Supplementary Information

Optimization of the process parameters for fabrication of polymer coated layered double hydroxide-methotrexate nanohybrid for possible treatment of osteosarcoma.

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Drug entrapment efficiency of the optimized batches of LDH-MTX, PLGA-LDH-MTX and PLGA-MTX nanoparticles

a.) LDH-MTX: 100 mg of sample LDH-MTX was dispersed in 10 ml solution of PBS and sonicated for 15 min to extract the MTX drug in the same. The extract was filtered and diluted with PBS to quantify the drug loading by HPLC (820 Metrohm, Herisau, Switzerland, using column, X-Bridge C18, 4.6×150 mm, Waters Corporation, USA) analysis as following:

Drug loading in LDH-MTX (%) =

$$\frac{\text{Weight of MTX/ mg LDH-MTX} \times \text{Total weight of LDH-MTX}}{\text{Initial weight of MTX}} \times 100 \qquad \text{(S1)}$$

b.) PLGA-LDH-MTX and PLGA-MTX: Both the freeze dried (~100 mg by weight) PLGA-MTX and PLGA-LDH-MTX nanoparticles were added in 10 ml of acetone to dissolve the polymer and the drug was extracted in phosphate buffer saline solution at pH 7.4. The resulting solution was then centrifuged at 2739 g rcf for a period of 5 min and the supernatant was filtered through membrane filter (0.22 µm, Merck-Millipore, USA) and analyzed by HPLC analysis as above to determine the drug loading as,

i.) Drug loading in PLGA-MTX (%) =

$$\frac{\text{Weight of MTX/ mg PLGA-MTX} \times \text{Total weight of PLGA-MTX}}{\text{Initial weight of MTX}} \times 100 \qquad \text{(S2)}$$

ii.) Drug loading in PLGA-LDH-MTX (%) =

$$\frac{\text{Weight of MTX/ mg PLGA-LDH-MTX} \times \text{Total weight of PLGA-LDH-MTX}}{\text{Weight of MTX in LDH-MTX}} \times 100 \qquad \text{(S3)}$$

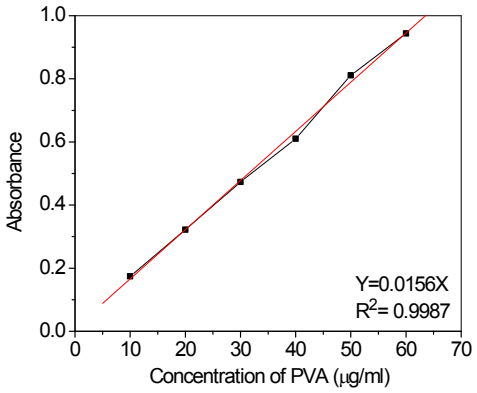

Fig. S1 Calibration curve of PVA.

Table S1 Effect of PLGA concentration in the internal organic phase (DCM/Acetone)

Formulation	Polymer concentration / ml	Mean diameter of particle (nm)	EE (%)
PLGA-MTX-1	20	129±2.27	19.49±2.98
PLGA-MTX-2	30	188±1.41	34.23±4.64
PLGA-MTX-3	40	236±1.09	48.78±2.32
PLGA-MTX-4	50	288±2.43	54.52±2.39
PLGA-LDH-MTX-1	20	212±2.21	39.21±0.98
PLGA-LDH-MTX-2	30	323±1.37	44.45±1.41
PLGA-LDH-MTX-3	40	398±1.04	56.49±7.89
PLGA-LDH-MTX-4	50	413±2.89	58.82±2.90

Table S2 Effect of surfactant concentration (PVA/Tween 80) in the external aqueous phase

Formulation	Surfactant concentration (%)	Mean diameter of particle (nm)	Polydispersity index	EE (%)
PLGA-MTX-7	0.25	321±2.21	0.28±0.19	3.22±6.78
PLGA-MTX-8	0.5	293±1.31	0.241±1.39	7.65±5.92
PLGA-MTX-9	1	276±1.08	0.198±0.89	10.56±3.97
PLGA-MTX-10	1.5	212±2.19	0.164±0.67	32.32±2.89
PLGA-LDH-MTX-7	0.15	397±3.33	0.326±1.76	72.85±4.65
PLGA-LDH-MTX-8	0.25	365±2.09	0.310±1.07	68.39±3.89
PLGA-LDH-MTX-9	1	228±1.21	0.214±1.54	65.54±1.27
PLGA-LDH-MTX-10	2.5	147±1.67	0.148±0.48	62.11±3.42

Percentage yield of PLGA-MTX and PLGA-LDH-MTX

a.) Percentage yield of PLGA-MTX
Weight of PLGA-MTX ×100 /Total weight of PLGA and MTX used (S4)

b.) Percentage yield of PLGA-LDH-MTX
Weight of polymer encapsulated nanoparticles obtained/PLGA-LDH-MTX ×100 /Total weight of LDH-MTX and PLGA used (S5)

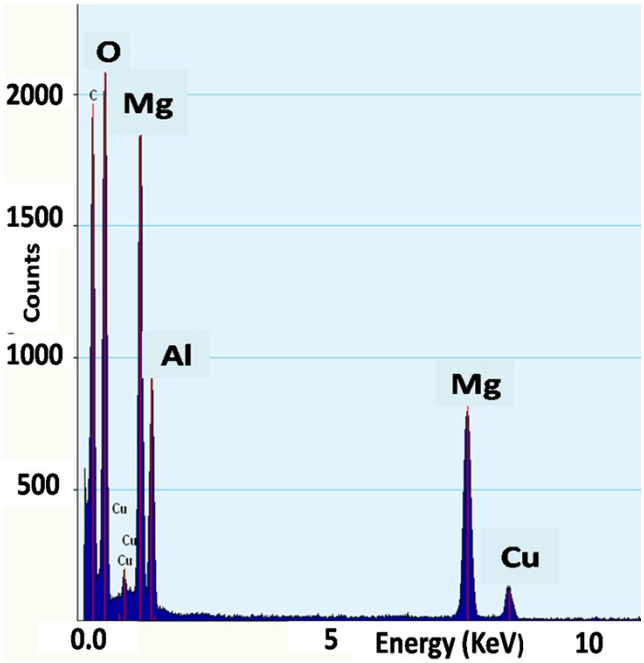

Fig. S2 TEM EDX (qualitative) of PLGA-LDH-MTX, showing the corresponding Mg and Al elements of LDH.

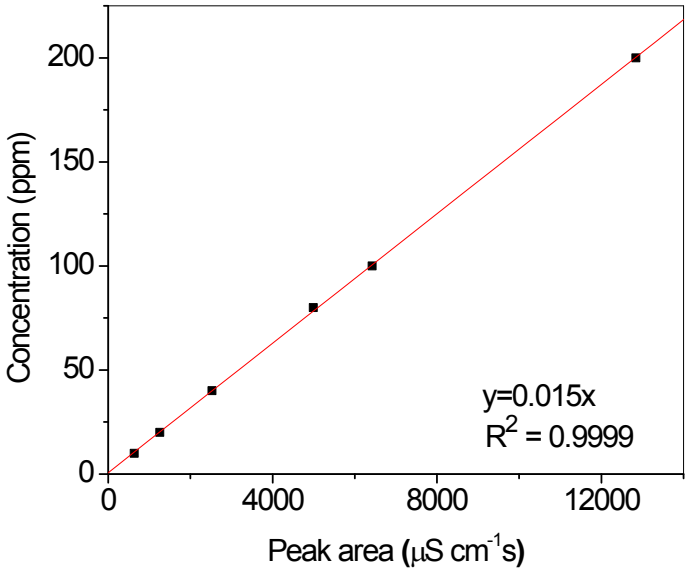

Fig. S3 Calibration curve of MTX in phosphate buffered saline at pH 7.4.

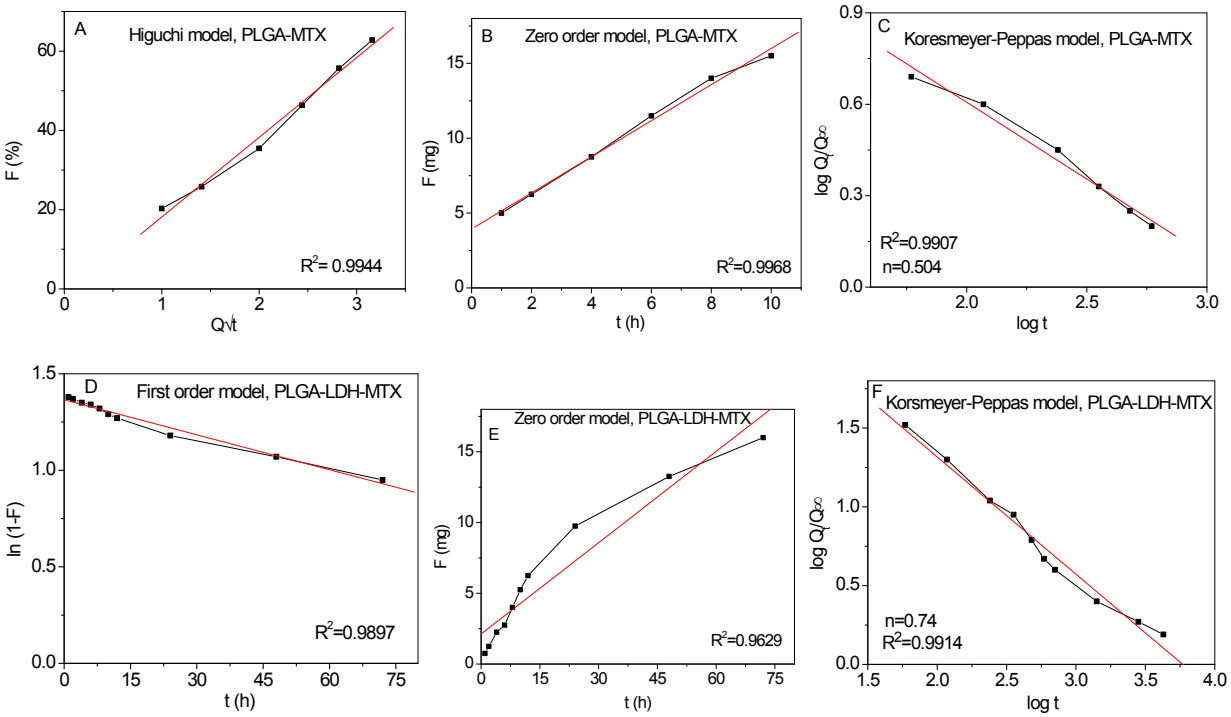

Fig. S4 Additional kinetic models and the fitting curves of release of MTX from optimized batch of PLGA-MTX (A to C) and PLGA-LDH-MTX (D to F).