volume ratio and the film was made after evaporation of the solvent mixture under vacuum at 40 °C. The film of PC and POx was hydrated with filtered phosphate buffered saline (PBS, 150 mM, pH 7.4). The solution was then processed 5 times with a high pressure homogenizer (Microfluidizer LV1, Microfluidics, USA) at 10 000 PSI. The size of MM was measured with a Zetasizer NanoZS apparatus (Malvern Instrument, UK) at 18 nm (PDI 0.3). Further structural characterization such as TEM and DSC as well as stability study were performed in [11].

To evaluate the impact of PC excipient on the nonbiological skin model surface properties, the phospholipids were prepared as liposome at the same concentration as in MM (10 g/L).

I.2.3 Preparation of lipid nanocapsules (LNC)

The lipid nanocapsules (LNC) were composed of POx (200 mg), caprylic acid triglycerides oil (Labrafac®) (150 mg) and water (650 mg) and additional phospholipids (Lipoid® S75) (15 mg) and NaCl (30 mg). The mixture was heated at 85 °C and cooled down to 30 °C three times and then sonicated for 4 min at 30 % amplitude with a sonication probe (Branson Ultrasonics Corporation, USA). The solution was heated and cooled down again and 2.5 mL of cold water was added under magnetic stirring. The resulting LNC size was about 30 nm and a PDI of 0.16 [12]. Further structural characterization such as TEM and AFM as well as stability study were performed in [12].

To evaluate the impact of S75 excipient on the nonbiological skin model surface properties, the phospholipids were prepared as liposome at the same concentration as in LNC (5 g/L).

I.2.4 Nonbiological skin surface model

The model was developed by Eudier et al. and the production of the NBSM was deeply explained in their article [8]. The NBSM was made of two parts, one polymeric material (biocompatible silicon) mimicking the surface topography and a coating of artificial sebum (28% free fatty acids, 32% triglycerides, 25% was esters, 10% squalene and 4% cholesterol) reproducing the lipid composition of skin face.

I.2.5 Advancing contact angle measurement

The contact angles were measured with a portative goniometer (PGX +, ScanGaule, France) associated to a high resolution camera and connected to a software (PGPlus). A syringe with an internal diameter of 0.77 mm was used to deposit the solution droplet on the surface. Five successive droplet deposition were performed to reach a final volume of droplet of 7 µL to measure the advancing contact angle. Five pictures were taken immediately after each deposition. Contact angles were determined with the software on both sides of the droplet, the maximum value of the

two sides mean from the five measurements was selected. This entire measurement procedure was repeated five times for each solution tested on the NBSM. All measurements were conducted at 20 °C.

The kinetics measurement over 5 minutes was performed with the same protocol. After deposition, a picture was taken every minute. The same kinetic measurement was performed with water and no significant modification of contact angle was observed showing that the evaporation is neglected.

I.2.6 Free surface energy determination

To determine the free surface energy, 20 µL of solution were dropped on the NBSM surface of 5 cm² and spread manually with 15 rotations and left untouched for 3 minutes. Contact angles with water, diiomethane and formamide were performed with the same procedure as described in 2.5. The Van Oss was employed to determine the free surface energy using the Lifschitz-Van Der Waals (γ_{LW}) components (interactions Keesom, Debye, London) and acid-base components (electron acceptor γ^+ and electron donor γ^-) [5], [4]. Each component was determined with the following Young equation:

$$(1+\cos\theta)\gamma L = 2\sqrt{\gamma_L^{LW}\gamma_S^{LW}} + 2\sqrt{\gamma_L^{+}\gamma_S^{-}} + 2\sqrt{\gamma_L^{-}\gamma_S^{+}}$$

The determination of this equation is further explained in Eudier et al. article [8].

I.2.7 Statistical analysis

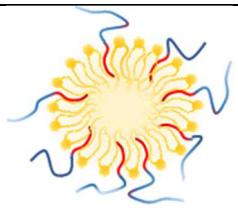
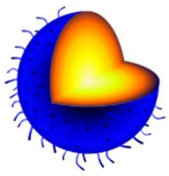
The statistical analysis of the data conducted on the contact angles and free surface energy were performed using the Origin Pro software 8.1 (OriginLab, USA). A two-sample t-test with equal variance was carried out to compare results one by one. The P value reflects the significance with *P < 0.05, **P < 0.01 and ***P < 0.001.

I.3 Results and discussion

I.3.1 Presentation of the mixed micelles and lipid nanocapsules

In order to understand the interactions of the MM and LNC with the surface of NBSM, the main features of the formulations are summarized in Table 1. This table reflected the difference of composition and morphology of the two formulations. MM is mainly composed of phospholipids whereas LNC has more POx and oil. Regarding their morphology, the hydrophobic core of MM correspond to the entanglement of phospholipids and POx alkyl chains [11] whereas for LNC, it is a rigid capsule of phospholipids and POx and a soft oil core. The mechanical properties due to the rigid capsule of LNC were confirmed with no deformation observed up to a constraint of 10-20 nN [12].

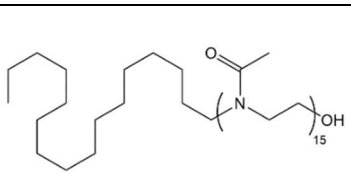
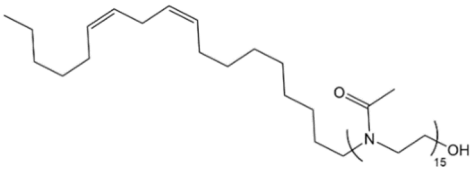
Table 1: Composition and concentration of MM and LNC

	Mixed micelles	Lipid nanocapsules
Structure		
Size (PDI)	20 nm (0.30)	30 nm (0.16)

Name	Composition	Concentration (g/L)	Name	Composition	Concentration (g/L)
Liposome PC	Phosphatidylcholine	10	**Liposome S75**	70% Phosphatidylcholine + 30% soy bean lecithin	5
POx	$C_{16}POx_{15}$ or $C_{18:2}POx_{15}$	0.5	**POx**	$C_{16}POx_{15}$ or $C_{18:2}POx_{15}$	62.5
			Labrafac®	Caprylic/ capric triglycerides	48

The characteristics of the two POx polymers: structure, molar mass, CMC, calculated HLB are summarized in Table 2 to evaluate the impact of the alkyl chain of the two different POx in formulation. These features were determined as described in a previous publication [11]. Even if their structures are different, their properties do not differ a lot. The other components such as the excipients used in the formulations were then examined as described in Table 1.

Table 2: Summary of $C_{16}POx_{15}$ and $C_{18:2}POx_{15}$ characteristics

Properties	**$C_{16}POx_{15}$**	**$C_{18:2}POx_{15}$**
Structure		
Molar mass (g/mol)	1520	1540
CMC (mg/L)	150	100
Effectiveness (mN/m)	34	32
Calculated HLB	17	16.7

I.3.2 Interactions of mixed micelles and lipid nanocapsules with the NBSM model

The contact angle of the two formulations on the NBSM was measured from 0 to 5 minutes to evaluate the spontaneous spreading of the solution on the surface after a droplet deposition and the potential kinetic effect over 5 minutes (Figure 1). Contact angle measurements characterize the wettability of a surface by a liquid which depends on the intermolecular interaction between the two. Thus, the smaller the angle is, the higher the liquid spreads, indicating a better intermolecular interaction.

The NBSM mimicking the physicochemical properties of the surface of the skin behaves as a basic monopolar surface. The contact angle of water is about 100° reflecting the hydrophobic surface properties of NBSM and confirming the properties of the model as in [8]. At T0, just after deposition on the NBSM all formulations behaved as water (due to the aqueous continuous medium) with a contact angle of about 100°. Afterwards, a kinetic effect occurred during the first minutes with a sharp decrease of the contact angle for formulations with $C_{16}POx_{15}$ (50° for MM and 55° for LNC) and with a slight decrease for $C_{18:2}POx_{15}$ (84° for MM and 87° for LNC). After 3 minutes, a plateau was

reached. A contact angle lower than 90° implies that the formulation has affinity with the surface. From this plateau, a significant difference between the two formulated POx was demonstrated but none between the type of formulations. To conclude, the POx alkyl chain rapidly dominates over the formulations effect or concentration of POx. Formulations with $C_{16}POx_{15}$ reacted quickly with the surface reflecting their affinity and leading to an enhanced spreading whereas $C_{18:2}POx_{15}$ had almost no impact.

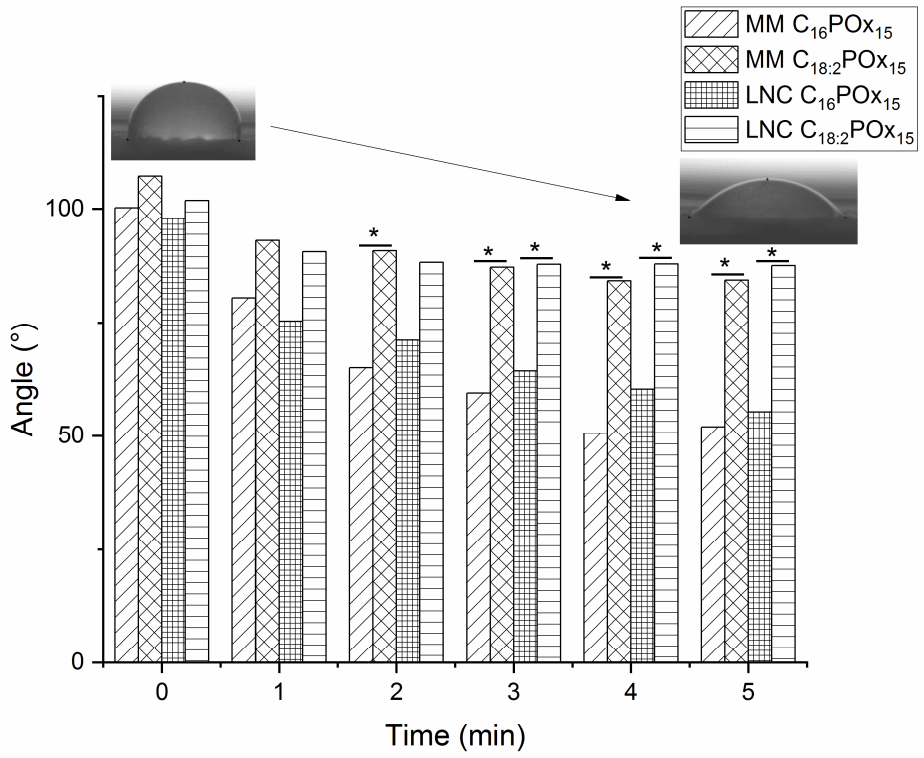


*Figure 1: Contact angle of MM and LNC with $C_{16}POx_{15}$ and $C_{18:2}POx_{15}$ over 5 minutes after deposition on the NBSM. Statistical analysis was performed by a two-sample t-test between the same formulation with different POx (*P < 0.05)*

To get rid of the kinetic effect and understand the interactions, the formulations were manually spread on the NBSM according to the procedure described. The surface was covered with a formulation residual film and left untouched for 3 minutes. It was noticed that the spreading was not completely homogenous on the surface as it is the case *in vivo* for application of aqueous products on human skin. This might be explained by the weak interaction of the hydrophobic surface with a hydrophilic water based solutions in addition to the lack of adsorption due to the polymeric nature of the NBSM. The total surface energy (γ_S) was composed of 3 components (γ_{LW}, γ^+, γ^-) (Figure 2). The surface energy of the NBSM with no formulation applied was measured and considered as a reference for statistical analysis.

The residual film after application on the NBSM was first characterized as described [8], after 3 minutes looking at the water contact angle. Before any product application, the NBSM had a water angle of 114.6 ± 1.7° whereas after formulations application, the value decreased as follows: LNC $C_{16}POx_{15}$ (41.2 ± 4.2°) > LNC $C_{18:2}POx_{15}$ (38.8 ± 2.3°) > MM $C_{18:2}POx_{15}$ (35.2 ± 4.9°) > MM $C_{16}POx_{15}$ (32.5 ± 4.9°). The residual film obtained after spreading both formulations deeply modified the surface

properties as Eudier et al. have previously described with standard emulsion [8]. The free surface energy might help understanding the ongoing interactions. All the following modifications were proved to be significant.

The total surface energy (γ_S) of NBSM (26.5 ± 2.1 mJ/m²) increased with both MM, $C_{16}POx_{15}$ (42.6 ± 13.5 mJ/m²) and $C_{18:2}POx_{15}$ (47.1 ± 8.2 mJ/m²). The apolar and electron donor components explained these modifications. For both MM, γ^- increased while γ_{LW} was lowered thus reflecting a higher basic monopolar behavior. MM $C_{16}POx_{15}$ led to a higher increase of γ^- (72.2 ± 11.6 mJ/m²) compared to MM $C_{18:2}POx_{15}$ (51.1 ± 17.8 mJ/m²). Overall, once spread on the surface both POx from MM behaved the same way with a modification of the surface energy through the electron donor component.

Regarding the LNC, γ_S significantly increased with $C_{16}POx_{15}$ (39.6 ± 7.1 mJ/m²) and slightly increased with $C_{18:2}POx_{15}$ (28.6 ± 4.9 mJ/m²). The higher electron donor component (γ^-) of $C_{16}POx_{15}$ (69.9 ± 10.9 mJ/m²) compared to $C_{18:2}POx_{15}$ (53.5 ± 11.6 mJ/m²) can explain this difference. Overall, the same trends were observed as with MM.

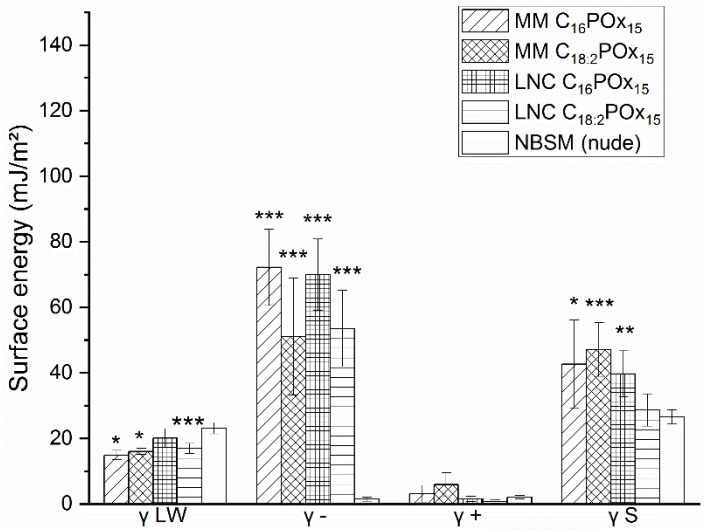


*Figure 2: Free surface energy of NBSM after application of MM and LNC with $C_{16}POx_{15}$ and $C_{18:2}POx_{15}$. Statistical analysis was performed by a two-sample t-test between the NBSM and formulations (*P < 0.05, **P < 0.01, ***P < 0.001)*

As a conclusion, with MM and LNC of both POx the NBSM became more monopolar basic meaning more hydrophilic inducing more interactions with polar molecules. Higher surface energy was reached with MM and LNC with saturated POx indicating a better affinity with hydrophilic and polar compounds and spreading on NBSM [8]. To better understand the formulation behavior regarding each surface energy components, the impact of each excipient was analyzed.

I.3.3 Impact of each component of the nanoformulations

Knowing that each component might influence the formulation behavior and explain the interactions observed in the previous part, all excipients were analyzed on their own.

First, the contact angle of both POx solutions over 5 minutes was measured at the concentration they were introduced in formulation: 0.5 g/L for MM and 62.5 g/L for LNC (Figure 3).

Overall, the contact angle did not decrease as much as for the formulations. However, two trends appeared at 0-1 minute where the concentration dominated over POx and the opposite (POx dominated over concentration) at 3-5 minutes when a plateau was reached. A significant difference ($P < 0.001$) for $C_{16}POx_{15}$ from 0.5 g/L (78°) to 62.5 g/L (88°) was observed at 5 minutes and none for $C_{18:2}POx_{15}$ (respectively 81° and 86°). Therefore, no effect of alkyl chain was clearly noticed and a slight effect of concentration with more hydrophilic modification at 0.5 g/L ($\Theta < 90°$).

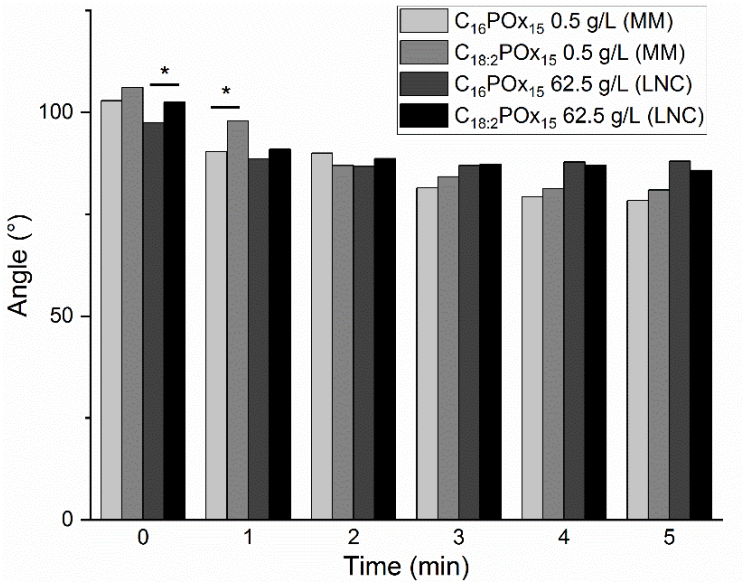


*Figure 3: Contact angle of $C_{16}POx_{15}$ and $C_{18:2}POx_{15}$ over 5 minutes as a function of time. Statistical analysis was performed by a two-sample t-test between the same formulation with different POx (*P < 0.05)*

Secondly, the free surface energy was evaluated separately for MM and LNC with the formulation, the POx and the excipients. Phospholipids were evaluated as liposomes at the concentration in formulation. The surface energy of the NBSM with no formulation applied was measured and considered as a reference for statistical analysis.

Study of each component for mixed micelles

Among the two MM formulations and their components ($C_{16}POx_{15}$, $C_{18:2}POx_{15}$, and liposomes PC), the PC give the higher surface energy γ_S (51.1 ± 2.9 mJ/m²). We do not observe any difference between the intermediate values of MM formulations related to the corresponding POx. From all these results we deduce that phospholipid PC was the component which mainly governed the interactions skin model/nanoformulation.

Looking at the other γ_S components: γ^- and γ_{LW}, both POx had no significant difference with NBSM reference. Nevertheless, $C_{16}POx_{15}$ seemed to slightly impact more the NBSM properties. On the other hand, liposome PC and both MM had significant increase of γ^- and slight of γ_{LW}. As a conclusion the MM behavior was ruled by the phospholipids PC modifying the properties to more basic monopolar. The main component of the formulation dominated the physicochemical interactions. This can be linked with the phospholipids surfactant characteristics and capacity to modify skin properties as demonstrated by Mavon et al. with the study on the sebum [6].

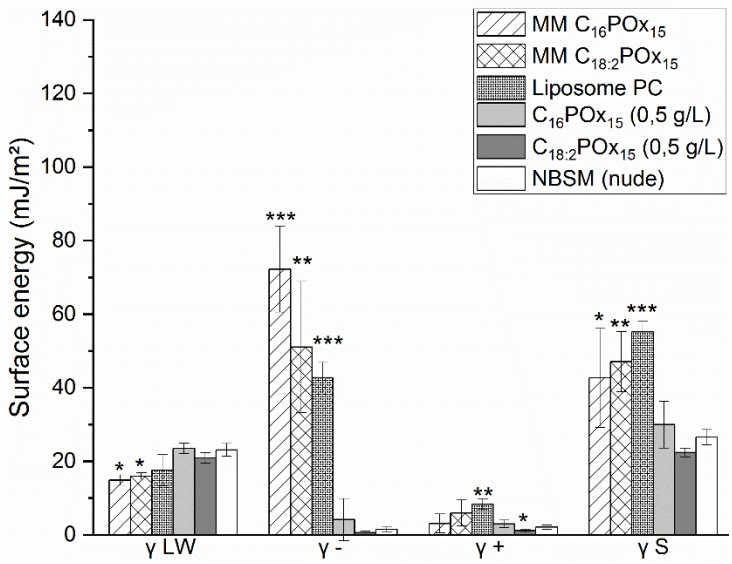


*Figure 4: Free surface energy of MM with each constituent for both POx. Statistical analysis was performed by a two-sample t-test between NBSM and excipients (*P < 0.05, **P < 0.01, ***P < 0.001)*

Study of each component for lipid nanocapsules:

In the case of LNC, almost all components have a significant impact the γ_S, increasing the value from (26.5 ± 2.1 mJ/m²) to (44.4 ± 11.7 mJ/m²) with phospholipid S75, (47.6 ± 0.3 mJ/m²) with Labarafac®, (39.7 ± 7.1 mJ/m²) with LNC $C_{16}POx_{15}$ and (40.4 ± 4.1 mJ/m²) with $C_{16}POx_{15}$.

This increase of surface energy was mostly due to the drastic rise of γ^- as illustrated for liposome S75 (122.1 ± 6.2 mJ/m²). The same trend was observed with LNC $C_{16}POx_{15}$ (69.9 ± 10.9 mJ/m²) and LNC $C_{18:2}POx_{15}$ (53.5 ± 11.6 mJ/m²). The electron donor behavior of LNC was this time governed by the POx as $C_{16}POx_{15}$ (72.1 ± 7.2 mJ/m²) and $C_{18:2}POx_{15}$ (57.1 ± 13.3 mJ/m²). A slight difference between the two polymers was observed with a higher impact with the linear saturated POx triggered by the very high electron donor behavior.

Regarding the apolar component, all excipients decreased value compared to NBSM except the oil (Labrafac®) that had a major rise due to its apolar nature.

To conclude, both POx governed the LNC behavior as the main formulation component.

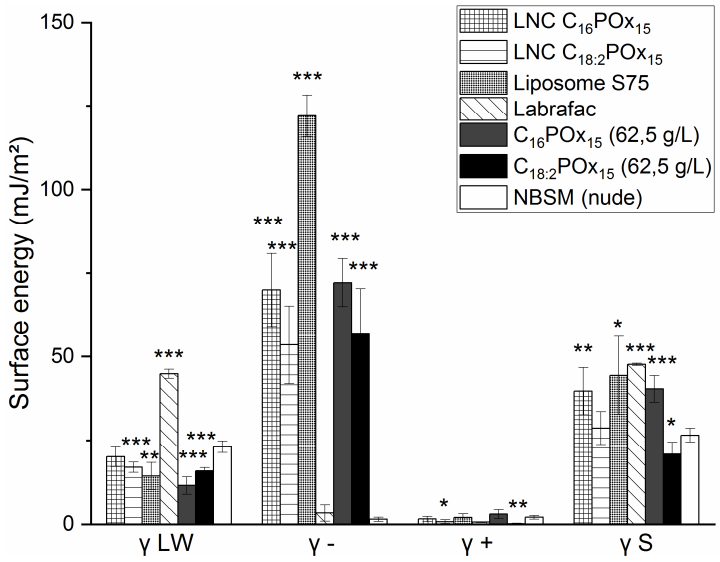


*Figure 5: Free surface energy of LNC with each constituent for both POx. Statistical analysis was performed by a two-sample t-test between NBSM and excipients (*P < 0.05, **P < 0.01, ***P < 0.001)*

The slight difference in between saturated and unsaturated POx might be explained by the air/liquid interfacial behavior with $C_{16}POx_{15}$ slightly more hydrophilic (CMC of 150 vs 100 mg/L) and a better effectivity (34 vs 32). Both formulations showed similar results in terms of surface energy and electron donor behavior. However, for MM the behavior was mainly governed by phospholipids and for LNC by POx. The difference might be explained with the difference of composition and structure. At high concentration (62.5 g/L), POx showed a pronounced electron donor behavior compared to none at low concentration (0.5 g/L). The morphology difference between MM and LNC might also impact the resulting formulation behavior. MM are made of an entanglement of phospholipids (PC) and POx that seem flexible and soft. On the contrary, LNC are highly ordered with oily core, phospholipids (S75) shell and POx corona therefore POx are localized in the outer part of the formulation, they interact directly with the surface. Moreover, the mechanical properties of LNC (rigid capsule) might lower the impact with less interaction capacity and during spreading on surface compared to MM. As the measured surface properties are very sensitive, a nanosized organization of each formulation might matter.

It is difficult to compare these results with the literature as POx alone and formulated POx have never been tested on skin or on the NBSM and the surface physicochemistry is seldom studied. However, we can discuss with the experiments conducted by Mouzouvi et al on the lipid nanocapsules surface properties evaluated with drop tensiometry measurement [13]. Even if these LNC were based on PEG, the formulations were proved to lower the surface tension (35 mN/m) compared to the polymer alone (38 mN/m). Moreover, the high impact of surfactant on the LNC physicochemical properties was demonstrated. These findings are in agreement with the LNC POx. Indeed, the physicochemical interactions were improved with LNC POx compared to POx alone and the POx were shown to play a key role in these interactions.

I.4 Conclusion

This work investigated for the first time polyoxazolines influence on surface physicochemical properties formulated as well as in solution. As polyoxazolines have never been evaluated for topical application, a nonbiological skin surface model mimicking the human skin topography and sebum composition was first used. This tool provides insight on the tested polyoxazolines based nanoformulations capacity to potentially modify and penetrate the skin barrier. To conduct this study, two complementary parameters were assessed. First the spontaneous spreading on the surface model was observed showing the formulation affinity with the surface. Then, the evaluation of residual film after manual application of the formulations, reproducing the use conditions of topical products, reflected the modification of the surface physicochemical properties. Overall, mixed micelles and lipid nanocapsules spontaneous spreading was enhanced with the saturated polyoxazolines ($C_{16}POx_{15}$) indicating the impact of the polymer architecture on formulation spreading. Both formulations produced residual films able to modify the NBSM properties to more hydrophilic behavior (more electron donor γ^-) thus enhancing the affinity with hydrophilic and polar molecules. Looking at the free surface energy components, both formulations behavior was governed by the main component. MM was led by the phospholipids (PC) and LNC by the POx, for this parameter, the structure of polyoxazolines had not influence. These new findings on amphiphilic polyoxazolines behavior on skin surface model highlighted their interesting capacity for topical delivery and these results will be confirmed on biological model such as human skin explant.

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