Lipid nanoparticles loaded with butamben and designed to improve anesthesia at inflamed tissues

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Supplementary Information

Table S1. Experimental composition of the NLC formulations according to the 2^3 factorial design and the corresponding responses using the lipids cetyl palmitate and Dhaykol 6040 (70:30), and Pluronic F-68 (P68) as excipients.

Symbol	**A**	**B**	**C**			
Sample	**Total lipid (% w/w)**	**P68 (% w/w)**	**BTB (% w/w)**	**Size (nm)**	**PDI**	**Zeta potential |mV|**
2	10	4	3	238.1	0.203	-27.8
8	15	4	3	252.3	0.187	-27.4
9	10	6	3	251.6	0.176	-24.4
7	15	6	3	233.9	0.207	-26.9
10	10	4	5	272.4	0.178	-28.5
1	15	4	5	271.7	0.175	-27.8
5	10	6	5	211.4	0.174	-24.4
3	15	6	5	227.8	0.169	-26.8
4	12.5	5	4	244.4	0.175	-25.3
6	12.5	5	4	239.3	0.178	-25.9
11	12.5	5	4	228.9	0.164	-25.8

Table S2. Results of DSC analysis.

Sample	**Melting point (^oC)**	**Enthalpy (J/g)**
CP	57.7	222.3
BTB	61.1	133.5
NLC_{CRTL}	55.4	84.58
NLC_{BTB}	55.2	108.6

CP, cetyl palmitate; BTB, butamben; Nanostructured lipid carriers without (NLC_{CRTL}) and with butamben (NLC_{BTB}).

Table S3. Analyses of the release kinetics of butamben from the optimized NLC and control samples (free BTB, SUS_{BTB}) using different mathematical models, in terms of linear regression coefficient (r^2).

	r^2					
Model	**Zero order**	**First order**	**Higushi**	**Hickson-Crowell**	**Korsmeyer-Peppas**	**Weibull**
Free BTB	0.2859	0.0704	-1.7021	0.1710	0.9692	0.9961
SUS_{BTB}	0.4973	0.1024	-0.7978	0.3522	0.9893	0.9963
NLC_{BTB}	0.8600	0.5700	0.8141	0.6699	0.9731	0.9984

Table S4 – Histopathological results. Score of damage and frequency, after 28 h of administration (n=5) of the formulations: SUS_{CTRL}, SUS_{BTB}, NLC_{CTRL}, NLC_{BTB}.

		Damage Type				
Sciatic nerve						
		Frequency				
Formulation	**Score of damage**	MII	NII	Edema	Hemorrhage	Necrosis
SUS_{CTRL}	**4**	-	-	-	-	-
	3	-	-	-	-	-
	2	-	-	-	-	-
	1	-	-	-	-	-
	0	5	5	5	5	5
SUS_{BTB}	**4**	-	-	-	-	-
	3	-	-	-	-	-
	2	-	-	-	-	-
	1	1	-	-	-	-
	0	4	5	5	5	5
NLC_{CTRL}	**4**	-	-	-	-	-
	3	5	-	-	-	-
	2	-	-	5	-	-
	1	-	5	-	-	-
	0	-	-	-	5	5
NLC_{BTB}	**4**	-	-	-	-	-
	3	5	-	-	-	-
	2	-	-	5	-	-
	1	-	5	-	-	-
	0	-	-	-	5	5
striated skeletal muscle						
		Frequency				
Formulation	**Score of damage**	MII	NII	Edema	Hemorrhage	Necrosis
SUS_{CTRL}	**4**	-	-	-	-	-
	3	-	-	-	-	-
	2	-	-	-	-	-
	1	-	-	-	-	-
	0	5	5	5	5	5
SUS_{BTB}	**4**	-	-	-	-	-
	3	-	-	-	-	-
	2	-	-	-	-	-
	1	1	-	-	-	-
	0	4	5	5	5	5
NLC_{CTRL}	**4**	-	-	-	-	-

	3	2	-	-	-	-
	2	-	-	-	-	-
	1	-	-	-	-	-
	0	3	5	5	5	5
	4	-	-	-	-	-
	3	2	-	-	-	-
NLC_{BTB}	**2**	-	-	1	-	-
	1	-	-	-	-	-
	0	3	5	4	5	5

MII: mononuclear inflammatory infiltrate. NII: neutrophilic inflammatory infiltrate. Class of damage: 0 = normal; 1 = minimal; 2 = slight; 3 = moderate; 4 = substantial.

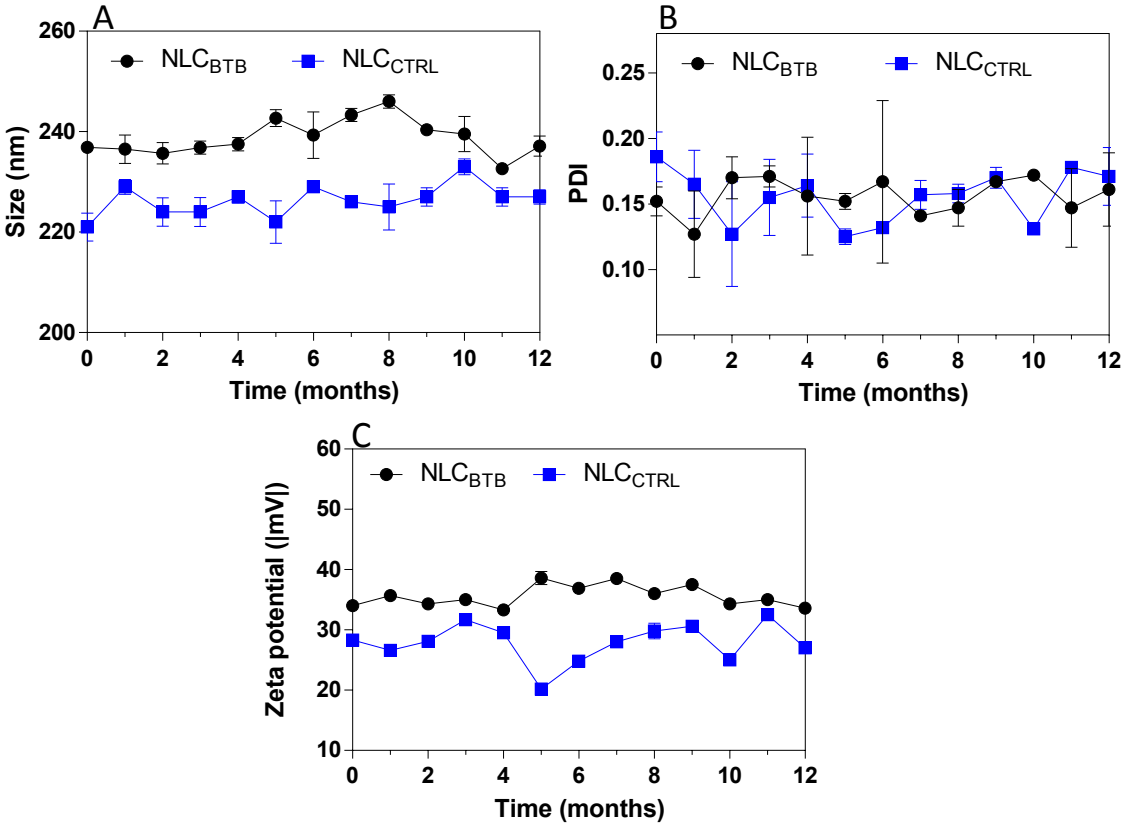

Figure S1. Physicochemical stability test. Variation of **A**) Size; **B**) PDI; and **C)** Zeta potential over 12 months of storage, at room temperature. NLC_{BTB} = Nanostructured lipid carriers with 3% butamben, NLC_{CTRL} = control NLC, without butamben. No statistical differences in the size, PDI and ZP values were found, in comparison to the values of freshly prepared samples (Two-way ANOVA plus Tukey–Kramer post hoc test).

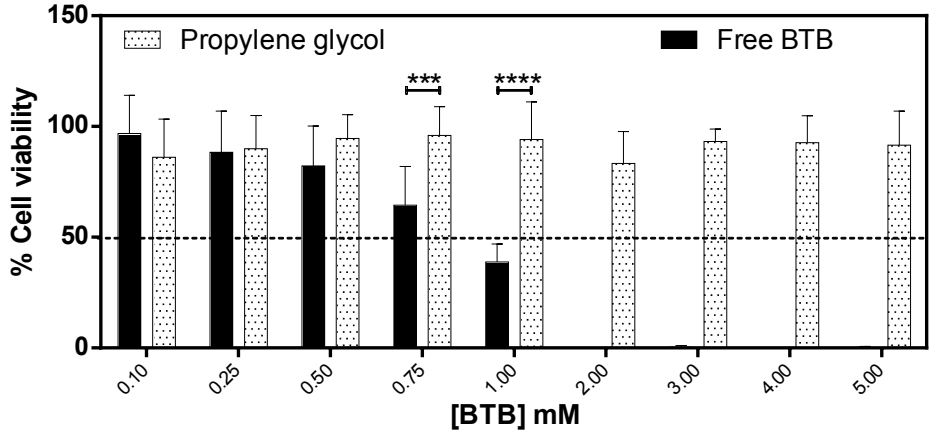

Figure S2. Cell viability of neuronal Schwann cells (ATCC® CRL-2765) after 2 h of treatment with propylene glycol and butamben in propylene glycol (free BTB). Statistical analysis: two-way ANOVA plus Tukey–Kramer post hoc test. ***p < 0.001; **** p <0.0001.

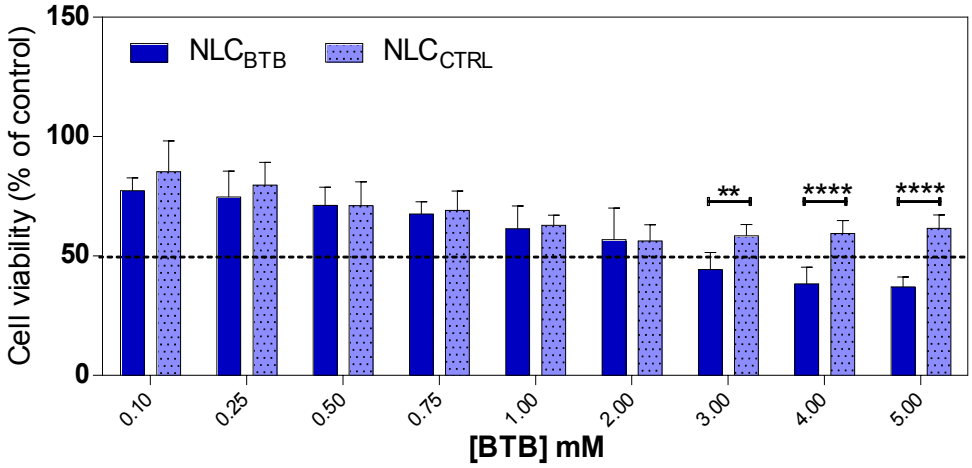

Figure S3. Cell viability of neuronal Schwann cells (ATCC® CRL-2765) after 2 h treatment with nanostructured lipid carriers without (NLC_{CRTL}) or with butamben (NLC_{BTB}). Statistical analysis: two-way ANOVA plus Tukey–Kramer post hoc test. **p < 0.005; **** p <0.0001.