Self-assembled ZnS nanospheres with nanoscale porosity as an efficient carrier for the delivery of doxorubicin

Vandana Kumari, Nabanita Chatterjee, Subhadip Das, Subhajit Bhunia, Krishna Das Saha and Asim Bhaumik*

Section S1	*Details of biological experimemental techniques*
Figure S1	*HRTEM image of the material MZnS-1E in PBS (a) and selected area electron diffraction (SAED) pattern of the sample MZnS-E in PBS (b).*
Figure S2	*Effect of MZnS-EDox on peripheral blood mononuclear cells*
Figure S3	*Effect of MZnS-EDox in mitochondrial membrane potential*
Figure S4	*Effect of MZnS-EDox in generation of reactive oxygen species*
Figure S5	***Effect of MZnS-EDox in different pro-and anti apoptotic proteins in K562 cells:*** *Effect of MZnS-EDox in K562 cells. Status of different apoptotic markers including Bax, Bad, Bcl-2 and caspase-3 were evaluated in K562 cells in time dependent manner (0, 12, 24 and 36 h) with the treatment of MZnS-EDox by flow cytometrical analysis.*
Figure S6	***Effect of MZnS-EDox in K562 cells.*** *Status of different apoptotic markers including Cyt C, AIF and ENDO G were evaluated in K562 cells in time dependent manner (0, 12, 24 and 36 h) with the*

	treatment of MZnS-EDox by flow cytometrical analysis.
Figure S7	***Drug release study:*** *The cumulative release of Dox from the of MZnS-EDox loaded complex was up to 40.44 % at pH 7.4 respectively, for a period of 72 days at 37° C.*

Section S1

(A) Cell culture

Different cancer cell line U937, HL60, K562, HepG2, A375 and A549 cells (American Type Culture Collection, ATCC) were used. The cells were cultured in RPMI or DMEM supplemented with 10% heat-inactivated FBS, 100 U/mL penicillin and 100 μg/mL streptomycin sulfate at 37 °C under a humidified atmosphere of 5% CO_2.[1]

(B) MTT assay

MTT assay was done to evaluate cell viability. The cells were plated in 96 well plates and treated with or without different concentrations of TEABP for 24 h. Four hours after the addition of MTT, cells were lysed and formazan was solubilized with acidic isopropanol and the absorbance of the solution was measured at 595 nm using an ELISA reader.[2]

(C) Assessment of cellular morphology

Cells (1×10^4/well) grown in 6-well plates in RPMI supplemented with 10% FBS for 24 h were treated with or without compound. Morphological changes were observed with an inverted phase contrast microscope (Model: OLYMPUS IX 70, Olympus Optical Co. Ltd.) and images were acquired.

(D) Quantification of apoptosis using Annexin-V

Apoptosis was assayed by using an Annexin-V FITC apoptosis detection kit (Calbiochem, CA, USA). Briefly, cells were treated with or without derivatives, then washed and stained with PI and Annexin-V-FITC in accordance with the manufacturer's instructions. The percentages of live, apoptotic and necrotic cells were determined by

flow cytometric method (Beckton Dickinson, San Jose, CA, USA). Data from 10^6 cells were analyzed for each sample.

(E) Cell cycle analysis

Upon treatment cells were collected and fixed in 70% ethanol for 24 h at 4°C. The cells were centrifuged (1500g); cell pellet was resuspended in PBS (400 µl), RNaseA (10 mg /ml, 50 µl) and PI (2 mg/ ml, 10 µl). The mixture was incubated in the dark at 37 °C for 30 min and was then acquired and analysed by flow cytometry.

(F) Reactive oxygen species (ROS) assay

ROS were detected using the cell-permeable fluorescent probe 2-7-dichlorofluorescein diacetate (H2DCFDA) (Sigma-Aldrich, USA), a non-fluorescent compound, which is converted into highly fluorescent dichlorofluorescein (DCF) by cellular peroxides. Briefly, cells were exposed to various agents for the indicated times and then loaded with H2DCFDA (20 mM). Following incubation at 37°C for 30 min, cells were washed with PBS and fluorescence accrued by flow cytometric method at excitation wavelength of 488 nm and emission wavelength of 530 nm.

(G) Mitochondrial membrane potential (MMP/Δψm) measurement

Upon cells were treated and MMP was measured with the voltage-sensitive lipophilic cationic fluorescent probe 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl-benzimidazolylcarbocyanine Iodide (JC-1). JC-1 monomers fluorescence red in stable mitochondria and upon exclusion combines to form green fluorescent dimers. Briefy, cells were collected; washed with cold PBS, incubated with JC-1 (5 m/ ml) for 15 min and analyzed by flow cytometric method.

(H) Analysis of different apoptotic proteins by flow cytometry

Analysis of apoptosis related proteins by flow cytometry. The expression levels of CytC (Cytochrome C), AIF (Apoptosis inducing factors), Endo G (Endonuclease G), and Bax, Bad, Bcl-2 and caspase-3 in K562 cells from each group were determined by flow cytometry. Briefly cell (1×10^6) were fixed and permeabilized with 70% ethanol and labelled with FITC, PE conjugated antibody (BD Pharmingen, USA) for 1 h at room temperature. Cells were washed thoroughly and analyzed on a flow cytometer (Becton–Dickinson) equipped .A total of 10,000 events were acquired.

(I) Drug release study

Doxorubicin conjugated ZnS was put into the dialysis tube and transferred to a beaker containing 50 mL of phosphate buffer solution at pH 7.4. The drug release study was conducted at 37 °C with continuous stirring at 100 rpm. To measure the drug release content, samples (1 mL) were periodically removed and replaced with an equivalent volume of the phosphate buffer solution. The amount of released DOX was analyzed with a spectrophotometer at 485 nm.

(J) Statistical analysis

Results were expressed as mean ±SD. Statistical analyses were performed with ANOVA, followed by Dunnett's test. $P < 0.05$ was considered as significant.

Figure S1.

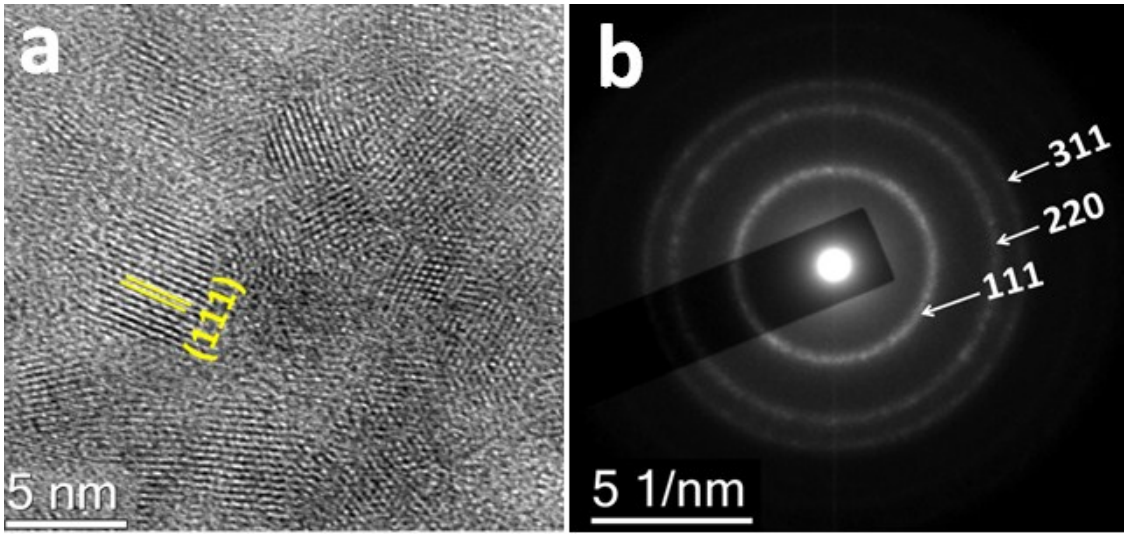

Figure S1. HRTEM image of the material MZnS-1E in PBS (a) and selected area electron diffraction (SAED) pattern of the sample MZnS-E in PBS (b).

Figure S2.

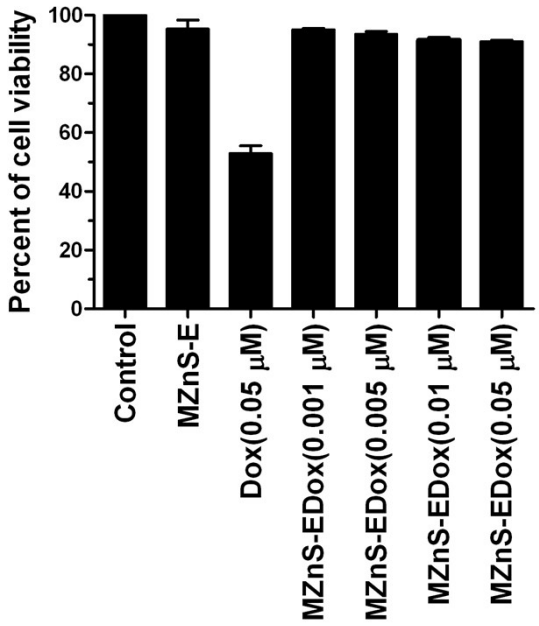

Figure S2. Cytotoxic effect of MZnS-EDox on peripheral blood mononuclear cells (PBMC): Cells were treated with different concentrations (0, 0.001, 0.005, 0.01 and 0.05μM) of the MZnS-EDox for 24 h and viability was measured by MTT assay. The data are represented as mean ± SD from triplicate independent experiments. No significant change was found.

Figure S3

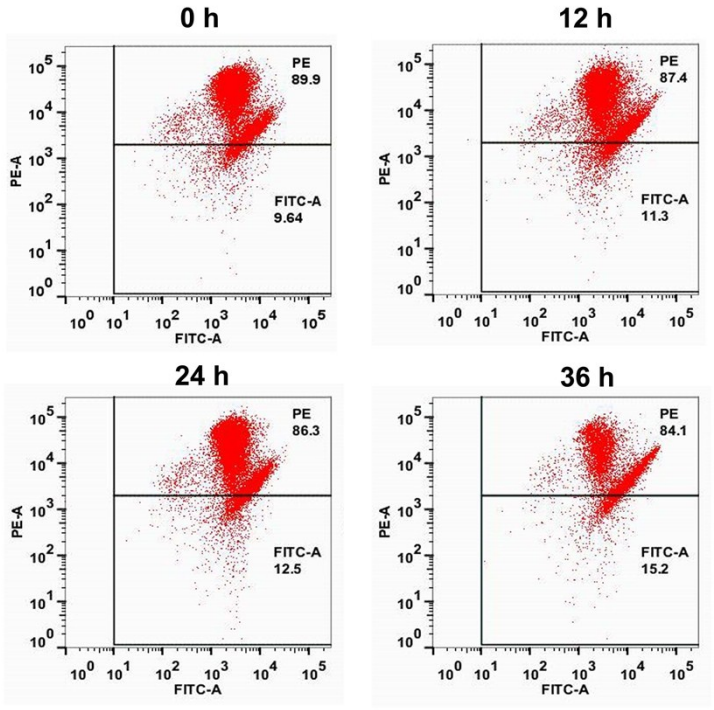

Figure S3. Effect of MZnS-EDox on K562 cells. Membrane potential evaluated with JC-1 dye reactively measured by flow cytometrical analysis in time dependent manner. The scattered plots are represented.

Figure S4

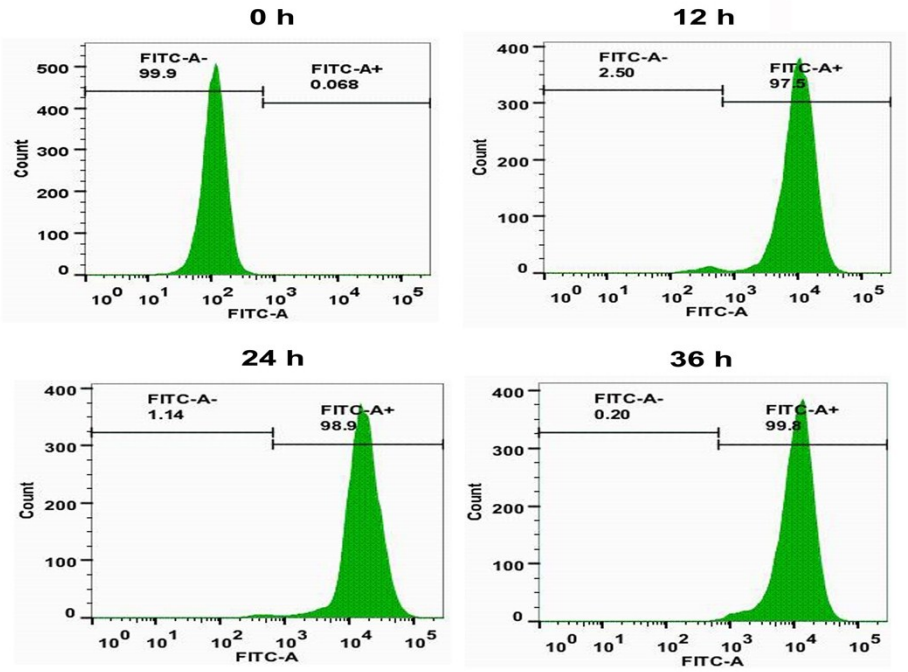

Figure S4. Effect of MZnS-EDox on K562 cells. Generation of reactive oxygen species evaluated by DCFDA dye measured by flow cytometrical analysis in time dependent manner. The scattered plots are represented.

Figure S5

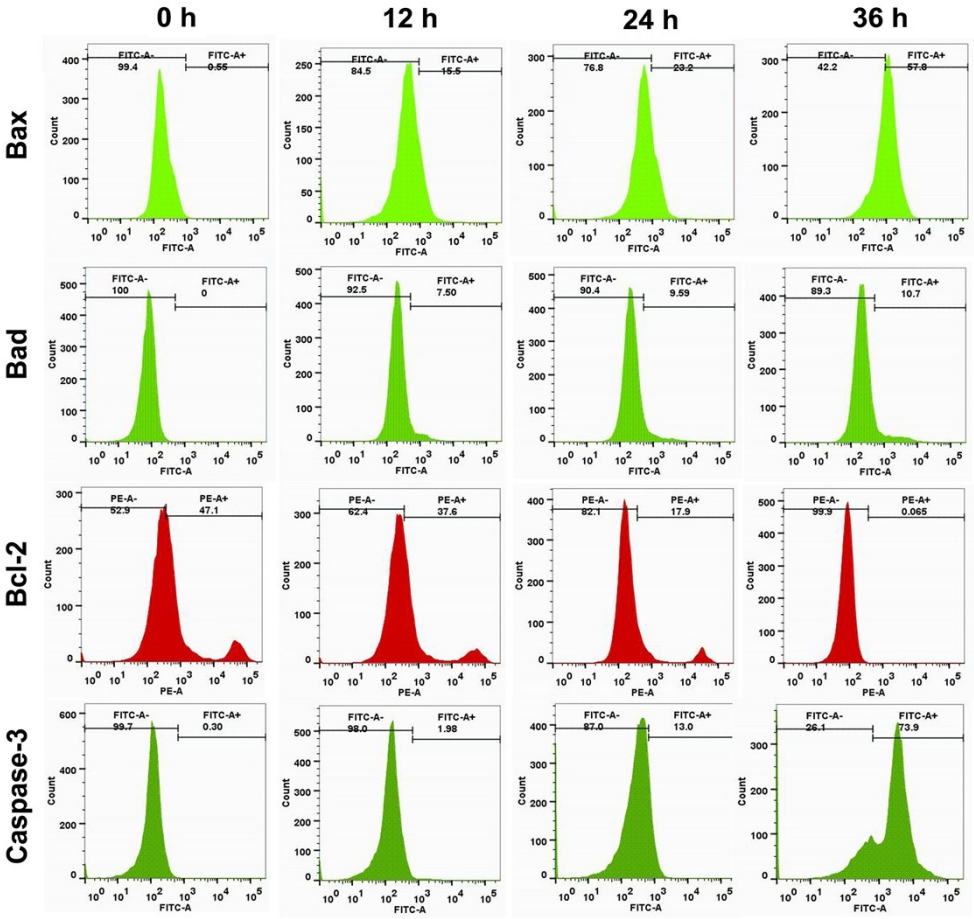

Figure S5. Effect of MZnS-EDox in K562 cells. Status of different apoptotic markers including Bax, Bad, Bcl-2 and caspase-3 were evaluated in K562 cells in time dependent manner (0, 12, 24 and 36 h) with the treatment of MZnS-EDox by flow cytometrical analysis.

Figure S6

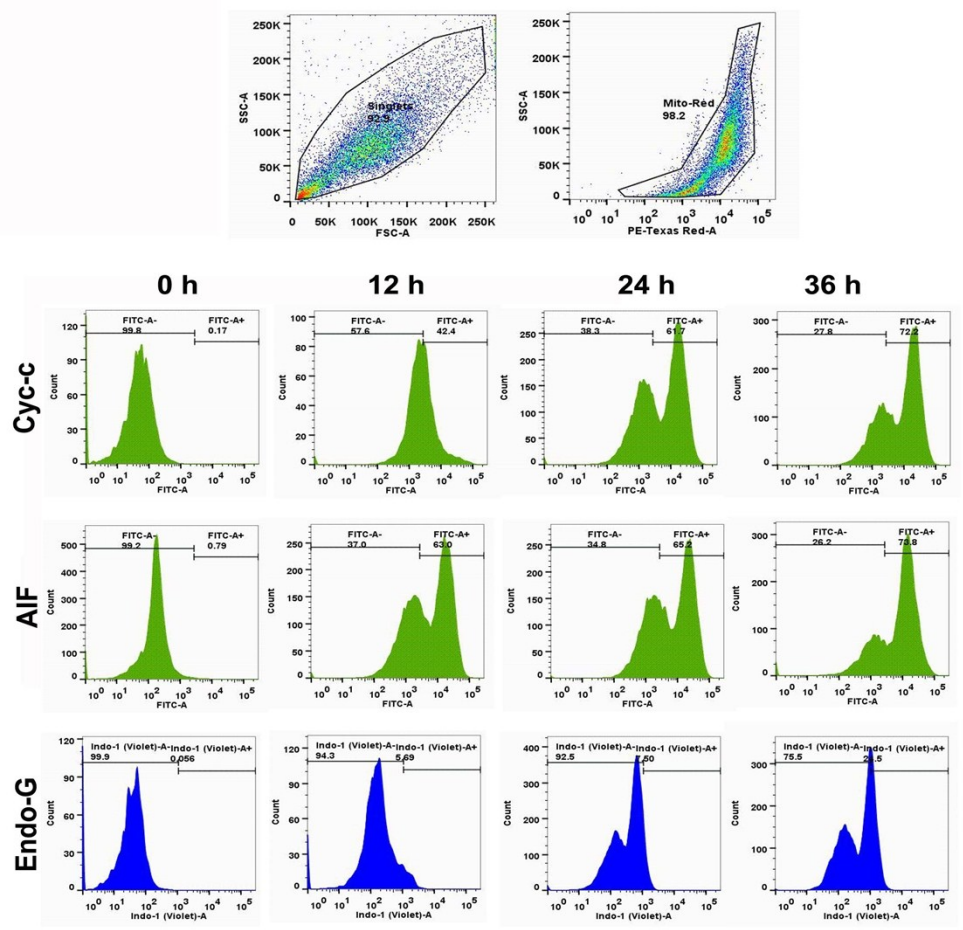

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Figure S7

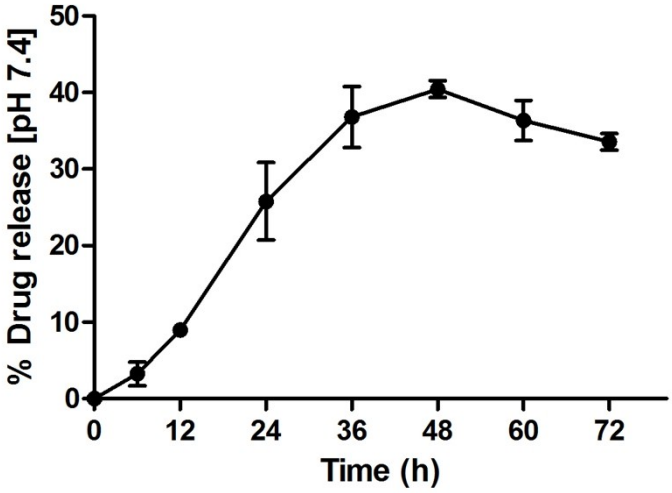

Figure S7: Drug release study: The cumulative release of Dox from the of MZnS-EDox loaded complex was up to 40.44 % at pH 7.4 respectively, for a period of 72 days at 37° C. These results suggest that the slow and prolonged release of the drug with respect to time possibly prevents acute toxicity, minimizing the side effects and thus may have important applications in drug delivery and chemotherapy.

References

1	S. Das, N. Chatterjee, D. Bose, S. Banerjee, T. Jha, K. D. Saha, *Tumor Biol.*, 2015 **36**, 3109–3118.
2	T. Mosmann, *J. Immunol Methods*, 1983, **65**, 55–63.