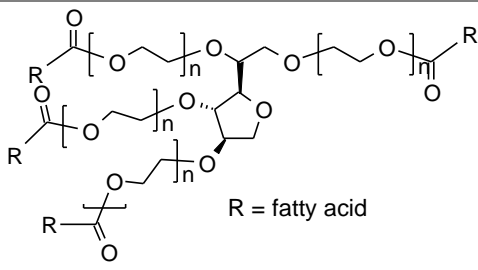
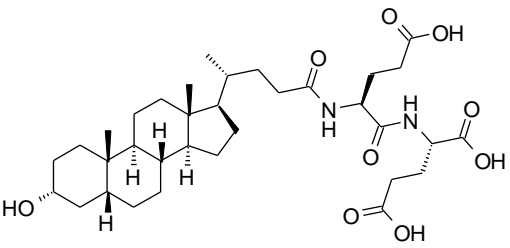
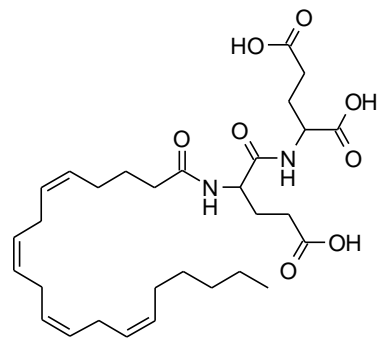
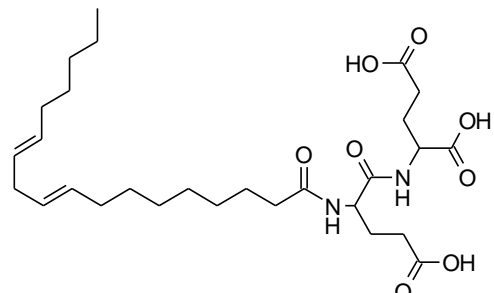
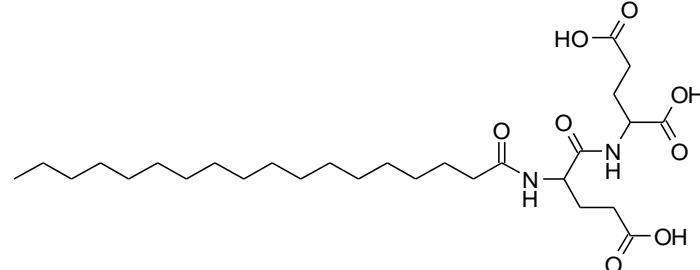
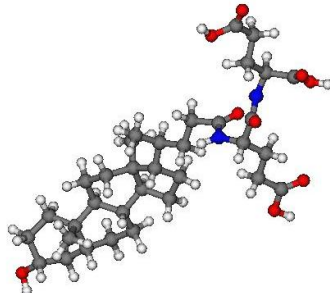
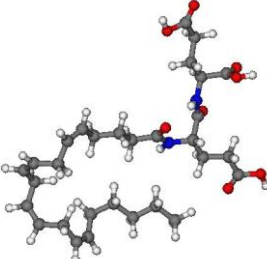
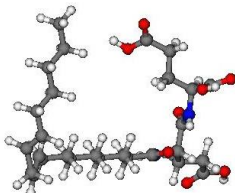
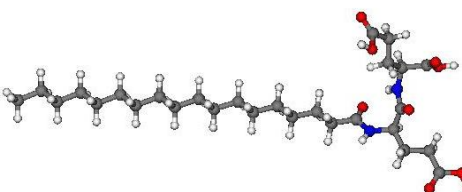


**Table 1** Surfactant composition, hydrophobic and hydrophilic moieties with molecular weights and their chemical structures.

Surfactant	Mw (g/mol)	Chemical structure
Polysorbate 80	1310	 <p>R = fatty acid</p>
Lithocholanoyl-diglutamic acid (DG)	635	
Arachidonoyl-diglutamic acid (DG)	563	
Linoleoyl-diglutamic acid (DG)	539	
Stearoyl-diglutamic acid (DG)	543	

**Table 2** Surfactant molecular modelling *in vacuo* and packing parameter values.

Surfactants	Packing parameters	Lower energy conformers
Lithocholanoyl-DG	0.13	
Arachidonoyl-DG	0.16	
Linoleoyl-DG	0.20	
Stearoyl-DG	0.20	

**Table 3** Comparison of innovative surfactants with polysorbate 80 in term of CMC, IC<sub>50</sub> (µg/mL) of HUVEC treated by surfactants determined with MTT and LDH assays. HC<sub>50</sub> (µg/mL) of rat RBCs treated by surfactants. NA: not applicable.

Surfactants tested	CMC (µg/mL)	CMC (mol/L)	MTT assay IC <sub>50</sub> (µg/mL)	LDH assay IC <sub>50</sub> (µg/mL)	Haemolytic assay HC <sub>50</sub> (µg/mL)
Polysorbate 80	14	1.1 x 10 <sup>-5</sup>	250	130	760
Lithocholanoyl-DG	780	1.23 x 10 <sup>-3</sup>	230	NA	4240
Arachidonoyl-DG	430	7.64 x 10 <sup>-4</sup>	450	NA	4600
Linoleoyl-DG	920	1.71 x 10 <sup>-3</sup>	400	NA	2920
Stearoyl-DG	200	3.69 x 10 <sup>-4</sup>	100	190	2150

**Table 4** Comparison of surfactants with polysorbate 80 in term of drug solubilization and drug loading with an extrapolated surfactant concentration of 40 mg/mL. Drug solubility in water is lower than 0.15 µg/mL.

Surfactants tested	Drug solubility in micelles (mg/mL)	Drug loading (mass balance, %)
Polysorbate 80	13.73	25.6
Lithocholanoyl-DG	0.00065	0.0016
Arachidonoyl-DG	0.017	0.042
Linoleoyl-DG	1.27	3.1
Stearoyl-DG	11.13	21.8

**Table 5** Synthesis of melting temperature and enthalpy of different drug/hydrophobic moieties mixtures determined from DSC profiles.

Drug/hydrophobic moiety % (w/w)	Stearic acid		Lithocholic acid		Linoleic acid	
	Melting point (°C)	Enthalpy (J/g)	Melting point (°C)	Enthalpy (J/g)	Melting point (°C)	Enthalpy (J/g)
0 %	72	198	192	99	- 3	97
10 %	69	183	185	74	- 8	39
30 %	ND	ND	181	28	- 5	8
37 %	68	28	ND	ND	ND	ND
50 %	69	27	183	26	-3	2
70 %	NA	0	NA	0	NA	0
90 %	ND	ND	NA	0	NA	0
100 %	NA	0	NA	0	NA	0

**Table 6** Macroscopic changes at the injection site (tail) after repeated intravenous administration of stearyl-DG. -: no animal affected, relevant changes are indicated in bold.

Injection site (Tail)	Male				Female			
	0	4.5	9	18	0	4.5	9	18
Dose (mg/kg)	0	4.5	9	18	0	4.5	9	18
Number of animals	3	3	3	3	3	3	3	3
Haematoma	-	2	1	2	-	-	-	1
Dark red discolouration	-	-	-	1	-	-	-	2
Oedema	-		<b>1</b>	<b>3</b>	-	-	-	-

**Table 7** Haematologic and clinical chemistry parameters after repeated intravenous administration of stearoyl-DG in rats for a minimum period of 5 days. Daily doses (DD) and cumulative doses (CD) are presented. Indicated values represent the minimum and maximum values of each group. Values in bold are out of the historical data and considered as significant changes, - : No noteworthy findings.

	Historical data		DD = 0 mg/kg CD = 0 mg/kg		DD = 4.5 mg/kg CD = 31,5 mg/kg		DD = 9 mg/kg CD = 45-63 mg/kg		DD = 18 mg/kg CD = 90-126 mg/kg	
	M	F	M	F	M	F	M	F	M	F
RBCs (T/L)	6.59- 8.68	6.66-8.80	-	<b>6.42-</b> 8.62	-	<b>6.14-</b> 8.06	-	6.93- 8.07	-	<b>6.39-</b> 7.04
Haemoglobin concentration (mmol/L)	8.2-10.6	8.5-10.8	-	<b>7.1-</b> 9.6	-	<b>7.1-</b> 9.0	-	8.6-9.4	-	7.3-8.4
Packed cell volume (%)	39.1- 49.1	39.1-49.0	-	<b>33.6-</b> 45.6	-	<b>33.5-</b> 42.6	-	40.5- 45.2	-	<b>34.7-</b> 39.9
Leukocytes (G/L)	1.76- 12.97	1.92-9.68	6.99- 11.54	-	-	-	-	-	4.49- <b>14.93</b>	-
Neutrophils (G/L)	0.23- 2.27	0.17-2.40	0.89- 1.56	-	-	-	-	-	1.52- <b>3.42</b>	-
Albumine (g/L)	36.4- 48.4	39.6-50.4	45.1- 50.2	-	-	-	-	-	36.5-40.3	-
GLDH (U/L)	3-10	4-9	7-10	4-6	-	-	-	-	<b>6-20</b>	<b>6-10</b>

**Table 8** Microscopic observations at the injection site (tail) after repeated intravenous administration of stearyl-DG. -: no animal affected, relevant changes are indicated in bold, §: parietal (one), those seen in dosed rats often seen in several veins, mainly occlusive.

Intravenous injection site (Tail)	Male				Female			
Dose (mg/kg)	0	4.5	9	18	0	4.5	9	18
Number of animals	3	3	3	3	3	3	2	3
Vascular thrombus	-	<b>3</b>	<b>3</b>	<b>2</b>	1§	<b>3</b>	<b>1</b>	<b>2</b>
Dilated veins with congestion	-	<b>1</b>	-	<b>1</b>	-	-	-	<b>1</b>
Exudate , perivenous	3	3	3	3	3	3	2	3
Minimal	3	1	-	-	3	2	2	1
Slight	-	<b>2</b>	<b>2</b>	<b>1</b>	-	<b>1</b>	-	<b>2</b>
Moderate	-	-	<b>1</b>	<b>2</b>	-	-	-	-
Haemorrhage , perivenous	3	3	2	2	1	3	2	3
Minimal	3	3	2	1	1	3	1	2
Slight	-			<b>1</b>	-	-	<b>1</b>	<b>1</b>
Inflammatory cell infiltrate , perivenous	3	3	3	3	3	3	2	3
Minimal	3	1	2	2	3	1	-	2
Slight	-	<b>2</b>	<b>1</b>	<b>1</b>	-	<b>2</b>	<b>2</b>	<b>1</b>
Ulceration (with erosion or vacuolar degeneration), overlying epidermis	-	-	-	<b>1</b>	-	-	-	<b>2</b>