## Supplementary file

# Polyglycerol Ester-Based Low Energy Nanoemulsions with Red Raspberry Seed Oil and Fruit Extracts: Formulation Development toward Effective In vitro/In vivo Bioperformance

### Formulation optimization - influence of the cosurfactants, oil and water phase composition

**Table S1.** Z-average droplet size (Z-ave) and polydispersity index (PDI) of blank nanoemulsions prepared with cosurfactant 1 (phenoxyethanol, ethylhexyl glycerol) and/or cosurfactant 2 (raspberry fragrance). Each nanoemulsion contained 10 wt% polyglycerol ester-based surfactant mix, 10 wt% oil phase (ethylhexyl pelargonate + cosurfactant), and 80 wt% water. The samples were stored at room temperature, and the measurements were taken 24 hours and one month after preparation.

| | Z-ave (nm) 24 h | Z-ave (nm) 1 m | PDI 24 h | PDI 1 m |
|---|---|---|---|---|
| **Cosurfactant 1** | | | | |
| 0.5 wt% | 131.700 ± 1.114 | 157.167 ± 2.060 | 0.226 ± 0.012 | 0.201 ± 0.004 |
| 1 wt% | 231.333 ± 5.525 | 2F, unstable | 0.361 ± 0.031 | 2F, unstable |
| **Cosurfactant 2** | | | | |
| 0.5 wt% | 89.760 ± 0.428 | 99.393 ± 0.840 | 0.112 ± 0.003 | 0.116 ± 0.010 |
| 1 wt% | 86.397 ± 0.708 | 103.167 ± 0.814 | 0.116 ± 0.016 | 0.141 ± 0.008 |
| 1.5 wt% | 89.627 ± 0.085 | 104.100 ± 1.153 | 0.078 ± 0.016 | 0.120 ± 0.019 |
| 2 wt% | 89.563 ± 0.533 | 90.917 ± 0.293 | 0.069 ± 0.019 | 0.098 ± 0.006 |
| 2.5 wt% | 85.153 ± 0.345 | 100.050 ± 1.516 | 0.099 ± 0.018 | 0.145 ± 0.021 |
| 3 wt% | 92.443 ± 0.725 | 109.633 ± 1.002 | 0.122 ± 0.009 | 0.133 ± 0.019 |
| 3.5 wt% | 101.100 ± 0.458 | 110.733 ± 1.159 | 0.105 ± 0.006 | 0.168 ± 0.012 |
| 4 wt% | 161.700 ± 5.810 | 159.667 ± 5.372 | 0.314 ± 0.037 | 0.360 ± 0.061 |
| **Cosurfactant mix** | | | | |
| cosurfactant 1, 0.5 wt + cosurfactant 2, 2 wt% | 216.000 ± 1.473 | 2F, unstable | 0.398 ± 0.003 | 2F, unstable |
| cosurfactant 1, 1 wt% + cosurfactant 2, 2 wt% | 328.410 ± 18.28 | 2F, unstable | 0.629 ± 0.116 | 2F, unstable |
| cosurfactant 1, 1 wt% + cosurfactant 2, 1 wt% | 255.212 ± 1.153 | 2F, unstable | 0.272 ± 0.006 | 2F, unstable |

2F –two phase system, i.e. phase separation in unstable formulations

**Table S2.** Z-average droplet size (Z-ave) and polydispersity index (PDI) of the red raspberry seed oil (RO)-loaded nanoemulsions with additional hydrophilic antioxidant extracts from red raspberry – RE or French oak – FE fruit in the nanoemulsion water phase. Each nanoemulsion contained 10 wt% polyglycerol ester-based surfactant mix, 10 wt% oil phase, 0 to 5 wt% hydrophilic extract and water phase (30 wt% glycerol in water), up to 80 wt%, relative to the nanoemulsion total mass. The formulations were stored at room temperature, and the measurements were taken 24 hours and three months after preparation.

| | Z-ave (nm) 24 h | Z-ave (nm) 2 m | PDI 24 h | PDI 2 m |
|---|---|---|---|---|
| ***F1*** *basic formulation* ***oil phase*** *contains: 2 wt% RO (lipophilic antioxidant), 0.5 wt% cosurfactant 1, 7.5 wt% EP* ***Water phase****:* | | | | |
| *NO hydrophilic antioxidant* | 64.328 ± 6.111 | 65.580 ± 1.074 | 0.063 ± 0.020 | 0.059 ± 0.008 |
| 1 wt% RE | 55.150 ± 0.710 | 63.747 ± 0.819 | 0.073 ± 0.012 | 0.071 ± 0.049 |
| 2 wt% RE | 54.917 ± 0.195 | 63.883 ± 1.115 | 0.074 ± 0.025 | 0.055 ± 0.006 |
| 3 wt% RE | 57.243 ± 0.245 | 64.040 ± 1.713 | 0.066 ± 0.112 | 0.056 ± 0.003 |
| 4 wt% RE | 62.633 ± 1.148 | 59.963 ± 0.963 | 0.049 ± 0.004 | 0.049 ± 0.021 |
| 5 wt% RE | 59.350 ± 0.850 | 66.967 ± 1.662 | 0.066 ± 0.013 | 0.061 ± 0.019 |
| 1 wt% FE | 52.237 ± 0.386 | 63.467 ± 1.290 | 0.082 ± 0.014 | 0.042 ± 0.019 |

| | | | | |
|---|---|---|---|---|
| 2 wt% FE | 53.187 ± 0.176 | 62.280 ± 0.951 | 0.097 ± 0.006 | 0.041 ± 0.018 |
| 3 wt% FE | 67.587 ± 1.192 | 65.743 ± 1.401 | 0.044 ± 0.012 | 0.060 ± 0.006 |
| 4 wt% FE | 66.630 ± 1.414 | 66.963 ± 1.589 | 0.063 ± 0.019 | 0.034 ± 0.027 |
| 5 wt% FE | 68.770 ± 2.192 | 62.400 ± 0.607 | 0.054 ± 0.012 | 0.054 ± 0.009 |
| ***F2*** *basic formulation* ***oil phase*** *contains: 2 wt% RO (lipophilic antioxidant), 2 wt% cosurfactant 2, 6 wt% EP* ***Water phase:*** | | | | |
| *NO hydrophilic antioxidant* | 58.980 ± 1.976 | 62.272 ± 4.054 | 0.079 ± 0.012 | 0.111 ± 0.016 |
| 1 wt% RE | 56.543 ± 0.087 | 56.657 ± 1.583 | 0.076 ± 0.010 | 0.081 ± 0.009 |
| 2 wt% RE | 52.853 ± 0.237 | 56.133 ± 1.793 | 0.119 ± 0.010 | 0.080 ± 0.015 |
| 3 wt% RE | 53.030 ± 0.344 | 58.490 ± 1.032 | 0.098 ± 0.022 | 0.077 ± 0.016 |
| 4 wt% RE | 54.100 ± 0.674 | 53.933 ± 1.851 | 0.072 ± 0.009 | 0.037 ± 0.011 |
| 5 wt% RE | 56.700 ± 0.292 | 56.147 ± 1.925 | 0.109 ± 0.019 | 0.046 ± 0.014 |
| 1 wt% FE | 64.923 ± 1.760 | 67.557 ± 1.819 | 0.059 ± 0.012 | 0.060 ± 0.014 |
| 2 wt% FE | 55.560 ± 0.967 | 62.510 ± 1.886 | 0.063 ± 0.019 | 0.062 ± 0.021 |
| 3 wt% FE | 63.907 ± 0.942 | 67.980 ± 1.763 | 0.044 ± 0.019 | 0.052 ± 0.006 |
| 4 wt% FE | 64.697 ± 3.206 | 63.157 ± 1.783 | 0.042 ± 0.021 | 0.067 ± 0.013 |
| 5 wt% FE | 57.373 ± 2.037 | 61.880 ± 2.654 | 0.056 ± 0.014 | 0.089 ± 0.017 |

## Flow rheological measurements

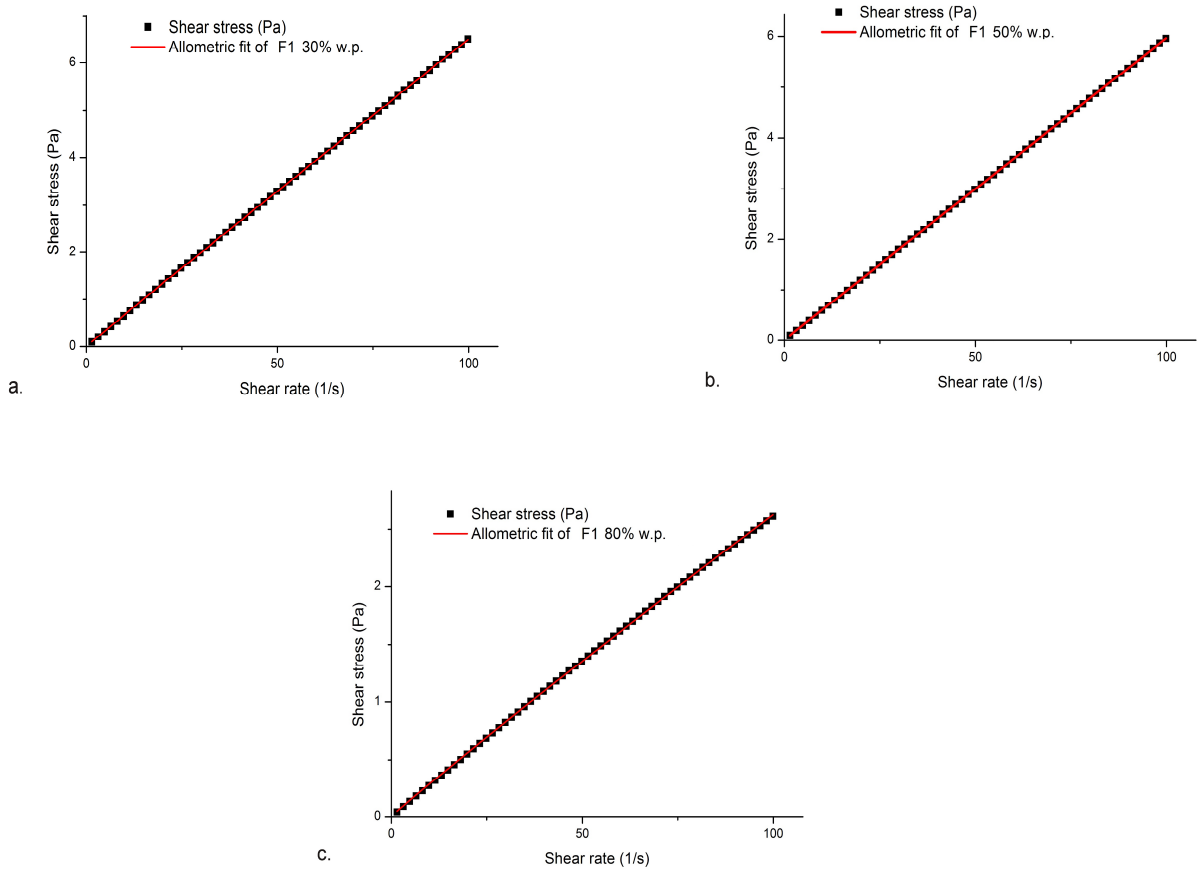


**Figure S1.** Representative flow curves of F1 formulations prepared with: (a) 30 wt% water phase (microemulsion phase) and low-energy nanoemulsions prepared with: (b) 50 wt% water phase and (c) 80 wt% water phase (30 wt% glycerin in water). The figures represent Newtonian flow behavior in all tested samples.

**In vitro cell viability assay – optical density measurements**

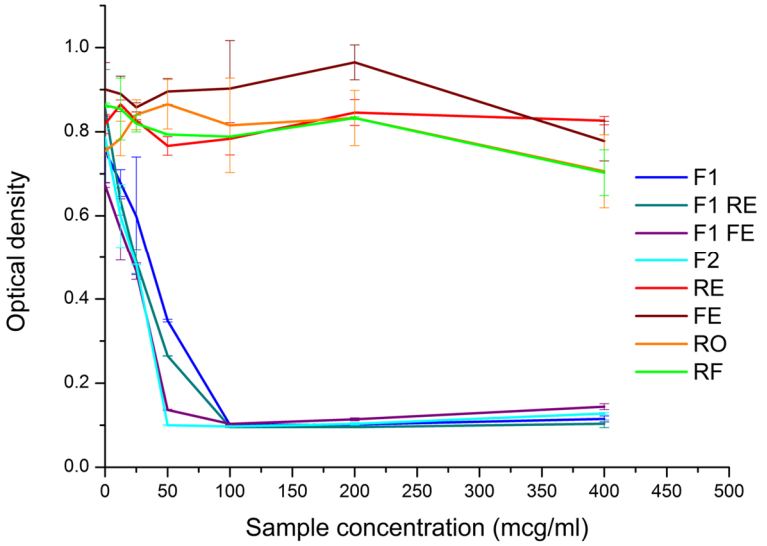


**Figure S2. Optical density at 570 nm of normal human keratinocytes (HaCaT cells):** effect of the raw materials and the corresponding nanoemulsions. Obtained data represent optical density of the cell culture depending on the sample concentration. Each experiment was repeated three times and the results were presented as the mean value ± SD.

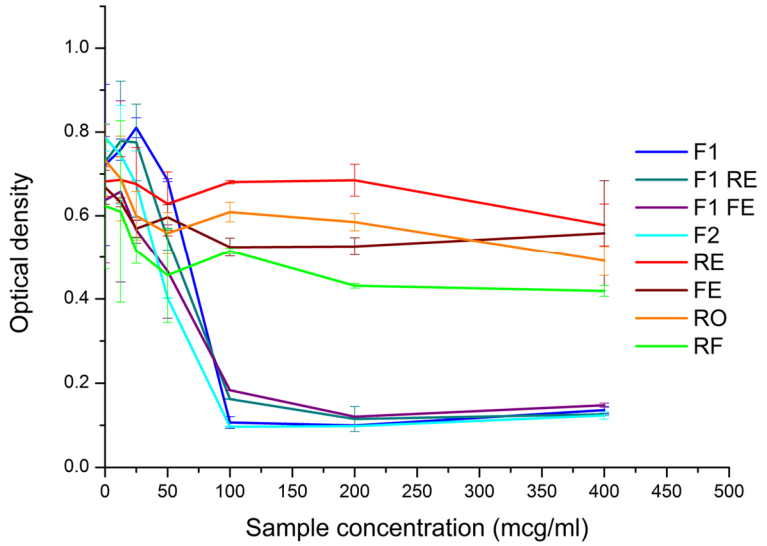


**Figure S3. Optical density at 570 nm of Fem-X human malignant melanoma cells:** effect of the raw materials and the corresponding nanoemulsions. Obtained data represent optical density of the cell culture depending on the sample concentration. Each experiment was repeated three times and the results were presented as the mean value ± SD.

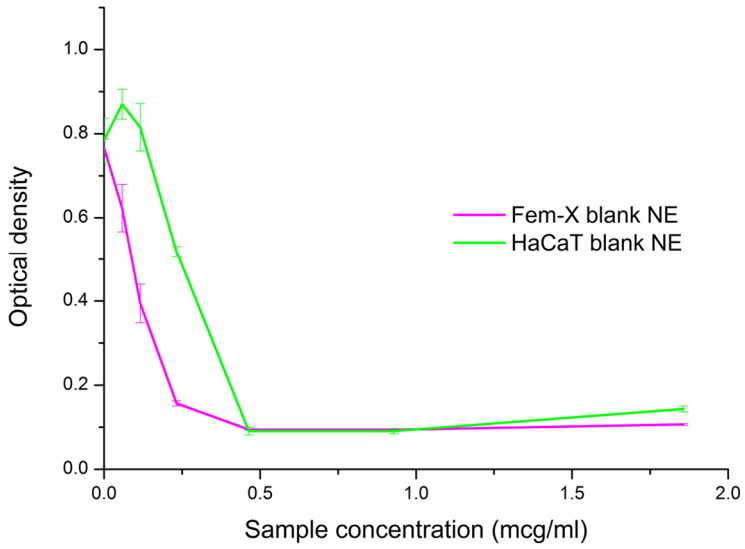


**Figure S4. Optical density at 570 nm of the blank nanoemulsion on HaCaT and Fem-X cells.** Obtained data represent optical density of the cell culture depending on the sample concentration. Each experiment was repeated three times and the results were presented as the mean value ± SD.