Supplementary material

Formulation and *ex vivo* evaluation of ivermectin within different nano-drug delivery vehicles for transdermal drug delivery

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Composition of nano-emulsion formulae

All four nano-emulsions (**NE1–NE4**) contained the same amount of oil phase (evening primrose oil (EPO) at 20%). The total surfactant concentration was 10% for **NE1** and **NE2** and 12% for **NE3** and **NE4**. The Span® 60: Tween® 80 ratios for **NE1–NE4** were 1:6, 1:4, 1:4, and 1:2, respectively.

Table S1. Formulas used for the four various **NEs**

	Phase	Excipients	Quantity (g; %*w*/*w*)
NE1	Oil phase	EPO	10.000 g (20.00%)
		Ivermectin	1.000 g (2.00%)
		Span® 60	1.000 g (2.00%)
	Aqueous phase	Tween® 80	4.000 g (8.00%)
		UP water	34.000 g (68.00%)
NE2	Oil phase	EPO	10.000 g (20.00%)
		Ivermectin	1.000 g (2.00%)
		Span® 60	0.715 g (1.43%)
	Aqueous phase	Tween® 80	4.285 g (8.57%)
		UP water	34.000 g (68.00%)
NE3	Oil phase	EPO	10.000 g (20.00%)
		Ivermectin	1.000 g (2.00%)
		Span® 60	1.200 g (2.40%)
	Aqueous phase	Tween® 80	4.800 g (9.60%)
		UP water	33.000 g (66.00%)
NE4	Oil phase	EPO	10.000 g (20.00%)
		Ivermectin	1.000 g (2.00%)
		Span® 60	2.000 g (4.00%)
	Aqueous phase	Tween® 80	4.000 g (8.00%)
		UP water	33.000 g (66.00%)

Preformulation characterization results of the nano-emulsions

Table S2. Summary of the characterization results for all four pre-formulated o/w **NEs**

	pH	Droplet size (nm)	PDI	Zeta-potential (mV)	Viscosity (cP)
NE1	5.508±0.008	167.233±0.998	0.141±0.026	-27.2±1.4	56.6±1.4
NE2	5.385±0.004	104.567±2.167	0.201±0.018	-26.6±0.8	107.3±1.3
NE3	5.548±0.004	57.157±0.455	0.165±0.014	-30.6±1.3	25.9±0.6
NE4	5.490±0.004	69.633±0.957	0.207±0.007	-29.0±0.2	56.5±1.3

From Table S2, it can be observed that **NE3** and **NE4** exhibited smaller droplet sizes than **NE1** and **NE2**. This difference is likely due to **NE3** and **NE4** containing a total surfactant concentration of 12%, while **NE1** and **NE2** contained a lower total surfactant concentration of 10%. Chang et al. [116] found that higher surfactant concentrations in a nano-emulsion are associated with smaller droplets.

Nano-emulsions are known to have limited drug-loading capacity. Therefore, achieving a 2% (w/w; 1 000 mg of ivermectin in 50 ml) concentration fully entrapped within the oil phase of these nano-emulsions would likely be challenging without significantly increasing the oil phase, which is already at 20%. Research conducted by An et al. [117] indicated that larger droplet sizes are formed when increasing the oil phase, potentially resulting in instability, including flocculation, creaming and sedimentation [118]. Furthermore, larger droplets could reduce the effectiveness of transdermal delivery, since nanosized droplets are vital for enhancing skin permeation [119]. Therefore, incorporating a combination of lipophilic surfactants, like Span® 60, and hydrophilic surfactants, such as Tween® 80, can improve entrapment efficacy. Lipophilic surfactants enhance entrapment in the oil phase, while hydrophilic surfactants improve solubility of the active pharmaceutical ingredient (API) in the aqueous phase [120], which alleviates instability, since no aggregation or sedimentation was observed, while maintaining a homogenous (low PDI) formulation across all four nano-emulsions.

Despite the relatively low entrapment efficiency observed in Table S3, the high concentration of surfactants (10–12% w/w) stabilized the nano-sized droplets and improved API solubility in the water phase. This enabled the inclusion of a high percentage free ivermectin in the nano-emulsions (**NE1**–**NE4**) without compromising stability or homogeneity, allowing nearly the entire 2% (w/w) of ivermectin to be incorporated in the nano-emulsion,

which is likely due to the high (and favorable) Tween® 80 to Span® 60 ratio.

Table S3. Entrapment efficiency (%) and unentrapped (free) percentage of ivermectin within the respective pre-formulated o/w **NEs**

	Entrapment efficiency (%)	**Unentrapped percentage (%)**
NE1	17.87	81.39
NE2	17.88	81.22
NE3	18.36	80.98
NE4	18.11	81.12

Composition of nano-emulgel formulae

Table S4. Formulas used for the four various **NEGs**

	Phase	Excipients	Quantity (g; %*w*/*w*)
NEG1	Oil phase	EPO	10.00 g (20.0%)
		Ivermectin	1.00 g (2.0%)
		Span® 60	1.20 g (2.4%)
	Aqueous phase	Carbopol® Ultrez 20	0.35 g (0.7%)
		Tween® 80	4.80 g (9.6%)
		UP water	32.65 g (65.3%)
NEG2	Oil phase	EPO	10.00 g (20.0%)
		Ivermectin	1.00 g (2.0%)
		Span® 60	1.20 g (2.4%)
	Aqueous phase	Carbopol® Ultrez 20	0.40 g (0.8%)
		Tween® 80	4.80 g (9.6%)
		UP water	32.60 g (65.2%)
NEG3	Oil phase	EPO	10.00 g (20.0%)
		Ivermectin	1.00 g (2.0%)
		Span® 60	1.20 g (2.4%)
	Aqueous phase	Carbopol® Ultrez 20	0.45 g (0.9%)
		Tween® 80	4.80 g (9.6%)
		UP water	32.55 g (65.1%)
NEG4	Oil phase	EPO	10.00 g (20.0%)
		Ivermectin	1.00 g (2.0%)
		Span® 60	1.20 g (2.4%)
	Aqueous phase	Carbopol® Ultrez 20	0.30 g (0.6%)
		Tween® 80	4.80 g (9.6%)
		UP water	32.70 g (65.4%)

Preformulation characterization results of the nano-emulgels

Table S5. Summary of the characterization results for all four pre-formulated o/w **NEGs**

	pH	Droplet size (nm)	PDI	Zeta-potential (mV)	Viscosity (cP)
NEG1	6.201±0.006	106.900±0.490	0.287±0.037	-40.4±1.3	16,858±237.8
NEG2	6.221±0.009	116.100±1.883	0.354±0.026	-47.3±1.1	19,098±57.4
NEG3	6.256±0.006	137.433±1.901	0.285±0.019	-48.4±0.9	24,740±130.2
NEG4	6.259±0.014	129.233±1.488	0.272±0.018	-32.7±0.2	16,548±51.1