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1 Study of the physicochemical interactions of nanoformulations based on 2 polyoxazolines with a skin surface model

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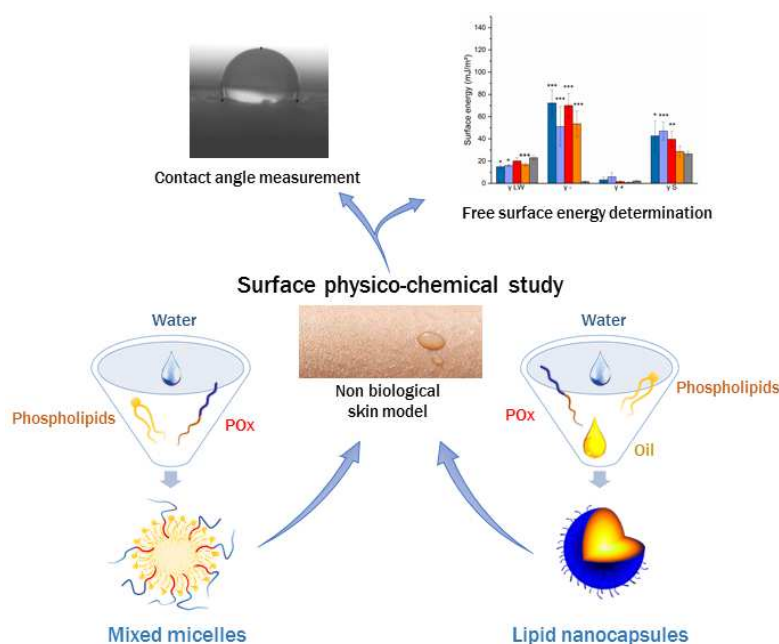
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8 9 **Abstract**

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

24
25 **Key words:** Skin surface model, physicochemical properties, polyoxazoline, lipid nanocapsule, mixed
26 micelles, free surface energy, contact angle

27 28 **Graphical abstract**



29
30

31 I.1 Introduction

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

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

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

75 I.2 Materials and Methods

76 -For POx synthesis:

77 2-Methyl-2-oxazoline (MOx, Sigma Aldrich, 99.0%) was dried over CaH₂, distilled at reduced pressure
78 and stored under nitrogen atmosphere. Iodo-hexadecane (95.0 %), potassium hydroxide (KOH),
79 lithium aluminum hydride (LiAlH₄, 95 %), trimethylamine (98 %), methanesulfonyl chloride (99.7 %)
80 acetonitrile, diethyl ether, anhydrous tetrahydrofuran (99 %), methanol and acetone were bought
81 from Sigma Aldrich (Germany). Linseed oil was brought from Bioplanète (France).

82 -For POx based nanoformulations:

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

89 -For contact angle measurements:

90 Three reference liquids were used to perform surface free energy calculations: ultrapure water
91 (resistivity of 18 M Ω .cm⁻¹ at 25 °C) from Merck Millipore (Germany), diiodomethane (analytical
92 grade, 99% purity) and formamide (analytical grade, 99% purity) from Sigma Aldrich (Germany).

93

94 I.2.1 Amphiphilic polyoxazolines (POx) synthesis: C₁₆POx_n and C_{18:2}POx_n

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

97 The polymerization of C₁₆POx_n was initiated with the commercialized iodo-hexadecane. Regarding
98 C_{18:2}POx_n, the initiator of the polymerization was produced from the linseed oil in two steps. First the
99 linseed oil (4 g, 4.55 mmol) was reduced by lithium aluminum hydride (9 eq, 41 mmol) under
100 nitrogen atmosphere in dry tetrahydrofuran for 8 h. The organic phase was then solubilized in
101 dichloromethane and washed with a solution of sodium chloride saturated. The corresponding fatty
102 alcohol (NMR spectra Figure SI 1) was obtained with a yield of 98 %. Then, methanesulfonyl chloride
103 (1.5 eq, 22.5 mmol) and trimethylamine (1.5 eq, 22.5 mmol) were added to the fatty alcohol in dry
104 diethyl ether for 8 h. After washing with water and dried on MgSO₄, the initiator of the
105 polymerization (C_{18:2}OMs) was synthesized (NMR spectra Figure SI 2) with a yield of 93 %.

106 C₁₆POx_n was synthesized after dissolving iodo-hexadecane (3.564 g, 10.1 mmol) and MOx (15 eq,
107 151.7 mmol) in dry acetonitrile (0.5 M). After strong agitation at 80 °C for 8 h, the solution was
108 quenched with potassium hydroxide dissolved in methanol (5 eq, 50.5 mmol, 5M). The mixture was
109 left under magnetic stirring at 40 °C for 8 h. The C₁₆POx₁₅ was obtained after precipitation dropwised
110 in cold diethyl ether and filtration [11]. C₁₆POx₁₅ had a molar mass of 1500 g/mol and a critical
111 aggregation concentration of respectively 150 mg/L. C_{18:2}POx₁₅ synthesis occurred in the same
112 conditions with the initiator (C_{18:2}OMs) (2.5 g, 7.8 mmol) left to react with MOx (15 eq, 117 mmol) in
113 dry acetonitrile (0.5 M). The polymerization and purification conditions remained the same as
114 C₁₆POx₁₅. C_{18:2}POx₁₅ (NMR spectra Figure SI 3) had a molar mass of 1500 g/mol and a critical
115 aggregation concentration of respectively 100 mg/L.

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

$$118 \quad \frac{\text{molar mass of hydrophilic part}}{\text{total molar mass}} \times 20$$

119

120 **I.2.2 Preparation of mixed micelles (MM)**

121 The mixed micelles (MM) were produced using the thin film method with POx and
122 phosphatidylcholine (PC) at the molar ratio 1:40. The components were dissolved in chloroform:
123 acetone in 1:1 volume ratio and the film was made after evaporation of the solvent mixture under
124 vacuum at 40 °C. The film of PC and POx was hydrated with filtered phosphate buffered saline (PBS,
125 150 mM, pH 7.4). The solution was then processed 5 times with a high pressure homogenizer
126 (Microfluidizer LV1, Microfluidics, USA) at 10 000 PSI. The size of MM was measured with a Zetasizer
127 NanoZS apparatus (Malvern Instrument, UK) at 18 nm (PDI 0.3). Further structural characterization
128 such as TEM and DSC as well as stability study were performed in [11].

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

131 **I.2.3 Preparation of lipid nanocapsules (LNC)**

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

139

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

142 **I.2.4 Nonbiological skin surface model**

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

148 **I.2.5 Advancing contact angle measurement**

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

155 two sides mean from the five measurements was selected. This entire measurement procedure was
 156 repeated five times for each solution tested on the NBSM. All measurements were conducted at 20
 157 °C.

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

161 I.2.6 Free surface energy determination

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

$$168 \quad (1 + \cos \theta)\gamma_L = 2\sqrt{\gamma_L^{LW}\gamma_S^{LW}} + 2\sqrt{\gamma_L^+\gamma_S^-} + 2\sqrt{\gamma_L^-\gamma_S^+}$$

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

170 I.2.7 Statistical analysis

171 The statistical analysis of the data conducted on the contact angles and free surface energy were
 172 performed using the Origin Pro software 8.1 (OriginLab, USA). A two-sample t-test with equal
 173 variance was carried out to compare results one by one. The P value reflects the significance with *P
 174 < 0.05, **P < 0.01 and ***P < 0.001.

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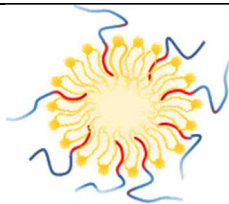
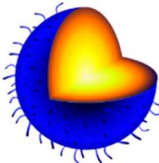
176 I.3 Results and discussion

177 I.3.1 Presentation of the mixed micelles and lipid nanocapsules

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

185

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

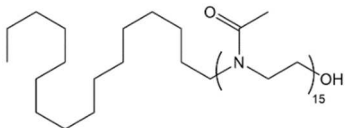
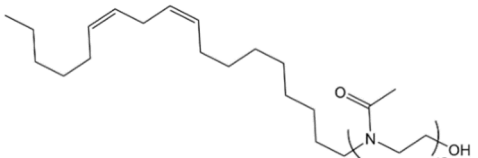
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187 The characteristics of the two POx polymers: structure, molar mass, CMC, calculated HLB are
 188 summarized in Table 2 to evaluate the impact of the alkyl chain of the two different POx in
 189 formulation. These features were determined as described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

192

193

Table 2: Summary of C₁₆POx₁₅ and C_{18:2}POx₁₅ 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

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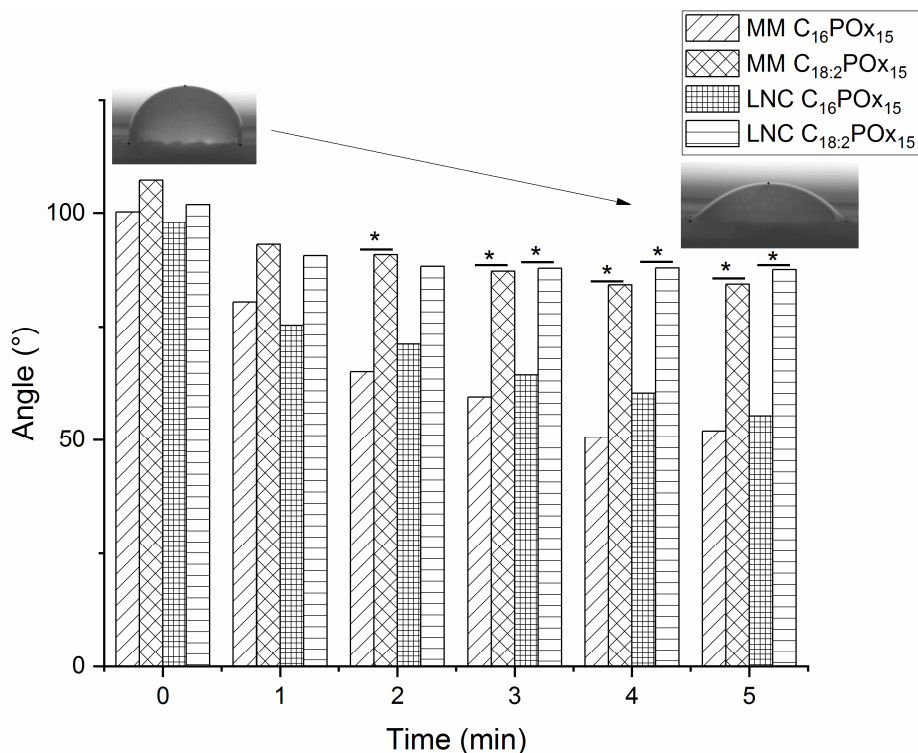
195 1.3.2 Interactions of mixed micelles and lipid nanocapsules with the NBSM model

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

202 The NBSM mimicking the physicochemical properties of the surface of the skin behaves as a basic
 203 monopolar surface. The contact angle of water is about 100° reflecting the hydrophobic surface
 204 properties of NBSM and confirming the properties of the model as in [8]. At T0, just after deposition
 205 on the NBSM all formulations behaved as water (due to the aqueous continuous medium) with a
 206 contact angle of about 100°. Afterwards, a kinetic effect occurred during the first minutes with a
 207 sharp decrease of the contact angle for formulations with C₁₆POx₁₅ (50° for MM and 55° for LNC) and
 208 with a slight decrease for C_{18:2}POx₁₅ (84° for MM and 87° for LNC). After 3 minutes, a plateau was

209 reached. A contact angle lower than 90° implies that the formulation has affinity with the surface.
 210 From this plateau, a significant difference between the two formulated POx was demonstrated but
 211 none between the type of formulations. To conclude, the POx alkyl chain rapidly dominates over the
 212 formulations effect or concentration of POx. Formulations with C₁₆POx₁₅ reacted quickly with the
 213 surface reflecting their affinity and leading to an enhanced spreading whereas C_{18:2}POx₁₅ had almost
 214 no impact.

215



216

217

218 *Figure 1: Contact angle of MM and LNC with C₁₆POx₁₅ and C_{18:2}POx₁₅ over 5 minutes after deposition on the*
 219 *NBSM. Statistical analysis was performed by a two-sample t-test between the same formulation with different*
 220 *POx (*P < 0.05)*

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

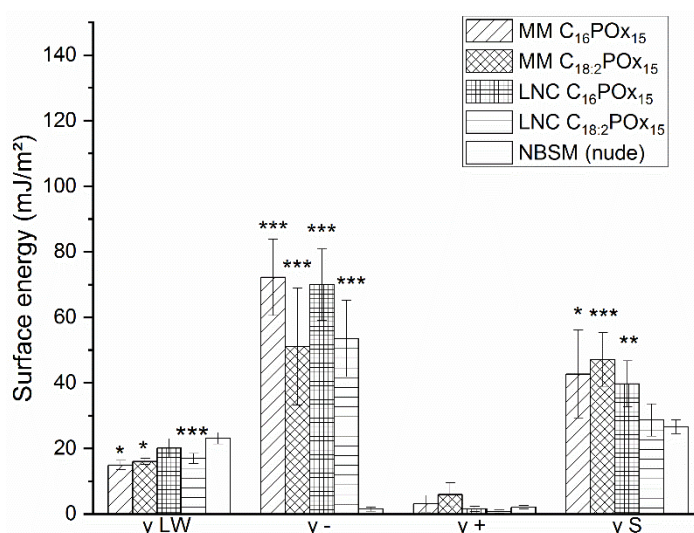
230 The residual film after application on the NBSM was first characterized as described [8], after 3
 231 minutes looking at the water contact angle. Before any product application, the NBSM had a water
 232 angle of $114.6 \pm 1.7^\circ$ whereas after formulations application, the value decreased as follows: LNC
 233 C₁₆POx₁₅ ($41.2 \pm 4.2^\circ$) > LNC C_{18:2}POx₁₅ ($38.8 \pm 2.3^\circ$) > MM C_{18:2}POx₁₅ ($35.2 \pm 4.9^\circ$) > MM C₁₆POx₁₅ (32.5
 234 $\pm 4.9^\circ$). The residual film obtained after spreading both formulations deeply modified the surface

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

238 The total surface energy (γ_s) of NBSM (26.5 ± 2.1 mJ/m²) increased with both MM, C₁₆POX₁₅ ($42.6 \pm$
 239 13.5 mJ/m²) and C_{18:2}POX₁₅ (47.1 ± 8.2 mJ/m²). The apolar and electron donor components explained
 240 these modifications. For both MM, γ^- increased while γ_{LW} was lowered thus reflecting a higher basic
 241 monopolar behavior. MM C₁₆POX₁₅ led to a higher increase of γ^- (72.2 ± 11.6 mJ/m²) compared to
 242 MM C_{18:2}POX₁₅ (51.1 ± 17.8 mJ/m²). Overall, once spread on the surface both POx from MM behaved
 243 the same way with a modification of the surface energy through the electron donor component.

244 Regarding the LNC, γ_s significantly increased with C₁₆POX₁₅ (39.6 ± 7.1 mJ/m²) and slightly increased
 245 with C_{18:2}POX₁₅ (28.6 ± 4.9 mJ/m²). The higher electron donor component (γ^-) of C₁₆POX₁₅ (69.9 ± 10.9
 246 mJ/m²) compared to C_{18:2}POX₁₅ (53.5 ± 11.6 mJ/m²) can explain this difference. Overall, the same
 247 trends were observed as with MM.

248



249
 250 *Figure 2: Free surface energy of NBSM after application of MM and LNC with C₁₆POX₁₅ and C_{18:2}POX₁₅. Statistical*
 251 *analysis was performed by a two-sample t-test between the NBSM and formulations (*P < 0.05, **P < 0.01,*
 252 ****P < 0.001)*

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

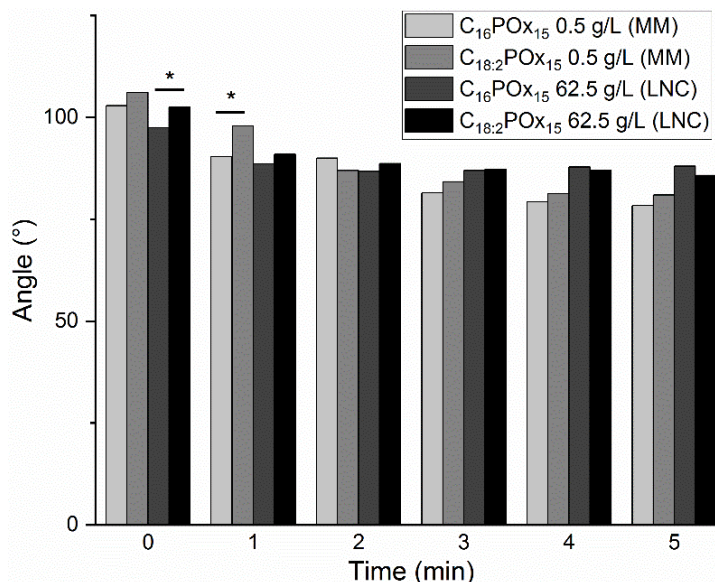
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259 1.3.3 Impact of each component of the nanoformulations

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

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

264 Overall, the contact angle did not decrease as much as for the formulations. However, two trends
 265 appeared at 0-1 minute where the concentration dominated over POx and the opposite (POx
 266 dominated over concentration) at 3-5 minutes when a plateau was reached. A significant difference
 267 ($P < 0.001$) 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
 268 $C_{18:2}POx_{15}$ (respectively 81° and 86°). Therefore, no effect of alkyl chain was clearly noticed and a
 269 slight effect of concentration with more hydrophilic modification at 0.5 g/L ($\theta < 90^\circ$).



270
 271 *Figure 3: Contact angle of $C_{16}POx_{15}$ and $C_{18:2}POx_{15}$ over 5 minutes as a function of time. Statistical*
 272 *analysis was performed by a two-sample t-test between the same formulation with different POx (* $P <$*
 273 *0.05)*

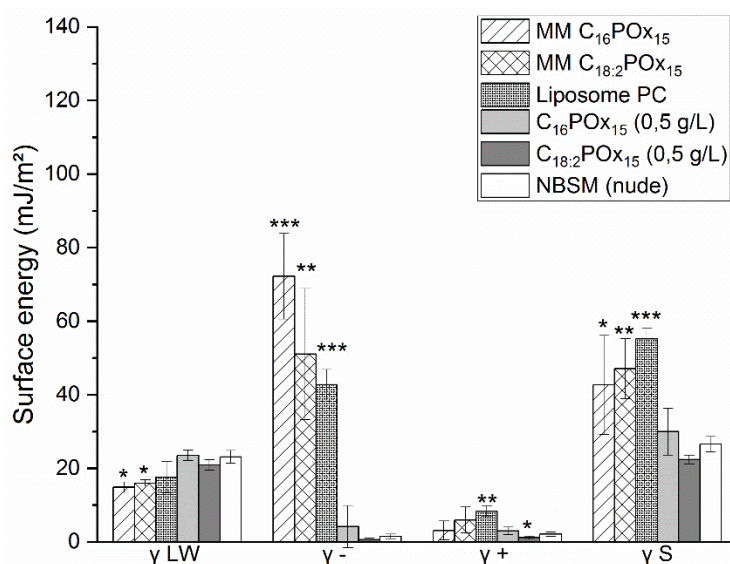
274 Secondly, the free surface energy was evaluated separately for MM and LNC with the formulation,
 275 the POx and the excipients. Phospholipids were evaluated as liposomes at the concentration in
 276 formulation. The surface energy of the NBSM with no formulation applied was measured and
 277 considered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

278

279 **Study of each component for mixed micelles**

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

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



292
 293 *Figure 4: Free surface energy of MM with each constituent for both POx. Statistical analysis was performed by a*
 294 *two-sample t-test between NBSM and excipients (*P < 0.05, **P < 0.01, ***P < 0.001)*

295
 296 **Study of each component for lipid nanocapsules:**

297 In the case of LNC, almost all components have a significant impact the γ_s , increasing the value from
 298 $(26.5 \pm 2.1 \text{ mJ/m}^2)$ to $(44.4 \pm 11.7 \text{ mJ/m}^2)$ with phospholipid S75, $(47.6 \pm 0.3 \text{ mJ/m}^2)$ with Labrafac®,
 299 $(39.7 \pm 7.1 \text{ mJ/m}^2)$ with LNC C₁₆POx₁₅ and $(40.4 \pm 4.1 \text{ mJ/m}^2)$ with C₁₆POx₁₅.

300 This increase of surface energy was mostly due to the drastic rise of γ^- as illustrated for liposome S75
 301 $(122.1 \pm 6.2 \text{ mJ/m}^2)$. The same trend was observed with LNC C₁₆POx₁₅ $(69.9 \pm 10.9 \text{ mJ/m}^2)$ and LNC
 302 C_{18:2}POx₁₅ $(53.5 \pm 11.6 \text{ mJ/m}^2)$. The electron donor behavior of LNC was this time governed by the
 303 POx as C₁₆POx₁₅ $(72.1 \pm 7.2 \text{ mJ/m}^2)$ and C_{18:2}POx₁₅ $(57.1 \pm 13.3 \text{ mJ/m}^2)$. A slight difference between
 304 the two polymers was observed with a higher impact with the linear saturated POx triggered by the
 305 very high electron donor behavior.

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

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

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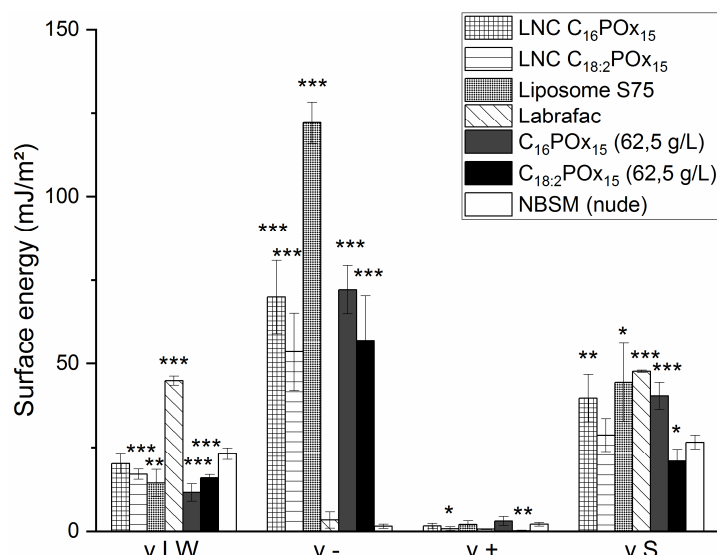


Figure 5: Free surface energy of LNC with each constituent for both POx. Statistical analysis was performed by a two-sample t-test between NBSM and excipients (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

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The slight difference in between saturated and unsaturated POx might be explained by the air/liquid interfacial behavior with C₁₆PO_{x15} 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.

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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.

340 **I.4 Conclusion**

341 This work investigated for the first time polyoxazolines influence on surface physicochemical
342 properties formulated as well as in solution. As polyoxazolines have never been evaluated for topical
343 application, a nonbiological skin surface model mimicking the human skin topography and sebum
344 composition was first used. This tool provides insight on the tested polyoxazolines based
345 nanoformulations capacity to potentially modify and penetrate the skin barrier. To conduct this
346 study, two complementary parameters were assessed. First the spontaneous spreading on the
347 surface model was observed showing the formulation affinity with the surface. Then, the evaluation
348 of residual film after manual application of the formulations, reproducing the use conditions of
349 topical products, reflected the modification of the surface physicochemical properties. Overall, mixed
350 micelles and lipid nanocapsules spontaneous spreading was enhanced with the saturated
351 polyoxazolines (C₁₆POx₁₅) indicating the impact of the polymer architecture on formulation
352 spreading. Both formulations produced residual films able to modify the NBSM properties to more
353 hydrophilic behavior (more electron donor γ^-) thus enhancing the affinity with hydrophilic and polar
354 molecules. Looking at the free surface energy components, both formulations behavior was
355 governed by the main component. MM was led by the phospholipids (PC) and LNC by the POx, for
356 this parameter, the structure of polyoxazolines had not influence. These new findings on amphiphilic
357 polyoxazolines behavior on skin surface model highlighted their interesting capacity for topical
358 delivery and these results will be confirmed on biological model such as human skin explant.

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