Supplementary Information

pH dependent chemical stability and release of methotrexate from a novel nanoceramic carrier.

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Determination of MTX loading in samples B, C and D by HPLC

The amount of methotrexate present in samples B, C and D was quantified by HPLC analysis (HPLC 820 Metrohm, Herisau, Switzerland) using column, X-Bridge C18, 4.6×150 mm, Waters Corporation, USA, and the drug loading as above was calculated according to the following equation:

Sample B: 50 mg of sample B was dispersed in 10 ml solution of PBS and sonicated for 15 min to extract the MTX in the same. The extract was filtered and diluted with PBS to quantify the drug loading by HPLC analysis as following:

$$\text{Drug loading (\%)} = \frac{\text{Weight of methotrexate per mg of sample B} \times \text{Total weight of sample B}}{\text{Initial weight of MTX taken for loading in sample A}} \times 100 \quad \text{(S1)}$$

where, sample A is LDH
sample B is the drug loaded LDH-MTX nanohybrid

Sample C: 50 mg of sample C was dispersed in 5 ml of dichloromethane and shaken vigorously for 1h to dissolve the PLGA coating. A 10 ml solution of PBS was added to the above solution and sonicated further for 15 min to extract the MTX in the same. The extract was filtered and diluted with PBS to quantify the drug loading by HPLC analysis as following:

$$\text{Drug loading (\%)} = \frac{\text{Weight of methotrexate per mg of sample C} \times \text{Total weight of sample C}}{\text{Initial weight of MTX taken for loading in sample B}} \times 100 \quad \text{(S2)}$$

where, sample B is LDH-MTX and sample C is PLGA coated LDH-MTX

Sample D: 50 mg of sample D was dispersed in 5 ml of dichloromethane and shaken vigorously for 30 min to dissolve the PLGA coating. A 10 ml solution of PBS was added to the above solution and sonicated further 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 analysis as following:

$$\text{Drug loading (\%)} = \frac{\text{Weight of methotrexate per mg of sample D} \times \text{Total weight of sample D}}{\text{Initial weight of MTX taken for loading in sample D}} \times 100 \quad \text{(S3)}$$

where, sample D is PLGA-MTX.

Determination of percentage yield of samples B, C and D

The respective percentage yield by weight of the samples as above was calculated using the following formula:
sample B = Weight of LDH-MTX nanoparticles obtained (Sample B) ×100 /Total weight of LDH and MTX used.
sample C = Weight of polymer encapsulated nanoparticles obtained (Sample C) ×100 /Total weight of LDH-MTX and PLGA used.
sample D =Weight of PLGA encapsulated MTX nanoparticles obtained (Sample D) ×100 /Total weight of PLGA and MTX used.

Table S1: Comparative results of samples A, B, B′, C and D

Formulation	Yield (% by weight)	Encapsulation efficiency (%)	Particle size D_{50} (nm)	Zeta potential (mV)	PDI
LDH	16.70	NA	60 ± 2.78	(+) 43.2	0.21
LDH-MTX	49.74	32.30	80 ± 4.38	(+) 19.01	0.24
LDH-MTX	43.87	13.10	109 ± 3.54	(+) 11.06	0.26
PLGA-LDH-MTX	67.03	56.60	200 ± 6.89	(-) 23.05	0.35
PLGA-MTX	76.60	14.20	140 ± 0.78	(-) 34.20	0.23

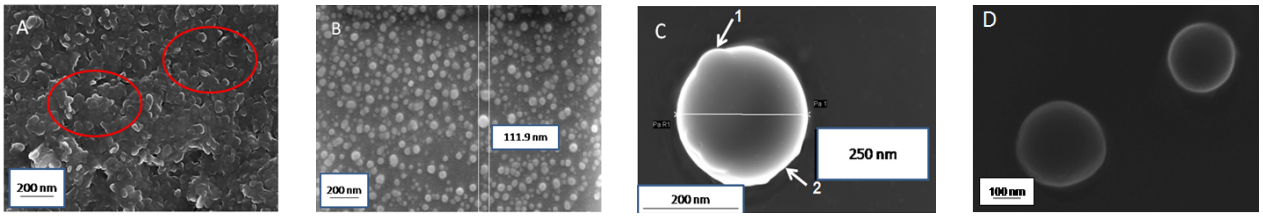


Fig. S1 FE-SEM images of A.) dense aggregates of LDH-MTX nanoparticles (in the circles) B.) aggregation free LDH-MTX nanoparticles after PLGA coating C.) a discrete LDH-MTX-PLGA nanoparticle at higher magnification, showing the small hump (arrow marked, No. 1) and a part of the planar nanocrystal of LDH-MTX, outside PLGA coating (arrow marked, No. 2) D.) discrete and aggregation free nanoparticles (dia~120-250 nm) of PLGA-MTX.

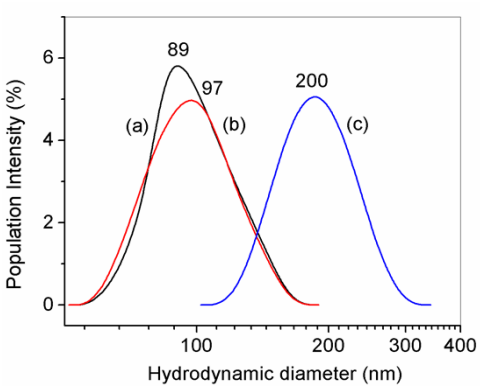
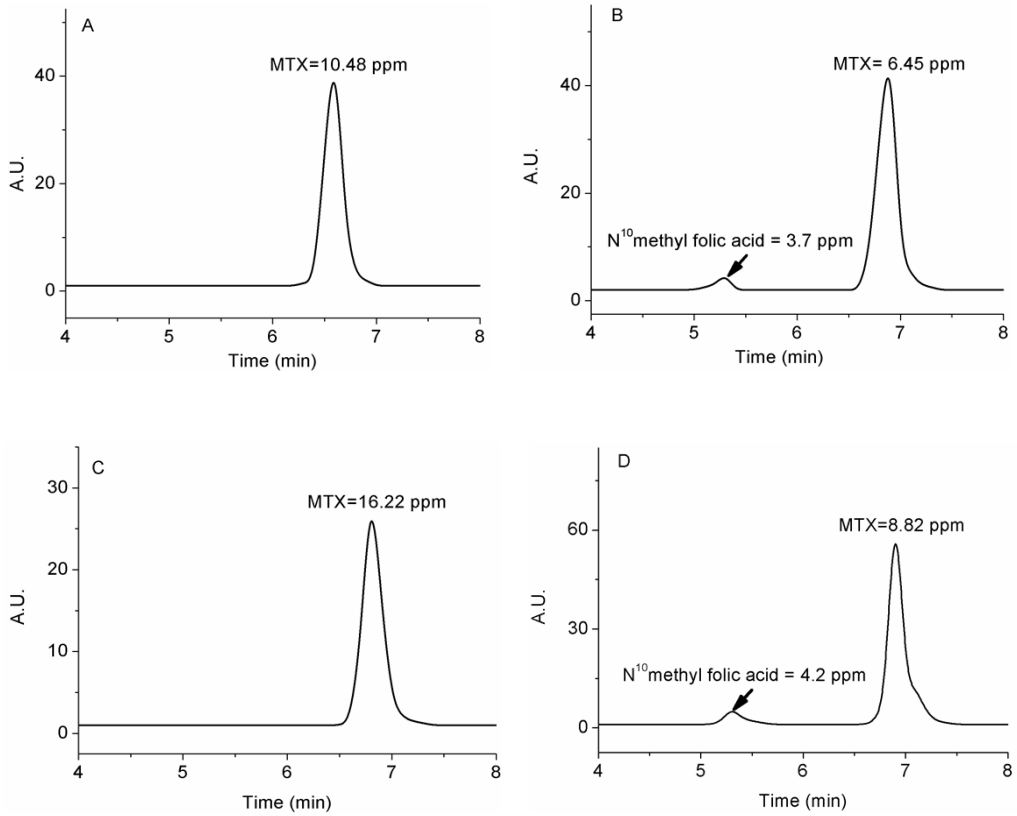


Fig. S2 Particle size distribution (intensity) of (a) pristine LDH (b) LDH-MTX and (c) PLGA coated LDH-MTX, having polydispersity index in the range 0.20-0.40, measured by dynamic light scattering technique.



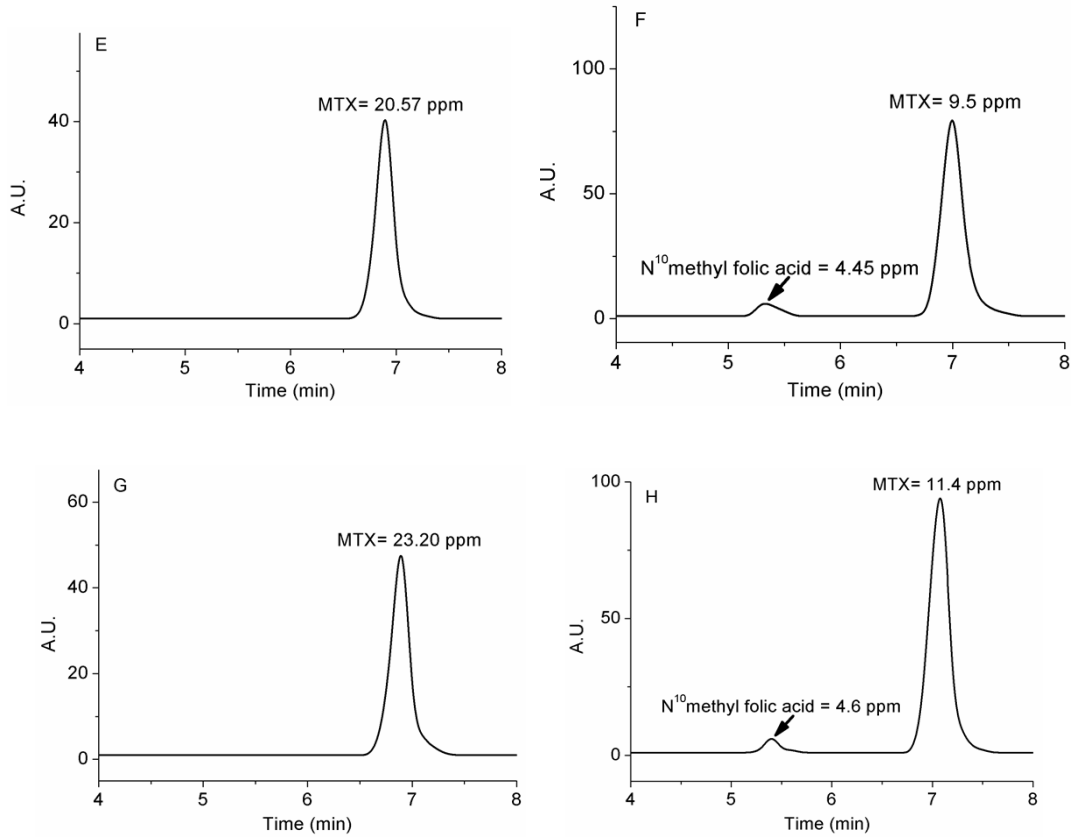


Fig. S3 Chromatograms showing the retention time in between 6.5-7.0 min for MTX release from LDH-MTX nanoformulation in PBS medium at pH 7.4: panel A showing the absence of any degradation peak (N^{10} methyl folic acid) after 2 h of release of MTX from sample B. Panel B showing the presence of the peak as above for sample B′ (arrow marked). Panels C, E and G showing the absence of any degradation peak as above at 4, 6 and 8 h of MTX release from sample B. Panels D, F and H showing the increased concentration of the degradation peaks corresponding to N^{10} methyl folic acid of sample B′ (arrow marked) at 4, 6 and 8 h of release. **Table S1** below demonstrates all the relevant parameters of the study as above.

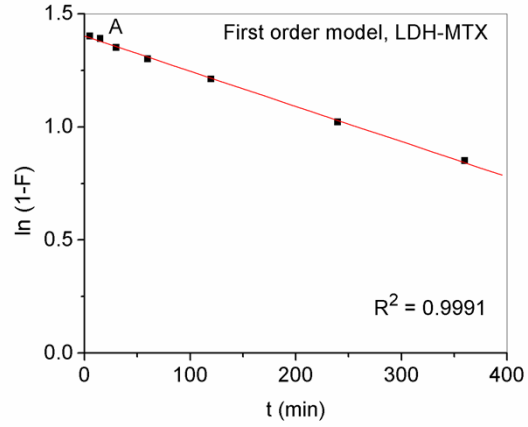
Table S2 Variation in concentration and peak area, versus the retention time of the samples B and B′ as a result of *in vitro* dissolution study at the time points, 2 h, 4 h, 6 h, 8 h.

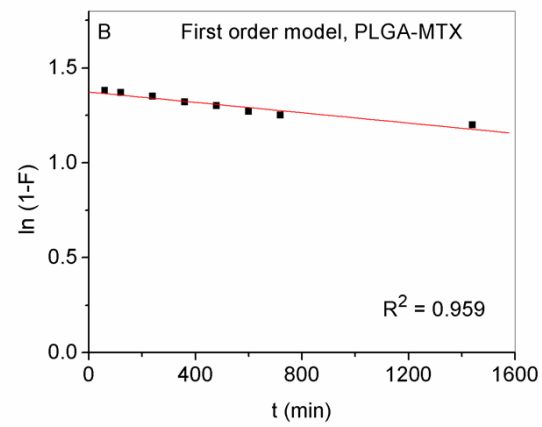
(a) Methotrexate

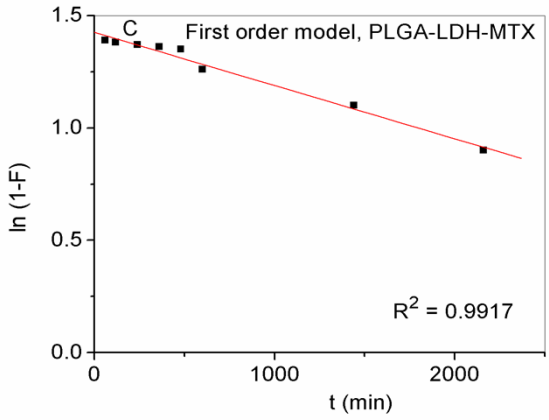
Sample name	Concentration (ppm)				Peak area (mV s)				Retention time (min)			
	2 h	4 h	6 h	8 h	2 h	4 h	6 h	8 h	2 h	4 h	6 h	8 h
B	10.48	16.22	20.57	23.20	655.2	1013 .8	1285.6	1449.7	6.9	6.9	7	7
B′	6.05	8.82	9.5	11.4	381.7	556.02	575.21	719.26	6.5	6.8	6.8	6.9

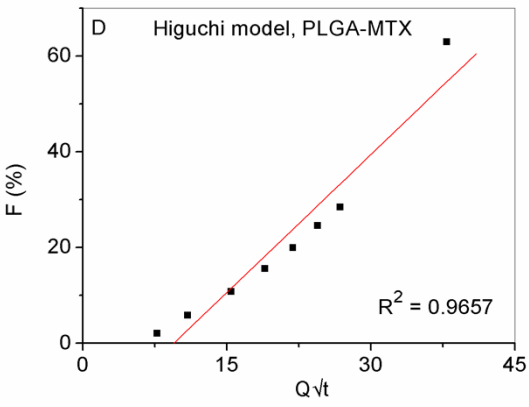
(b) N^{10} methyl folic acid

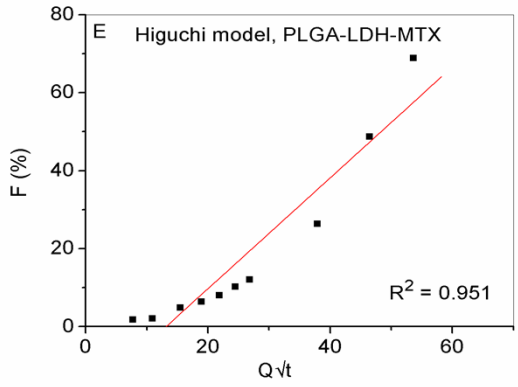
Sample name	Concentration (ppm)				Peak area (mV s)				Retention time (min)			
B	2 h	4 h	6 h	8 h	2 h	4 h	6 h	8 h	2 h	4 h	6 h	8 h
	3.7	5.5	5.2	5.25	6.4	6.5	6.9	7	5.3	5.3	5.3	5.4

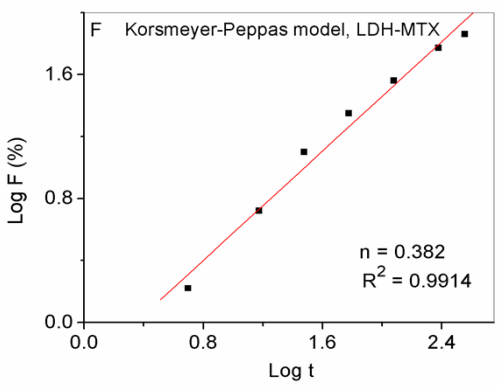

A
First order model, LDH-MTX
ln (1-F)
t (min)
R2 = 0.9991

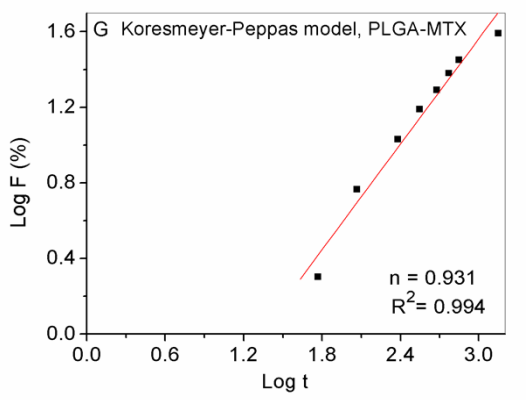

B
First order model, PLGA-MTX
ln (1-F)
t (min)
R2 = 0.959

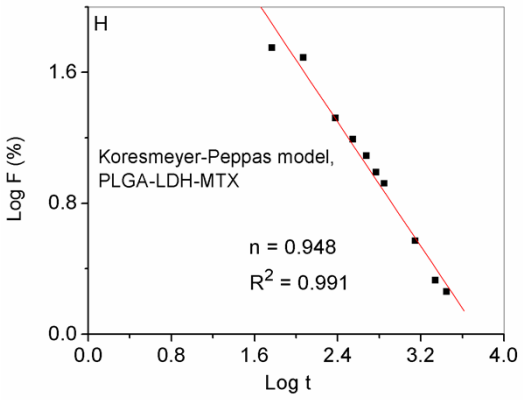

C
First order model, PLGA-LDH-MTX
ln (1-F)
t (min)
R2 = 0.9917


D
Higuchi model, PLGA-MTX
F (%)
Q√t
R2 = 0.9657


E
Higuchi model, PLGA-LDH-MTX
F (%)
Q√t
R2 = 0.951


F
Korsmeyer-Peppas model, LDH-MTX
Log F (%)
Log t
n = 0.382
R2 = 0.9914


G
Koresmeyer-Peppas model, PLGA-MTX
Log F (%)
Log t
n = 0.931
R2= 0.994


H
Koresmeyer-Peppas model,
PLGA-LDH-MTX
Log F (%)
Log t
n = 0.948
R2 = 0.991

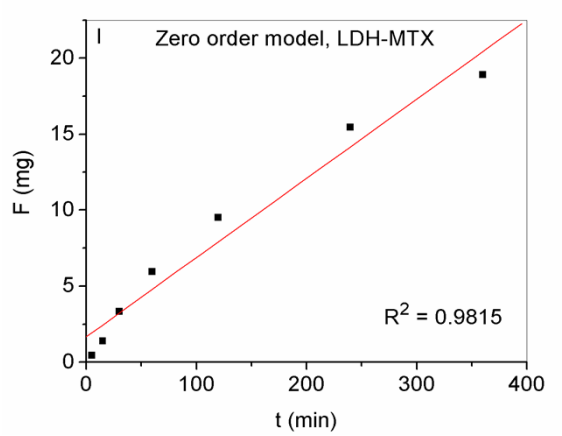


Fig. **S4** Additional kinetic models and the fitting curves of release of MTX from the samples B, C and D.