Supporting Information

Natural Salep/PEGylated Chitosan Double Layer towards a More Sustainable *p*H-Responsive Magnetite Nanocarrier for Targeted Delivery of DOX and Hyperthermia Application

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An introduction to the reported nanocarriers based magnetic core/polysaccharide shells

There are many reports on the use of different types of carbohydrates in the structure of drug nanocarriers; however, most of them are liposomes, hydrogels, micelles, vesicles, and nanocapsules (some related references are provided in the introduction of the main article). In

addition, many core-shell nanocarriers have been reported that most of them are nanocarriers with magnetic core. Silica, porous carbon, or quantum QDs cores are of the other most reported inorganic cores for drug nanocarriers. It is important to note that magnetic core in these core-shell nanocarriers are also varied from Fe-based cores to iron metal and other types of magnetic inorganic cores.

However, there are some similarities in the use of magnetic core/polysaccharide shell, but the present work are different from the reported works in details. The present work describes the synthesis of a magnetic nanocarrier that is double layered. The second layer is PEGylated chitosan that is coated on the surface of one-layered DOX-loaded nanocarrier using layer-by-layer technique. The effect of second layer in the targeted release of DOX is investigated and compared to the DOX-loaded nanocarrier without second layer. Salep and PEGylated chitosan shells are natural and only minor modifications were made on them. Nanocarrier reported in the present work are based on Fe_3O_4 core and we believe that it is truly different from the reported magnetic-carbohydrate core-shell nanocarriers in the type of shells (one- and double-layer), the technique of shell coating, investigation methods, hyperthermia application, and some biological results such as MTT, hemolysis, coagulation, and cellular uptake.

There are also few reports on the use of salep polysaccharide that all of them are hydrogels or carbon-based hydrogels.[1-8] The use of chitosan or PEGylated chitosan or polysaccharide, solely, in drug nanocarriers are also reported which is differ from the present work in the identity of being doubled layer.[9-15]

There are some related articles with the present work.[2, 16-23] However, the present work is different from these in one or more aspects such as the type of shell, biocompatibility of shells, type of polysaccharide shell, being one- or double-layered carrier, type of *in-vitro* biological analyses or their results. In addition, in most of these reported carriers, the second polymeric shell is grafted from the first shell by chemical reactions and the desired drug is loaded in the final step whereas the second shell in the present work is loaded using layer-by-layer technique.

Characterization of nanocarriers 1 and 2 using FT-IR analysis

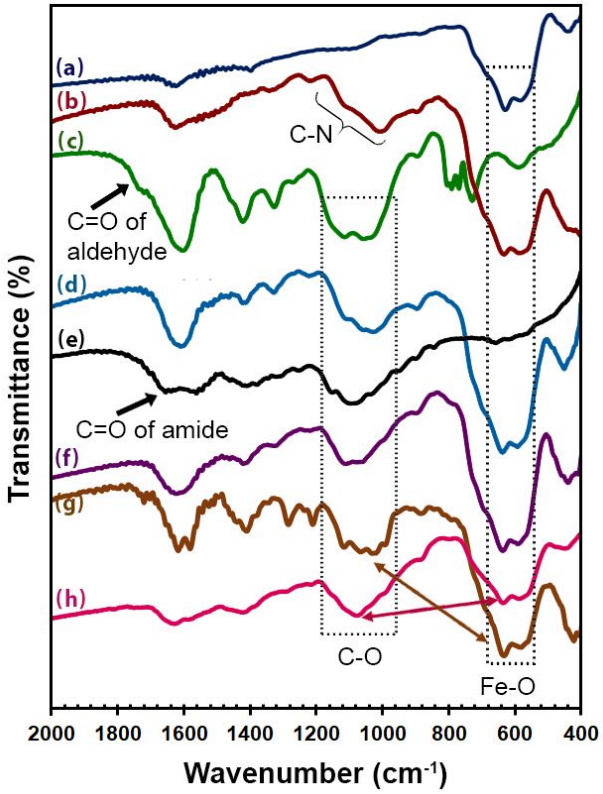


Fig. S1 FT-IR spectra of MNP (a), MNP@NH_2 (b), DAS (c), MNP@DAS (d), CSP (e), MNP@DAS@CSP (f), MNP@DAS@DOX (g), and MNP@DAS@DOX@CSP (h).

Characterization of DAS and CSP

Oxidation of salep to dialdehyde salep (DAS) was followed by FT-IR. As shown in Fig. S2-a and b, appearance of a distinct shoulder higher than 1700 cm^{-1} in the IR spectrum of DAS related to the aldehyde C=O, confirmed the oxidation process. ^1H NMR also confirmed the formation of DAS through Oxidation process (Fig S3). Synthesis of CSP was also followed by FT-IR. Esteric C=O of mPEG-CO_2Me is clearly appeared in Fig. S2-d compared to Fig. S2-c confirming

oxidation and esterification steps. However, FT-IR spectrum of CSP (Fig. S2-f) showed no visible change compared to chitosan (Fig. S2-e), mPEG-CO_2Me grafting on chitosan was followed by GPC analysis (Fig. S4). Increasing in molar mass of CPS compared to chitosan confirmed successful grafting process. ^{1}H NMR spectrums of chitosan, mPEGCO_2Me, and CSP (Fig. S5) also confirmed the grafting of mPEG-CO_2Me on chitosan.

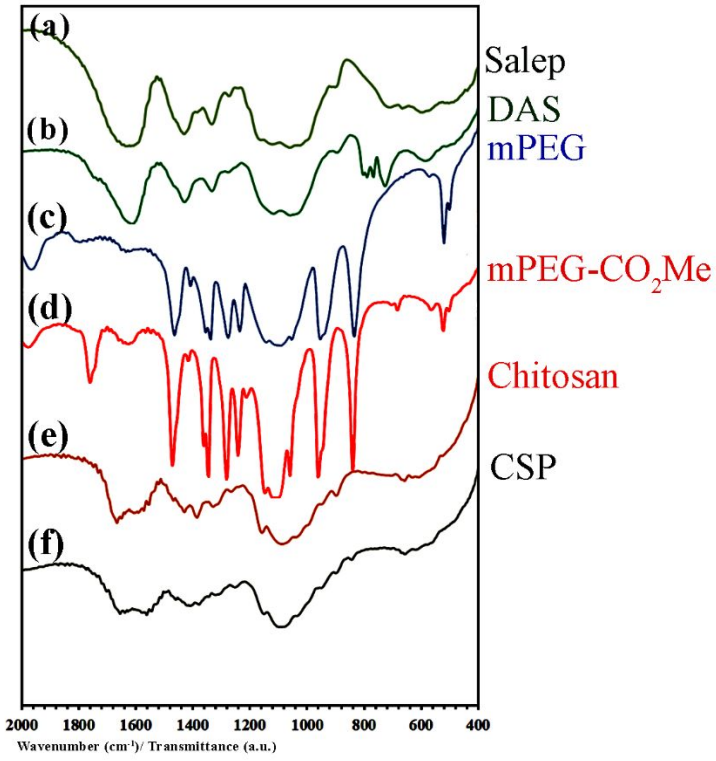


Fig. S2 FT-IR spectra of DAS and CSP and their precursors

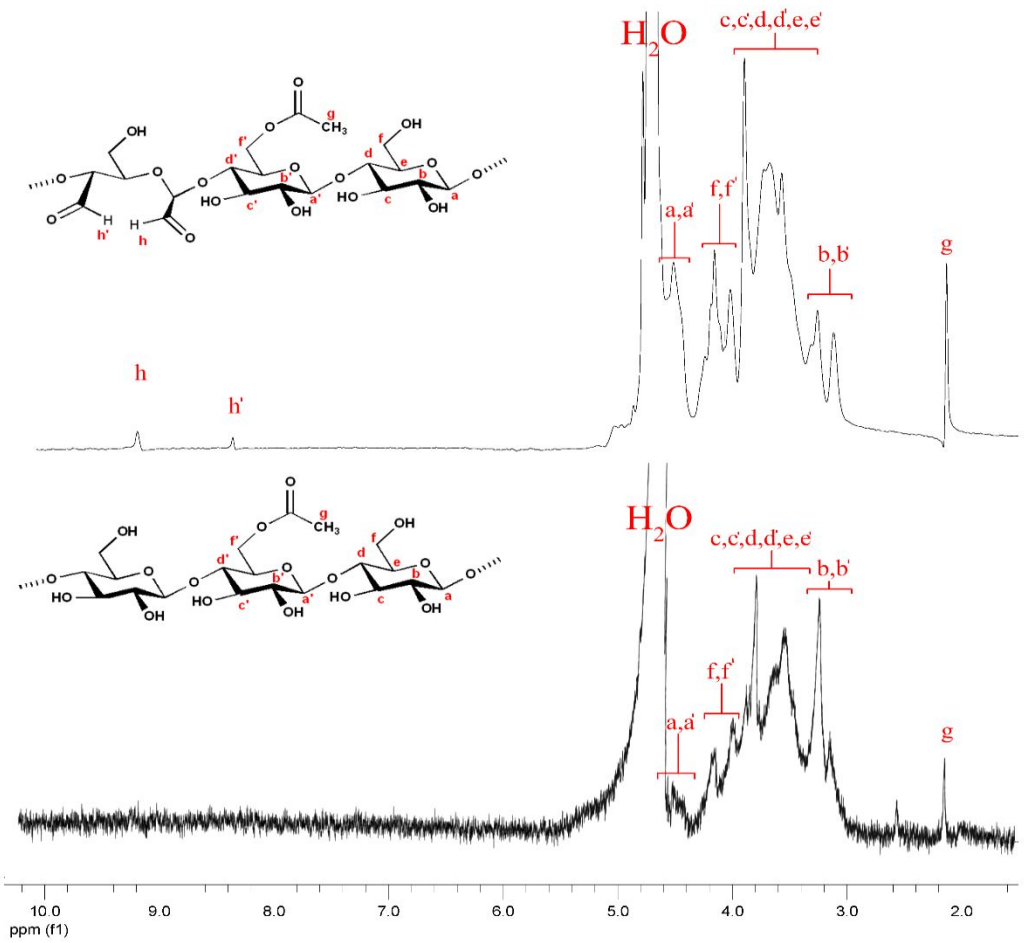


Fig. S3 ^{1}H NMR spectrum of salep and DAS.

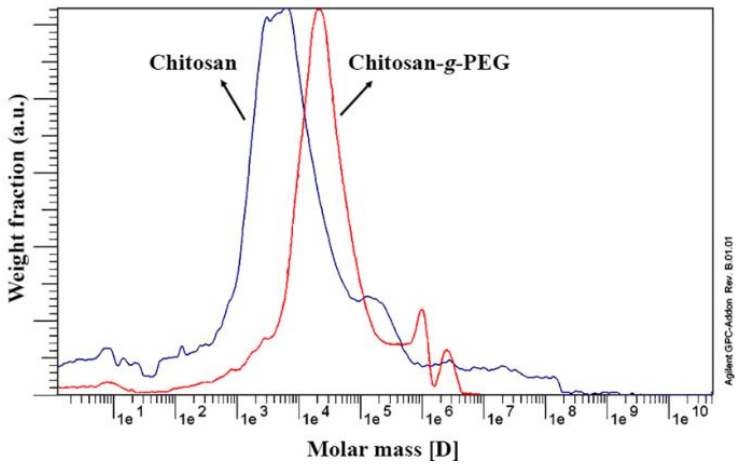


Fig. S4 GPC analysis of chitosan and PEGylated chitosan (CSP).

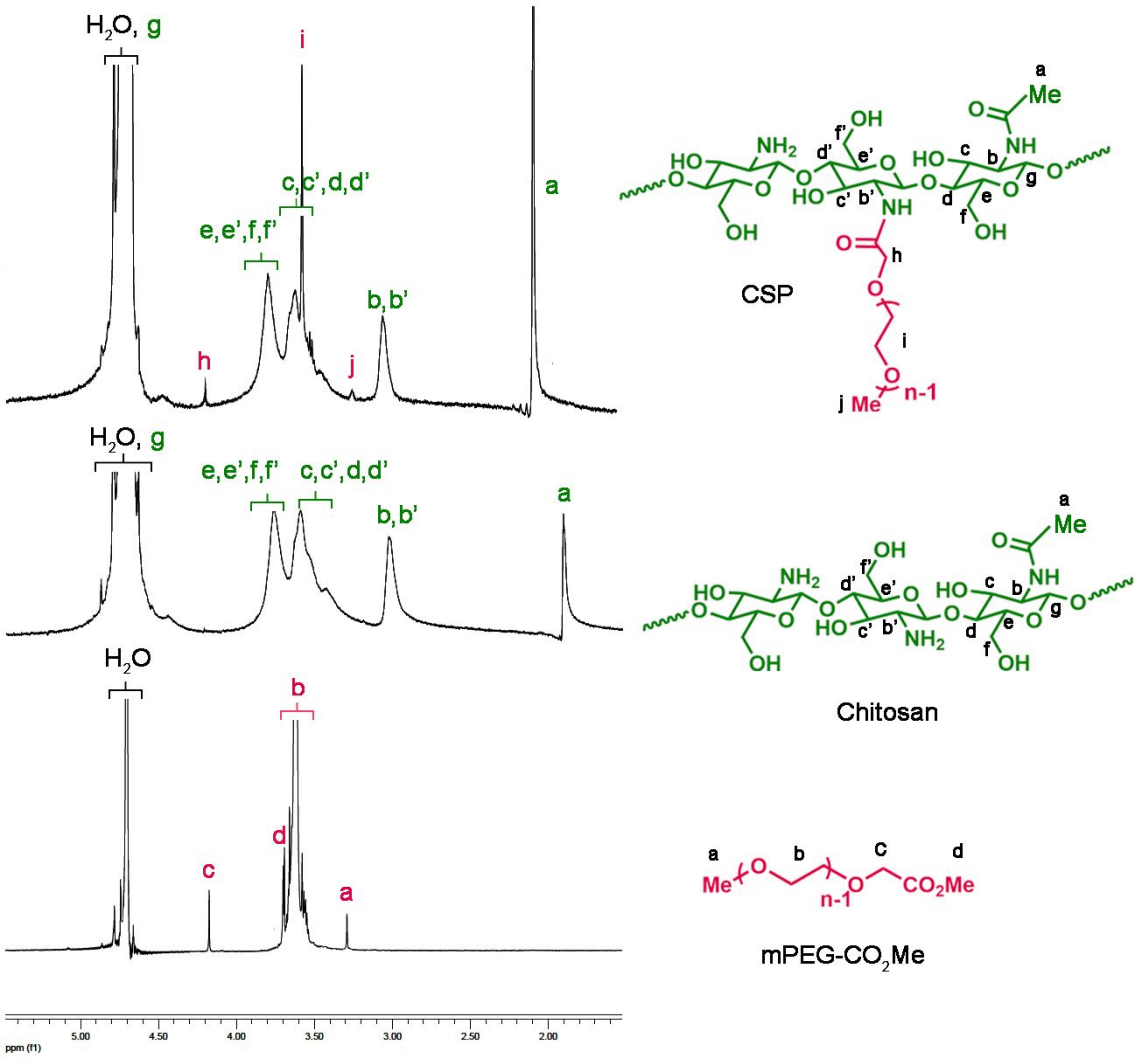


Fig. S5 NMR spectrum of mPEG-CO2Me, chitosan, and CSP.

Analyses for optimization of DAS loading on MNP@NH_2

Table S1: Optimization condition for DAS loading

Entry	MNP@APTS:DAS (*w*:*w*)	*T* (°C)	Time (h)	V_{water} (mL)	IR (Fig. S2)	TGA (Fig. S3)
1	1:1[a]	r.t	5	100	a	a
2	**1:1[a]**	**r.t**	**5**	**10**	**b**	**b**
3	1:2[b]	50	5	5	c	c
4	1:2[b]	r.t	5	5	d	d
5	1:4[a]	r.t	24	10	e	e
6	2:1[c]	r.t	24	10	f	f

[a]Fe_3O_4@APTS (200 mg). [b]Fe_3O_4@APTS (100 mg). [c]Fe_3O_4@APTS (50 mg).

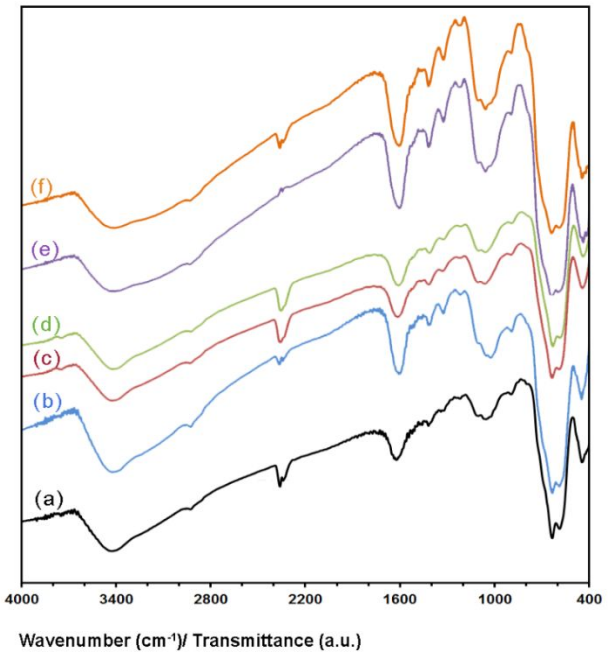


Fig. S6 FT-IR analyses for DAS loading related to Table 1.

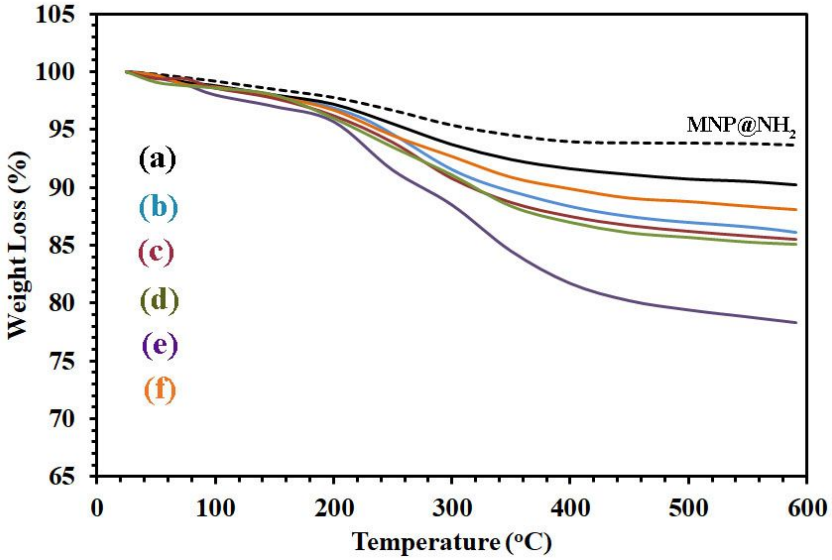
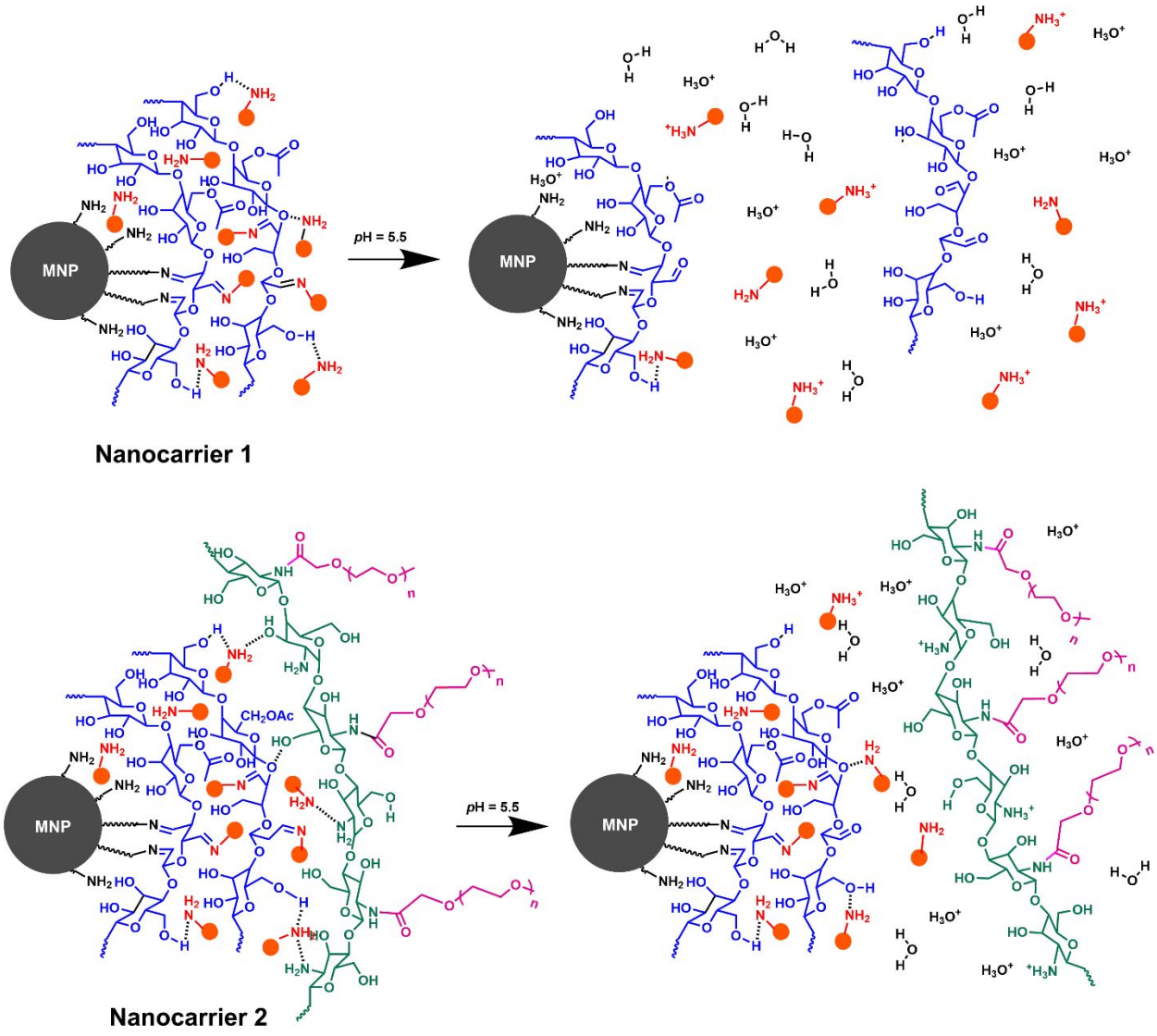


Fig S7 TGA analyses for DAS loading onto the surface of MNP@NH_2 related to Table S1.

Table S2 Comparison of DOX release from various nanocarrier at 37 °C.

Nanocarrier	Type of nanocarrier	% release at pH 7.4 (time)	The difference of release between pH 5.5 and 7.4 (%)	Reference No.
Fe_3O_4@OCMC@IRMOF-3/FA	Magnetic core, chitosan/MOF shell	35 (80 h)	30[a]	21
PLGA@PEG-g-CS[a]	Polymeric organic core, PEG/chitosan shell	50 (80 h)	n.r	24
MNP@CS	Magnetic core, chitosan shell	38 (48 h)	28	25
MTX-MagTSLs	Magnetic core, polymeric organic shell	42 (24 h)	n.r	26
MNP/MSN/p-NIBIM-AA	MNP/MSN core, polymeric organic shell	62 (80 h)	n.r	27
MagNanoGels	Magnetic nanogel	24 (6 h)	70	28
MNP-MSN-PF-127	Magnetic core, polymeric organic shell	32 (80 h)	n.r	29
Fe_3O_4@SiO2@SC-D	Magnetic core, polymeric organic double shell	72 (70 h)	9[b]	30
Fe_3O_4-CMC-AA-FA	Magnetic core, chitosan-FA shell	50 (80 h)	31	13
IFNγ-TSN (hyd)	Organic nanocapsule	40 (96 h)	42	31
RPAE-PEG	micelle	38 (48 h)	37	32-33
MNP@DAS@CSP	Magnetic core, organic polymer double shell	45 (80 h) 46 (135)	35 45	This work

[a]: epirubicin drug; b: *p*H of acid condition is 3.5



Scheme S1. The proposed mechanism for DOX release from nanocarrier **1** and **2.**

Table S3 Magnetic heating properties of magnetite nanoparticles and nanocarrier 2

Sample	Sample concentration (mg)	Fe_3O_4 component (mg)	Initial slope of related curve	SAR	ILP	Time required to reach 42 °C (s)
MNP	1	1	0.0726	303.5	1.32	201
MNP@DAS@CSP	1	0.87	0.0618	297	1.30	234
Nanocarrier **2**	1	0.75	0.0502	280	1.22	289
Nanocarrier **2**	2	1.5	0.0854	238	1.04	174
Nanocarrier **2**	0.5	0.375	0.0432	481.5	2.11	347
Nanocarrier **2**	0.3	0.225	0.0286	531	2.32	550
H = 300 Oe or 23.9 kAm^{-1}, f = 0.4 MHz						

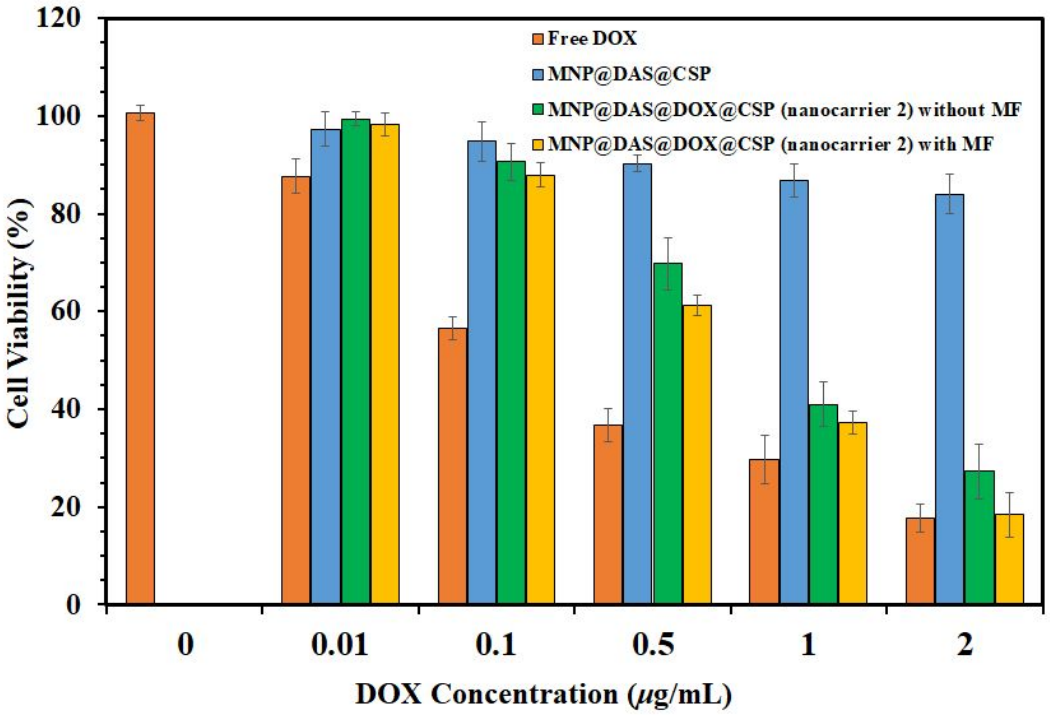


Fig. S8 Cell viability of Hela cells after 24 h incubation with free DOX, MNP@DAS@CSP, and nanocarrier **2**, with and without static magnetic field.

Table S4 Comparison of hemolysis assay with the results of reported works

Nanocarrier	Concentration (μgmL^{-1})	% of hemolysis	Reference
DCP@MNPs	5-400	0.1-0.9	[18]
MagTSLs	25-400	< 2	[26]
MWCNT/$CoFe_2O_4$-180	50-400	0-2	[34]
Dex-SA	39-625	0	[35]
MSNs-PPPFA	12.5-800	0.2-1.6	[36]
MNP-G2 MNP-G2@MTX	5-200	0-0.25 0.16-0.67	[37]
FA-Fe_3O_4@nGO	10-400	0.01-2.46	[38]
MNP@DAS@CSP MNP@DAS@DOX@CSP	15.5-500	0.07-0.35 0.05-0.36	This work

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