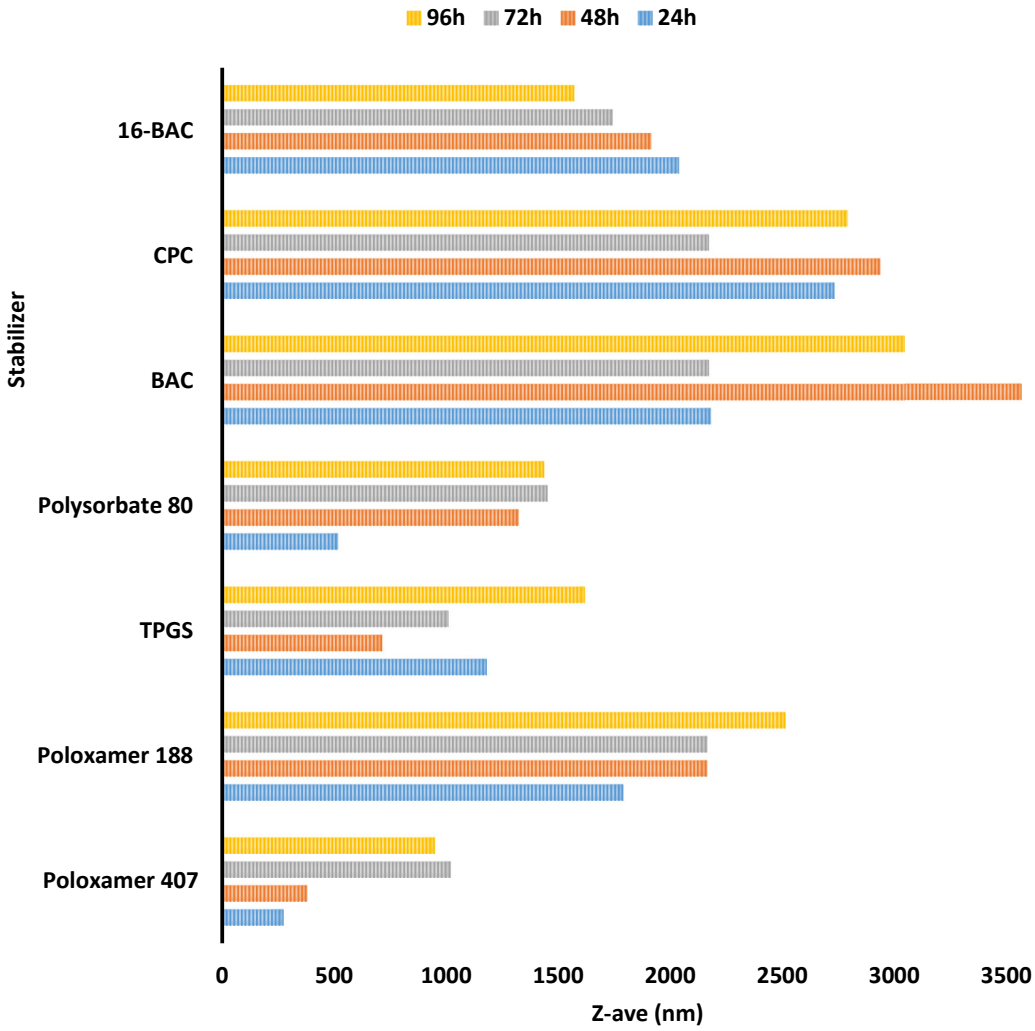

Figure S1. Determination of Z-ave (nm) for the first exploratory study. Those stabilizers conventionally used to obtain nanocrystals were tested. The determination of Z-ave was carried out at 24 hours intervals during the milling process (processing time: 96 hours). Conditions: 1200 RPM, 30% (wt%) zirconium beads. Concentration: stabilizer and besifloxacin 0.6 wt% (1:1). BAC: benzalkonium chloride; CPC: cetylpyridinium chloride; TPGS: tocopherol polyethylene glycol succinate; 16-BAC benzyldimethylhexadecylammonium chloride.

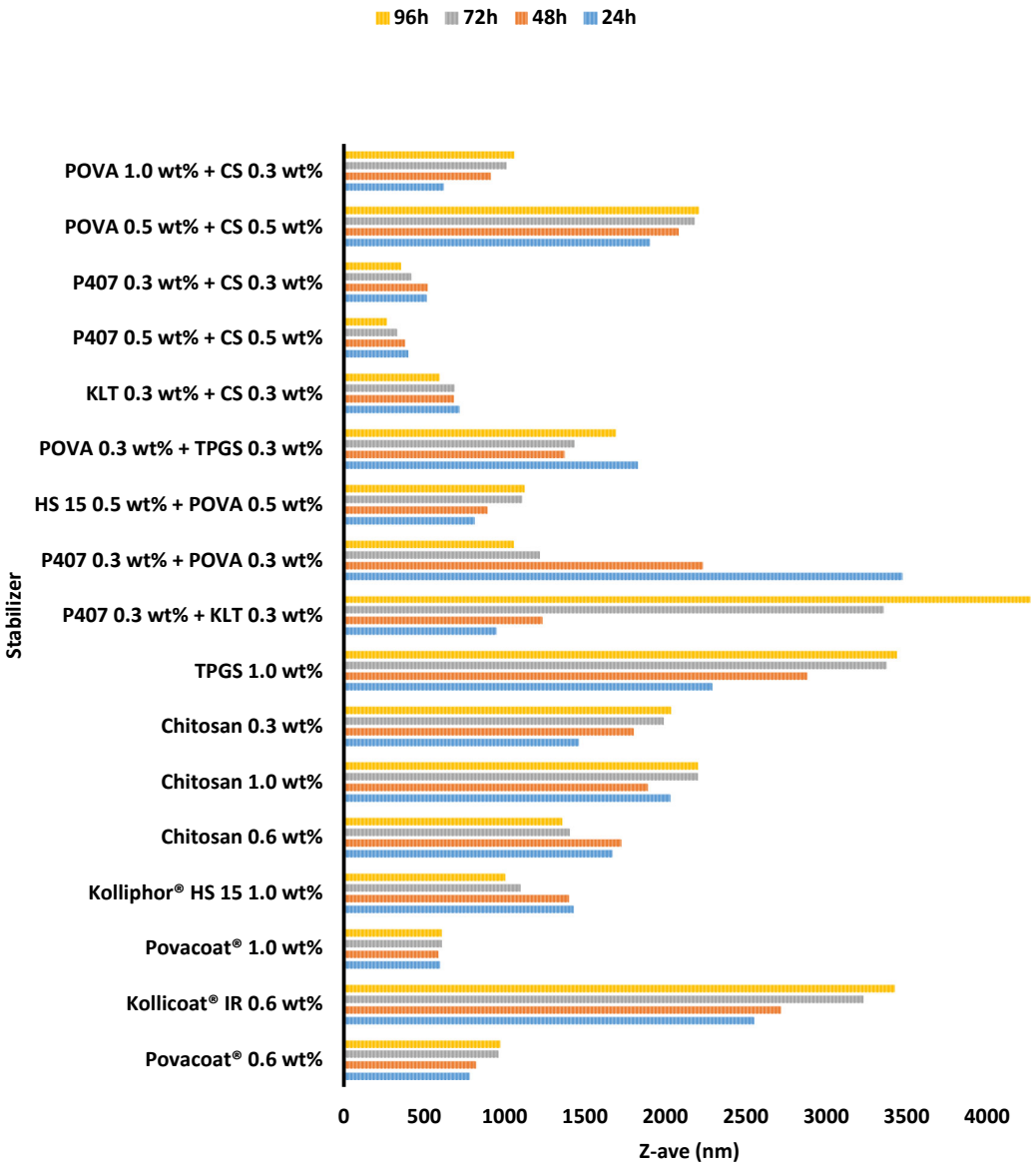

Figure S2. Determination of Z-ave (nm) for the second exploratory study. New and promising stabilizers, different combinations. The determination of Z-ave was carried out at 24 hours intervals during the milling process (processing time: 96 hours). Conditions: 1200 RPM and 30% (wt %) zirconium beads. Besifloxacin 0.6 wt %. TPGS: tocopherol polyethylene glycol succinate; P407: Poloxamer 407; KLT: Kollicoat® IR; POVA: Povacoat®; HS 15: Poloxamer HS 15; CS: chitosan.

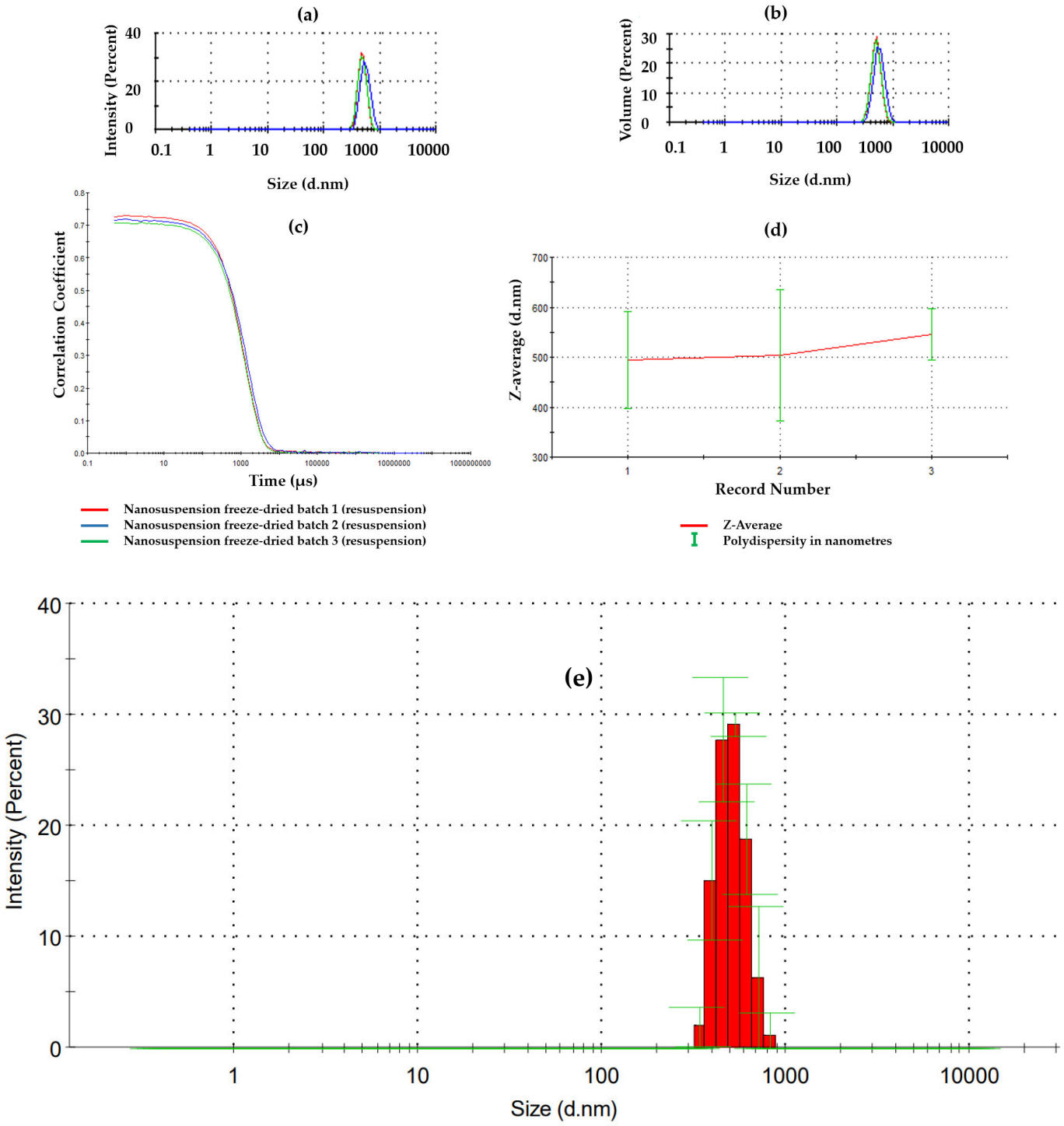

Figure S3: Freeze-dried besifloxacin nanosuspension DLS measurements: (a) Size distribution by intensity; (b) Size distribution by volume; (c) Correlogram; (d) Z-average trend; (e) Statistics graph *n* =3.

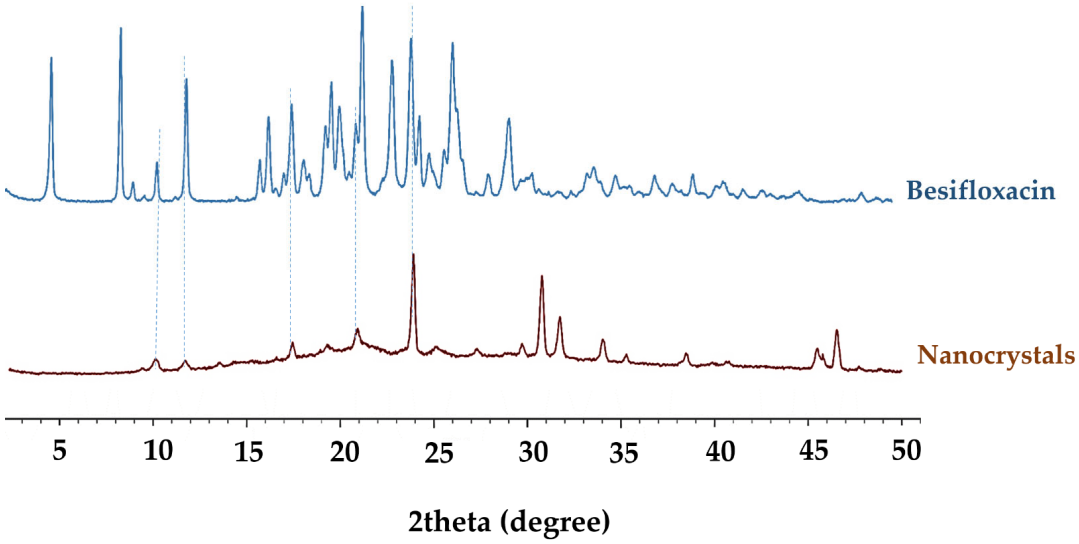

Figure S4: X-ray diffraction of besifloxacin (raw material) and nanocrystals (freeze-dried). *n* = 3.

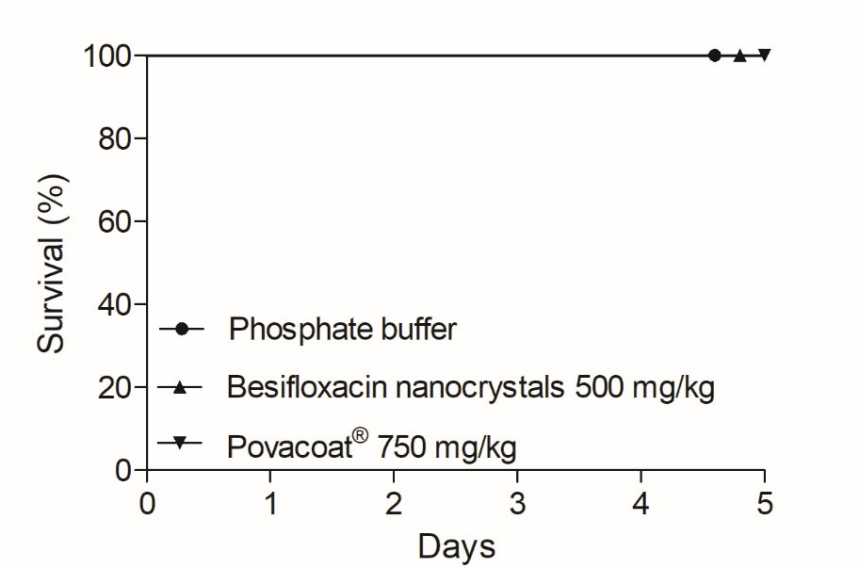

Figure S5. Toxicity of besifloxacin nanocrystals (500 mg/kg) and Povacoat® (750 mg/kg) in a larval model of *Galleria mellonella*.

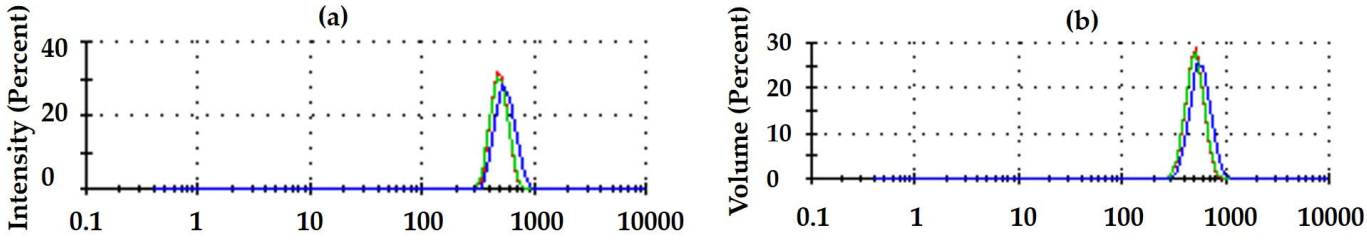

Figure S6: Particle distribution (monomodal), accelerated stability: 90 days. Study condition: 40°C ± 2°C / 75% RH ± 5% RH. (a) Size distribution by intensity; (b) Size distribution by volume. *n* = 3.

Table S1. Stability of the average hydrodynamic diameter (Z-ave) in nm, in the time intervals: immediately after milling (T0), 7 days, 15 days and 30 days. Process time 4 days. Storage condition: 25°C.

	Storage time (25°C)			
Stabilizer	T0	7 days	15 days	30 days
Povacoat® 1.0 wt%	607.2±11.56	662.2±10.7	660.9±15.5	679.4±1.8
Poloxamer P 407 0.6 wt%	337.7±14.6	418.5±9.9	415.5±7.9	450.7±10.3
KLT 0.3 wt% + CS 0.3 wt%	594.7±22.57	1205.0±173.9	-	-
Polysorbate 80 0.6 wt%	518.9±9.3	1100.0±33.5	-	-
TPGS	796.5±56.7	1570.0±191.3	-	-
P407 0.5 wt% + CS 0.5 wt%	267.6±1.5	398.0±5.6	428.8±10.8	-
P407 0.3 wt% + CS 0.3 wt%	355.3±7.1	570.1±18.67	479.2±19.35	-

(-): unmeasured; TPGS: tocopherol polyethylene glycol succinate; P407: Poloxamer 407; KLT: Kollicoat® IR; CS: chitosan.

Table S2: Nano-based besifloxacin delivery: type, composition, properties, antimicrobial activity and, physical-chemical stability condition.

Type	Components	Besifloxacin concentration	Size	PdI	Zeta potential (mV)	Antimicrobial Activity	Physical-chemical stability	Ref.
LP	LP PC LP PC: SPM	335.81 - 467.84 μg/mL	175.4 - 177.2 nm	0.019 ± 0.011 0.075 ± 0.021	+19.5 and - 5.7	*S. epidermidis:* MIC: 0.156 μg/mL *P. aeruginosa:* MIC: 0625 μg/mL	6 °C for 30 days	[17]
NE	Triacetin (oil), Cremophor® RH 40, and Transcutol®P	0.2 wt%	14.0 nm	0.145 - 0.217	-	comparative efficacy of lower drug-loaded NE (0.2%) versus 0.6% besifloxacin suspension against *P. aeruginosa* and *S. aureus*	heating–cooling (2–8 °C) and 40 °C for 48 h + 25 °C, at least 48 h, 13,000 rpm for 30 min	[18]
NLC	Sulfobutyl ether beta-cyclodextrin and chitosan	0.005 % (m/m)	13.6 – 16.1 nm	0.107 - 0.181	+33.8 ± 1.9	*P. aeruginosa:* MIC: 0.031 μg/mL	4 °C for 30 days	[19]
NF	HP-β-CD; PCL/PEG; sodium alginate or thiolated sodium alginate	40 μg / cm^2 fiber insert	thickness: 0.66 - 0.83 mm; diameter: 6.6-6.8 mm	-	-	bacterial keratitis in rabbit eyes was reduced upon single-dose application compared to multiple dosing with the commercial drug. Against *P. aeruginosa*	-	[20]

LP: Liposome; LP PC: Phosphatidylcholine; LP PC: SPM: Phosphatidylcholine and spermine; HP-β-CD: Hydroxypropyl-beta-cyclodextrin; PCL/PEG: Poly(caprolactone)/polyethyleneglycol MIC: Minimal inhibitory concentration; NE: Nanoemulsion; NF: Nanofibrous; NLC: Nanostructured lipid carrier; PdI: polydispersivity index, (-) not disclosed.