SUPPLEMENTARY INFORMATION

Dual Encapsulation of Hydrophobic and Hydrophilic Drugs in PLGA Nanoparticles by a Single-Step Method: Drug delivery and cytotoxicity assays

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1-Validation of HPLC method

Linear correlation was obtained between peak area and the concentration range, from 0.88 to 400.0 µg mL^{-1} and from 0.78 to 400.0 µg mL^{-1}, for DX and DS, respectively. The determination coefficient was better than 0.999. The detection and quantification limits were 2.77 µg mL^{-1} and 8.39 µg mL^{-1}; and 7.19 µg mL^{-1} and 21.79 µg mL^{-1} for DS and DX, respectively. The retention time are showed in the Figure S1.

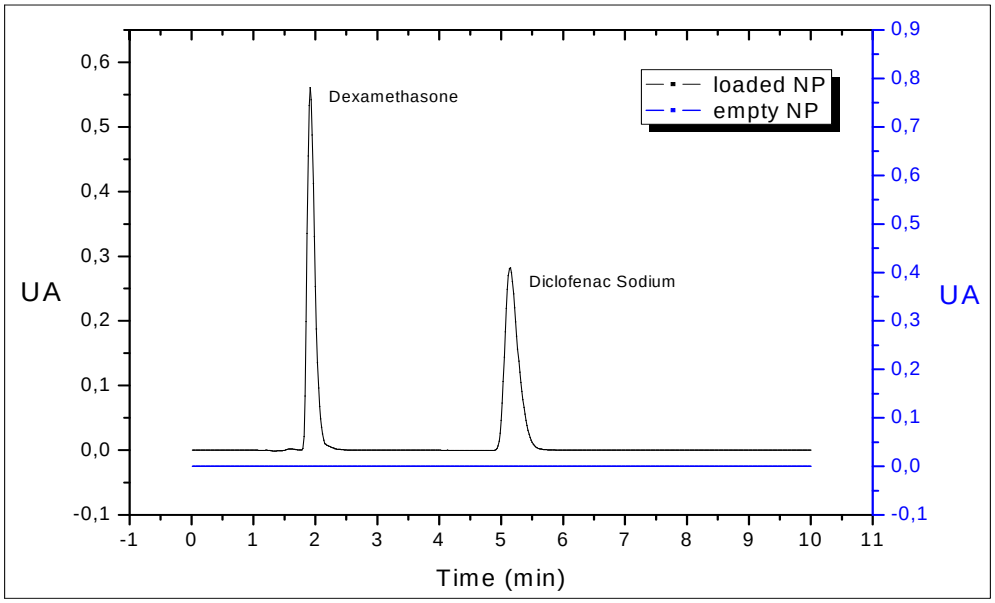

Figure S1. Representative chromatograms of supernatant from loaded NPs and supernatant from the non-drug loaded NPs

2- Validation of Micellar electrokinetic chromatography method.

Before using capillaries were conditioned with 1 M NaOH solution for 30 min, followed by deionized water during 20 min and a finally with the electrolyte buffer for 30 min. At the beginning of every working day the capillary was flushed with 1 M NaOH for 15 min, followed by deionized water for 10 min and then by electrolyte buffer for 15 min. Between each run, the capillary was rinsed with 1 M NaOH for 2 min, deionized water for 1 min and buffer solution for 2 min. Furosemide was used as internal standard. All assays were done in triplicate. To calculate the drug content in the nanoparticles, linear standard curves were constructed. The MEKC method was validated with respect to linearity, precision repeatability and the limits of quantification and detection.

In this research, the MEKC method was developed and validated for the quantitative determination of diclofenac sodium and dexamethasone in the resulting PLGA nanoparticles using furosemide as internal standard (Figure S2). The MEKC method was validated according to the International Conference on Harmonization (ICH) guideline. Linear analytical curves were obtained in the concentration ranges of 75.0 - 225.0 µg mL^{-1} and 100.0 - 300.0 µg mL^{-1}, for diclofenac sodium and dexamethasone, respectively. The determination coefficient (R^2) was better than 0.99. The detection and quantification limits were 0.18 µg mL^{-1} and 0.55 µg mL^{-1}; and 0.19 µg mL^{-1} and 0.56 µg mL^{-1} for DS and DX, respectively. The precision expressed as percentage of relative standard deviation (RSD) was less than 2.0%.

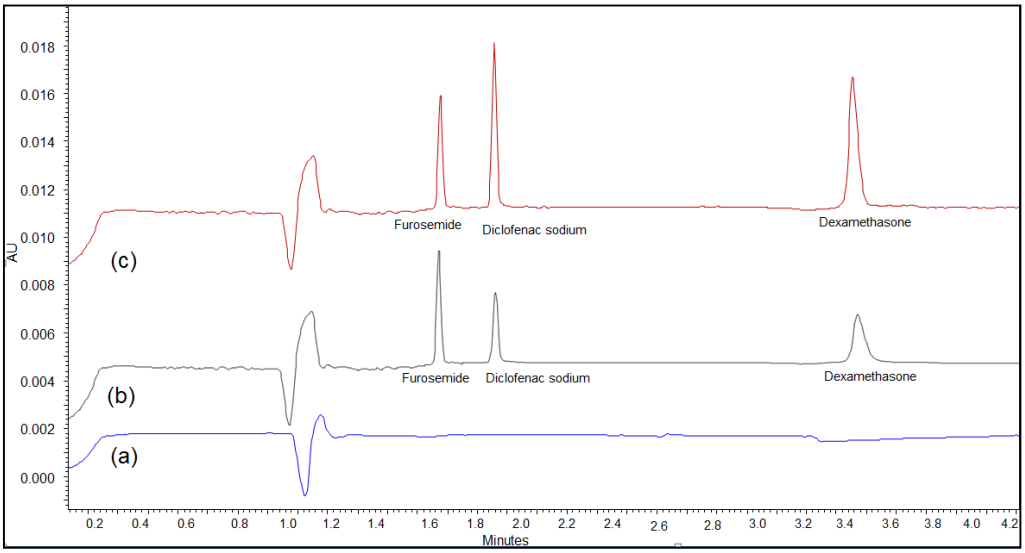

Figure S2. Electropherograms of (a) non-drug loaded nanoparticle, (b) loaded nanoparticle and (c) standard drugs (sodium diclofenac 100.0 µg mL^{-1}, dexamethasone 100.0 µg mL^{-1} and furosemide 100.0 µg mL^{-1}).

3-Optimization of DX-DS/PLGA NPs

Table S1. Analysis of variance for different responses.

	Particle size			Zeta potential			%EE DX			%EE DS		
Source	Sum of square	Mean square	P-value	Sum of square	Mean square	P-value	Sum of square	Mean square	P-value	Sum of square	Mean square	P-value
A:PLGA	227.91	227.91	0.031	12.5	12.5	0.161	690.06	690.06	0.029	380.88	380.88	0.016
B:Time	500.86	500.86	0.021	6.48	6.48	0.221	2.31	2.31	0.429	10.58	10.58	0.096
C:Pluronic	24.15	24.15	0.095	129.61	129.61	0.051	17.88	17.88	0.178	53.05	53.05	0.043
AB	2.31	2.31	0.289	16.25	16.25	0.143	3.43	3.43	0.369	1.45	1.45	0.248
AC	0.66	0.66	0.471	0.18	0.18	0.725	7.03	7.03	0.273	0.0	0.0	1.000
BC	0.55	0.55	0.500	27.38	27.38	0.111	11.95	11.95	0.215	21.78	21.78	0.067
Total error	0.55	0.55		0.85	0.85		1.48	1.48		0.25	0.25	
Total (corr)	756.99			193.24			734.15			467.98		
R^2	99.93			99.5627			99.79			99.95		
R^2 (adjusted for d.f.)	99.49			96.939			98.59			99.63		
Standard Error of Est	0.74			0.92			1.22			0.49		
Mean absolute error	0.26			0.33			0.43			0.18		

Table S2. Regression equations for the responses obtained by analyzing the experimentally determined input parameters

Response	Regression equation
Particle size (Y_1)	Size = 163.537 + 0.184*PLGA – 1.058*Time – 0.036*Pluronic + 0.002*PLGA*Time – 0.0002*PLGA*Pluronic + 0.0005*Time*Pluronic (1)
Zeta potential (Y_2)	ZP = 43.588 – 0.174*PLGA – 1.078*Time – 0.058*Pluronic + 0.006*PLGA*Time + 0.0001*PLGA*Pluronic + 0,004*Time*Pluronic (2)
Encapsulation efficiency DX (Y_3)	EE DX = 40.209 + 0.313*PLGA – 0.239*Time – 0.112*Pluronic - 0.003*PLGA*Time+0.001*PLGA*Pluronic+ 0.002*Time*Pluronic (3)
Encapsulation efficiency DS (Y_4)	EE DS = -8.6875 + 0.2165*PLGA + 0.6475*Time + 0.167*Pluronic + 0.0017*PLGA*Time + 0.0*PLGA*Pluronic – 0.0033*Time*Pluronic (4)

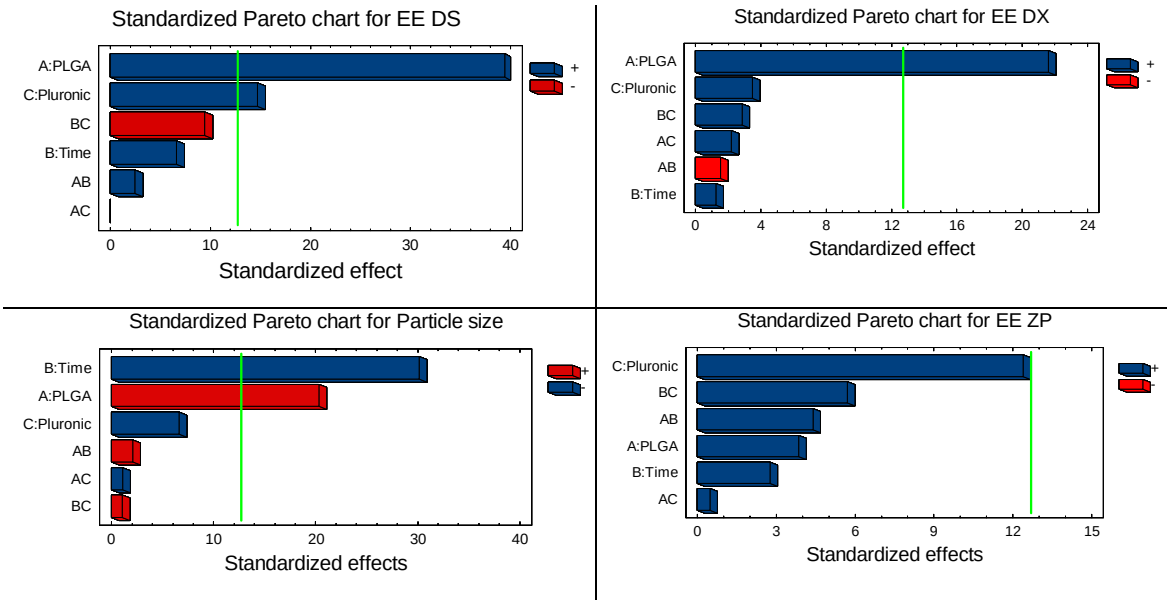

Figure S3. The Pareto chart showing the effects of X1, X2 and X3 on dependent variables.

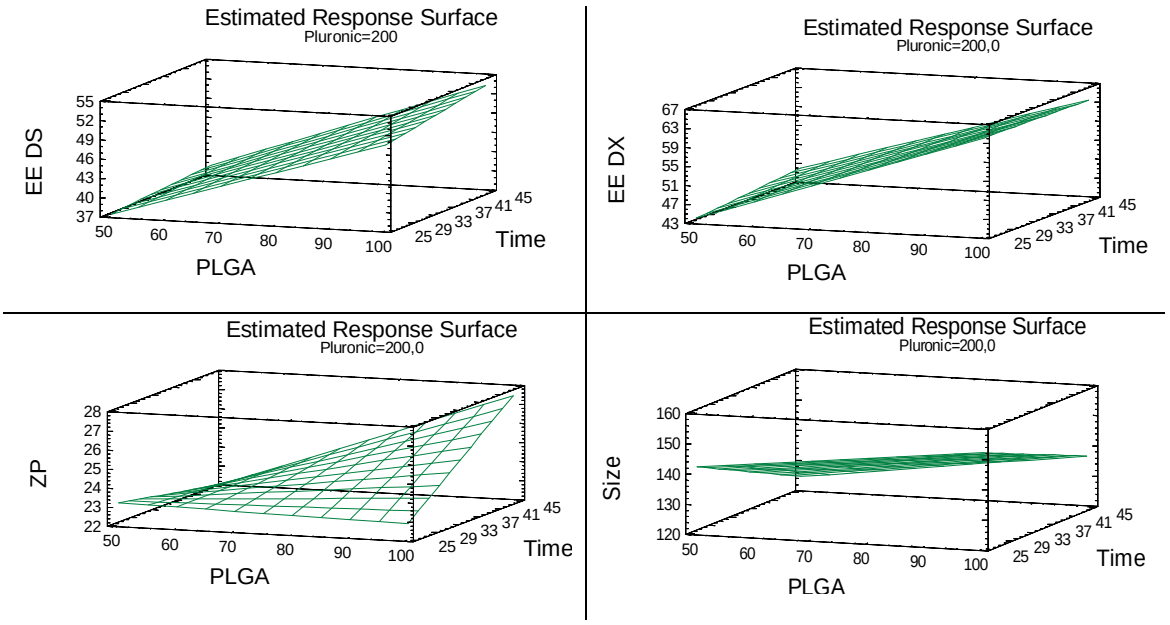

Figure S4. Surface plot showing the effect of X1 and X2 (at X3 = 200) on dependent variables.

4- Encapsulation efficiency

Table S3. Drug encapsulation efficiency for the different formulations of DX-DS-loaded PLGA NPs

	Encapsulation efficiency % by MEKC		Encapsulation efficiency % by HPLC	
Experiment	**Diclofenac Sodium**	**Dexamethasone**	**Diclofenac Sodium**	**Dexamethasone**
F1	30.4 ± 2	47.9 ± 3	33.3 ± 4	44.2 ± 8
F2	46.1 ± 8	59.3 ± 7	45.9 ± 9	63.1 ± 4
F3	35.3 ± 4	43.8 ± 4	37.7 ± 7	45.0 ± 7
F4	50.8 ± 5	61.7 ± 6	52.7 ± 5	59.5 ± 6
F5	46.4 ± 8	48.8 ± 8	41.4 ± 6	43.7 ± 4
F6	50.2 ± 2	60.4 ± 3	54.7 ± 6	64.6 ± 6
F7	43.4 ± 10	61.1 ± 3	39.9 ± 5	47.7 ± 5
F8	51.4 ± 5	66.8 ± 4	54.2 ± 2	67.7± 3

5-Detailed discussion about the effects of the independent variables used in the 2^3 factorial design model

An important factor, which has influence on the nanoparticle´s size, is the combination of the concentration values of PLGA and Pluronic. When the amount of PLGA is constant, the size of nanoparticles changes with the amount of surfactant. If the Pluronic concentration increases, the hydrodynamic diameter average of the nanoparticles decreases (Figure S3). Data showed that for any combination of values of PLGA concentration and sonication time, the size of the nanoparticles consistently decreased as the concentration of Pluronic increased. It has been reported that at high concentrations, more surfactant can be oriented to the organic/aqueous interface leading to an efficient reduction of the interfacial tension, resulting in a significant increase in the shear stress during emulsification. Consequently, there is a formation of smaller emulsion droplets.

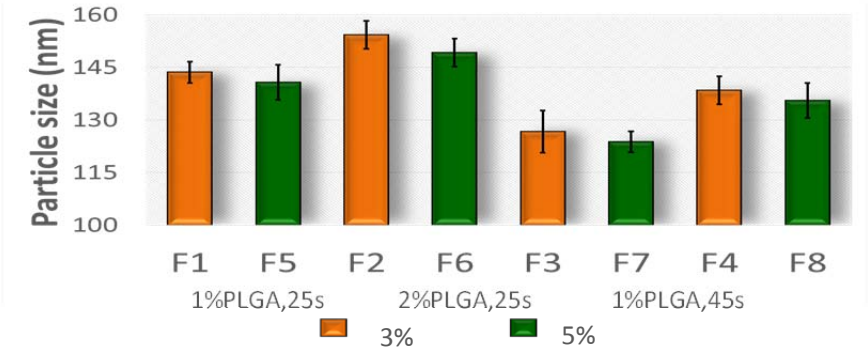

Figure S5. Effect of Pluronic concentration (3% and 5%) on particle size.

During the preparation of nanoparticles, the emulsification process critically influences the nanoparticle size. In this case, two different sonication times, 25 and 45 seconds, were tested under different concentrations of PLGA and Pluronic (Figure S4). As it could be expected, the increase in the sonication time caused a reduction in the hydrodynamic diameter in all the studied formulations.

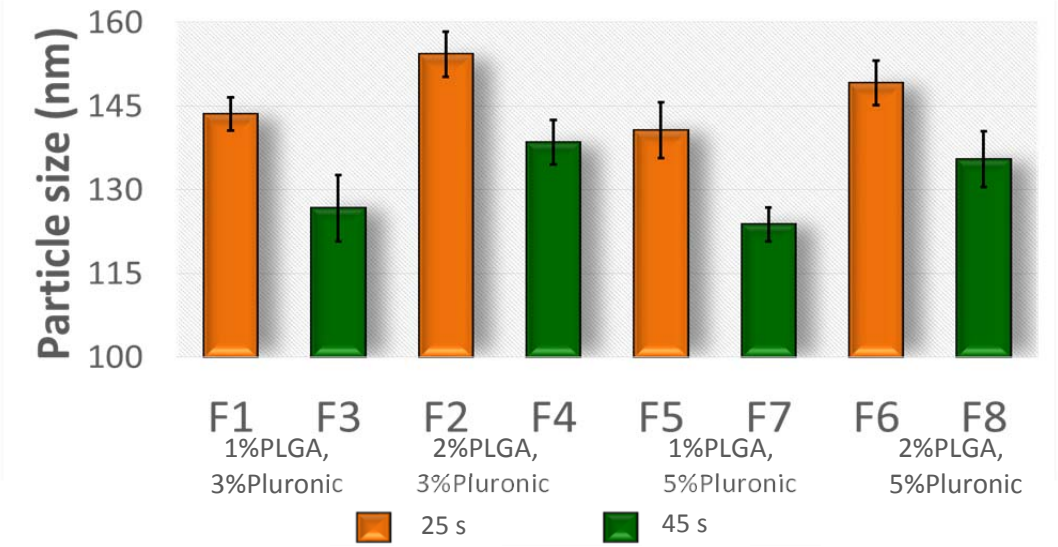

Figure S6. Effect of sonication time (45s and 25s) on particle size

An increase in the amount of PLGA (Figure S5) causes an increase in the percentage of encapsulation efficiency (%EE) of drugs for the NPs formulations. This effect can be explained by the fact that an increase in the amount of PLGA in the organic phase causes variation in the viscosity and surface tension of the organic phase, which consequently, affects the emulsification process

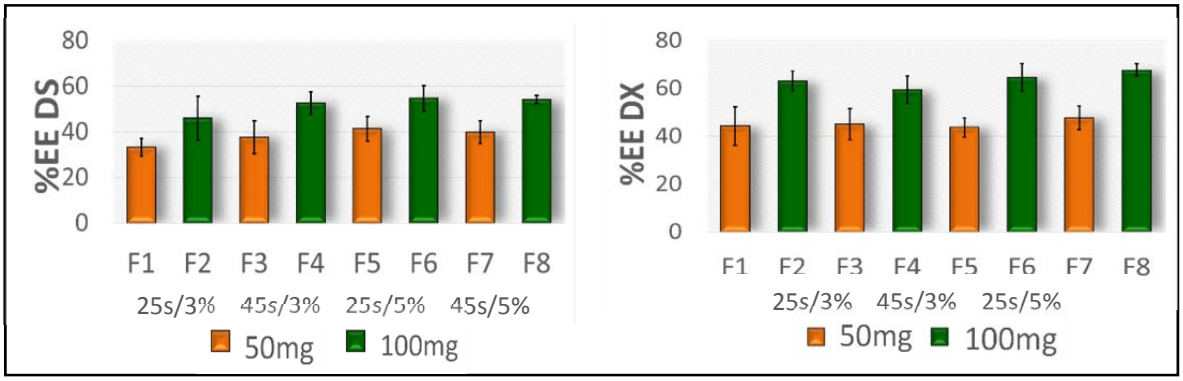

Figure S7. Effect of PLGA amount (mg) on the encapsulation efficiency (%EE) of dexamethasone (DX) and diclofenac sodium (DS). Pluronic concentration %w/v and sonication time were maintained constant.

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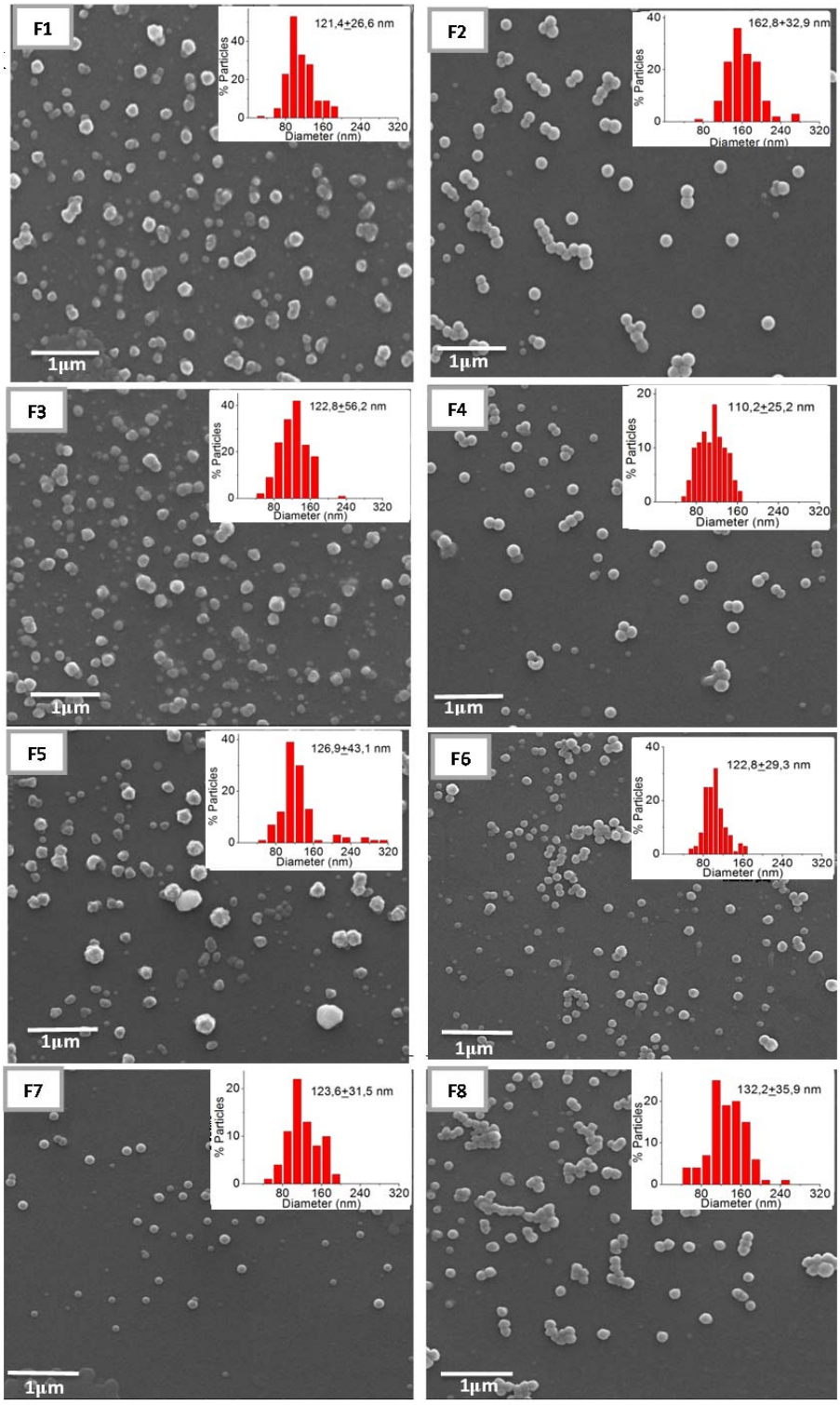

Figure S8. SEM images and particle size distributions for PLGA nanoparticles loaded with DS+DX. (In the Table 2 conditions of elaboration).