Supplemental Material

Development, optimization and *in vitro* evaluation of norcantharidin loaded self-nanoemulsifying drug delivery systems (NCTD-SNEDDS)

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Tables

Supplementary material Table S1 Emulsification ability of various non-ionic surfactants to emulsify the ethyl oleate (EO).

Surfactant	%Transmittance	Droplet Size (nm)	PDI	Emulsification time (sec)
Cremophor-EL [®]	92.21 ± 0.01	73.47 ± 1.55	0.18 ± 0.04	221 ± 15
Cremophor RH 40 [®]	93.39 ± 0.01	66.79 ± 0.40	0.15 ± 0.06	151 ± 8.5
Tween 20	77.38 ± 0.20	179.2 ± 7.31	0.29 ± 0.02	39 ± 3.0
Tween 80	68.03 ± 0.04	122.73 ± 3.09	0.16 ± 0.03	>300

Data are expressed as mean ± S.D. (n=3).

Supplementary material Table S2 Emulsification ability of various non-ionic surfactants to emulsify the isopropyl myristate (IPM).

Surfactant	%Transmittance	Droplet Size (nm)	PDI	Emulsification time (sec)
Cremophor-EL [®]	85.97 ± 0.01	59.03 ± 0.01	0.18 ± 0.07	194 ± 1.4
Cremophor RH 40 [®]	89.51 ± 0.03	50.36 ± 1.50	0.18 ± 0.02	61.5 ± 8.9
Tween 20	-	-	-	-
Tween 80	-	-	-	-

- means phase separation.

Data are expressed as mean ± S.D. (n=3).

Supplementary material Table S3 Emulsification ability of various non-ionic surfactants to emulsify the MCT.

Surfactant	%Transmittance	Droplet Size (nm)	PDI	Emulsification time (sec)
Cremophor-EL [®]	92.87 ± 0.01	78.88 ± 0.98	0.19 ± 0.01	152 ± 15
Cremophor RH 40 [®]	95.08 ± 0.01	65.12 ± 0.48	0.14 ± 0.03	97 ± 12
Tween 20	-	-	-	-
Tween 80	-	-	-	-

- means phase separation.

Data are expressed as mean ± S.D. (n=3).

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Supplementary material Table S4 Emulsification ability of various non-ionic surfactants to emulsify the Triacetin.

Surfactant	%Transmittance	Droplet Size (nm)	PDI	Emulsification time (sec)
Cremophor-EL [®]	100.08 ± 0.02	17.03 ± 0.83	0.33 ± 0.06	82.5 ± 3.12
Cremophor RH 40 [®]	99.96 ± 0.05	13.91 ± 0.55	0.17 ± 0.08	84.5 ± 6.26
Tween 20	100.36 ± 0.01	40.32 ± 18.08	0.52 ± 0.14	65 ± 7.07
Tween 80	99.38 ± 0.03	12.94 ± 0.89	0.28 ± 0.07	171 ± 12

Data are expressed as mean ± S.D. (n=3).

Supplementary material Table S5 Effect of co-surfactants on the characteristics of nanoemulsion obtained after dilution of SNEDDS prepared using Cremophor[®] EL/ EO.

Co-surfactants	%Transmittance	Droplet Size (nm)	PDI	Emulsification time (sec)
Ethanol	78.06 ± 0.06	56.34 ± 0.69	0.198 ± 0.004	<1min
Ethylene Glycol	95.23 ± 0.03	32.52 ± 1.40	0.094 ± 0.003	<1min
Propanol	63.94 ± 0.07	74.50 ± 0.35	0.161 ± 0.006	<1min
Propylene Glycol	54.63 ± 0.13	64.51 ± 0.78	0.282 ± 0.047	<1min
Isopropanol	83.90 ± 1.60	54.31 ± 1.43	0.150 ± 0.074	<1min
PEG-400	40.50 ± 0.30	33.58 ± 2.15	0.130 ± 0.002	<1min
Glycerol	29.44 ± 0.08	36.40 ± 0.71	0.051 ± 0.003	<1min
n-Butanol	86.89 ± 1.49	62.66 ± 0.11	0.231 ± 0.016	<1min

- means phase separation. The significance of bold typed is to emphasize the selected ingredient.

Data are expressed as mean ± S.D. (n=3).

Supplementary material Table S6 Effect of co-surfactants on the characteristics of nanoemulsion obtained after dilution of SNEDDS prepared using Cremophor[®] RH 40/ EO.

Co-surfactants	%Transmittance	Droplet Size (nm)	PDI	Emulsification time (sec)
Ethanol	52.39 ± 0.13	51.77 ± 0.78	0.205 ± 0.006	<1min
Ethylene Glycol	94.58 ± 0.05	31.50 ± 0.52	0.054 ± 0.004	<1min
Propanol	85.45 ± 0.22	46.71 ± 0.49	0.153 ± 0.018	<1min
Propylene Glycol	93.21 ± 0.07	36.92 ± 0.89	0.040 ± 0.001	<1min
Isopropanol	88.29 ± 0.25	47.07 ± 1.36	0.173 ± 0.006	<1min
PEG-400	33.36 ± 0.22	72.73 ± 0.64	0.274 ± 0.023	<1min
Glycerol	42.88 ± 0.22	73.45 ± 2.71	0.199 ± 0.003	<1min
n-Butanol	94.91 ± 0.19	38.52 ± 0.54	0.071 ± 0.006	<1min

- means phase separation. The significance of bold typed is to emphasize the selected ingredient.

Data are expressed as mean ± S.D. (n=3).

Supplementary material Table S7 Effect of co-surfactants on the characteristics of nanoemulsion obtained after dilution of SNEDDS prepared using Cremophor® EL/IPM.

Co-surfactants	%Transmittance	Droplet Size (nm)	PDI	Emulsification time (sec)
Ethanol	73.72 ± 2.49	56.32 ± 0.52	0.168 ± 0.008	<1min
Ethylene Glycol	92.88 ± 0.40	42.69 ± 1.18	0.023 ± 0.001	<1min
Propanol	72.19 ± 0.58	61.43 ± 0.60	0.050 ± 0.004	<1min
Propylene Glycol	81.61 ± 4.33	50.91 ± 0.52	0.133 ± 0.011	<1min
Isopropanol	56.79 ± 3.47	85.79 ± 0.59	0.228 ± 0.018	<1min
PEG-400	87.62 ± 1.53	48.55 ± 0.62	0.120 ± 0.005	<1min
Glycerol	68.61 ± 1.78	75.97 ± 0.52	0.170 ± 0.006	<1min
n-Butanol	77.59 ± 3.60	72.49 ± 0.52	0.139 ± 0.008	<1min

- means phase separation. The significance of bold typed is to emphasize the selected ingredient.

Data are expressed as mean ± S.D. (n=3).

Supplementary material Table S8 Effect of co-surfactants on the characteristics of nanoemulsion obtained after dilution of SNEDDS prepared using Cremophor® RH 40/IPM.

Co-surfactants	%Transmittance	Droplet Size (nm)	PDI	Emulsification time (sec)
Ethanol	41.18 ± 5.73	79.68 ± 0.52	0.244 ± 0.008	<1min
Ethylene Glycol	89.82 ± 0.32	43.55 ± 0.40	0.010 ± 0.008	<1min
Propanol	68.03 ± 3.59	68.13 ± 1.00	0.211 ± 0.009	<1min
Propylene Glycol	-	-	-	-
Isopropanol	79.23 ± 0.88	60.06 ± 1.34	0.020 ± 0.009	<1min
PEG-400	89.55 ± 1.67	48.13 ± 0.30	0.073 ± 0.071	<1min
Glycerol	75.93 ± 1.06	87.59 ± 1.64	0.187 ± 0.008	<1min
n-Butanol	87.72 ± 0.68	60.86 ± 0.49	0.169 ± 0.006	<1min

- means phase separation. The significance of bold typed is to emphasize the selected ingredient.

Data are expressed as mean ± S.D. (n=3).

Supplementary material Table S9 Effect of co-surfactants on the characteristics of nanoemulsion obtained after dilution of SNEDDS prepared using Tween 80/ Triacetin.

Co-surfactants	%Transmittance	Droplet Size (nm)	PDI	Emulsification time (sec)
Ethanol	100.39 ± 0.43	17.88 ± 10.63	0.263 ± 0.060	<1min
Ethylene Glycol	100.25 ± 0.03	54.23 ± 2.52	0.322 ± 0.145	<1min
Propanol	99.61 ± 0.11	42.41 ± 1.10	0.224 ± 0.009	<1min
Propylene Glycol	100.43 ± 0.16	8.395 ± 0.03	0.579 ± 0.045	<1min
Isopropanol	100.41 ± 0.10	10.95 ± 3.18	0.661 ± 0.141	<1min
PEG-400	100.30 ± 0.15	7.44 ± 1.71	0.357 ± 0.002	<1min
Glycerol	100.01 ± 0.84	45.79 ± 1.18	0.116 ± 0.025	<1min
n-Butanol	100.43 ± 0.06	8.60 ± 0.99	0.625 ± 0.163	<1min

Data are expressed as mean ± S.D. (n=3).

Supplementary material Table S10 Effect of co-surfactants on the characteristics of nanoemulsion obtained after dilution of SNEDDS prepared using Tween 20/ Triacetin.

Co-surfactants	%Transmittance	Droplet Size (nm)	PDI	Emulsification time (sec)
Ethanol	100.38 ± 0.42	5.47 ± 1.21	0.966 ± 0.049	<1min
Ethylene Glycol	100.20 ± 0.11	8.88 ± 0.22	0.670 ± 0.014	<1min
Propanol	99.79 ± 0.32	5.76 ± 1.06	0.435 ± 0.066	<1min
Propylene Glycol	100.44 ± 0.15	42.37 ± 2.03	0.245 ± 0.067	<1min
Isopropanol	100.38 ± 0.10	24.07 ± 0.23	0.264 ± 0.020	<1min
PEG-400	100.29 ± 0.15	44.84 ± 3.43	0.353 ± 0.023	<1min
Glycerol	100.01 ± 0.84	1824 ± 622.25	1	<1min
n-Butanol	100.22 ± 0.68	16.61 ± 3.22	0.244 ± 0.059	<1min

Data are expressed as mean ± S.D. (n=3).

Supplementary material Table S11 Effect of co-surfactants on the characteristics of nanoemulsion obtained after dilution of SNEDDS prepared using Cremophor® EL/ Triacetin.

Co-surfactants	%Transmittance	Droplet Size (nm)	PDI	Emulsification time (sec)
Ethanol	100.35 ± 0.39	7.50 ± 0.44	0.257 ± 0.087	<1min
Ethylene Glycol	100.16 ± 0.10	11.65 ± 1.94	0.438 ± 0.120	<1min
Propanol	99.92 ± 0.37	11.29 ± 2.79	0.407 ± 0.032	<1min
Propylene Glycol	100.40 ± 0.13	13.02 ± 1.48	0.314 ± 0.059	<1min
Isopropanol	100.37 ± 0.12	16.76 ± 0.25	0.177 ± 0.006	<1min
PEG-400	100.29 ± 0.14	12.89 ± 1.11	0.627 ± 0.095	<1min
Glycerol	100.44 ± 0.35	12.30 ± 1.70	0.643 ± 0.151	<1min
n-Butanol	100.35 ± 0.16	10.71 ± 2.30	0.454 ± 0.093	<1min

Data are expressed as mean ± S.D. (n=3).

Supplementary material Table S12 Effect of co-surfactants on the characteristics of nanoemulsion obtained after dilution of SNEDDS prepared using Cremophor® RH40/ Triacetin.

Co-surfactants	%Transmittance	Droplet Size (nm)	PDI	Emulsification time (sec)
Ethanol	100.36 ± 0.37	8.81 ± 0.72	0.275 ± 0.009	<1min
Ethylene Glycol	100.21 ± 0.17	56.67 ± 1.30	0.427 ± 0.014	<1min
Propanol	100.10 ± 0.27	8.50 ± 1.21	0.560 ± 0.051	<1min
Propylene Glycol	100.39 ± 0.13	8.91 ± 0.61	0.306 ± 0.021	<1min
Isopropanol	100.39 ± 0.13	8.34 ± 0.13	0.270 ± 0.010	<1min
PEG-400	100.37 ± 0.14	9.74 ± 0.39	0.297 ± 0.022	<1min
Glycerol	100.40 ± 0.31	8.87 ± 1.05	0.267 ± 0.054	<1min
n-Butanol	100.32 ± 0.13	8.02 ± 1.69	0.221 ± 0.005	<1min

Data are expressed as mean ± S.D. (n=3).

Supplement Figure 1 Pseudo-ternary diagrams of ethyl oleate (as oil phase) with Cremophor EL as surfactant and ethylene glycol as co-surfactant at different S_{mix} ratio as to get the nanoemulsion area (denoted by solid triangles).