Electronic Supplementary Material (ESI) for New Journal of Chemistry.

SUPPORTING INFORMATION

Functional Evaluation of Doxorubicin Decorated Polymeric Liposomal Curcumin: A Surface Tailored Therapeutic Platform for Combination Chemotherapy

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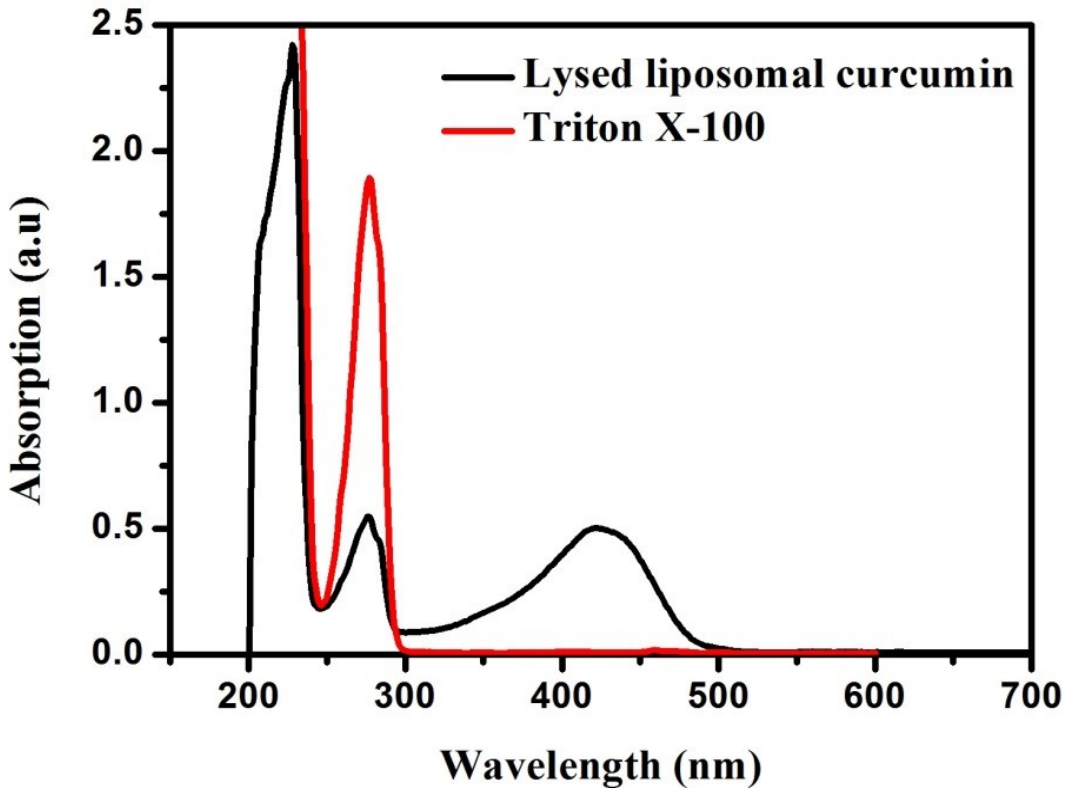
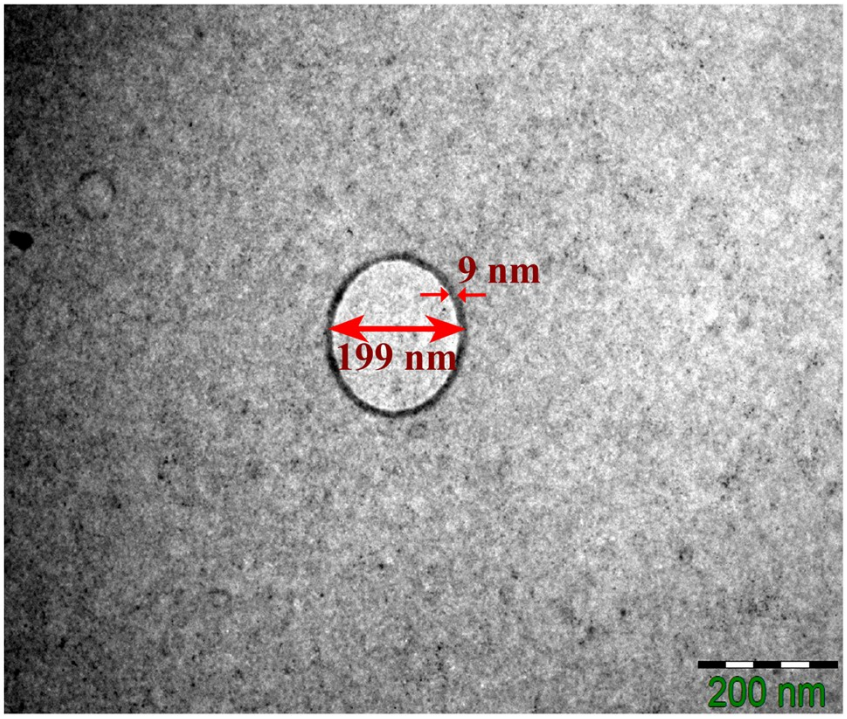


Figure S1. CUR quantification through lysing the liposomal surface by 1% Triton X-100



FigureS2. High magnification TEM profile representing the wall thickness which is 9 nm.

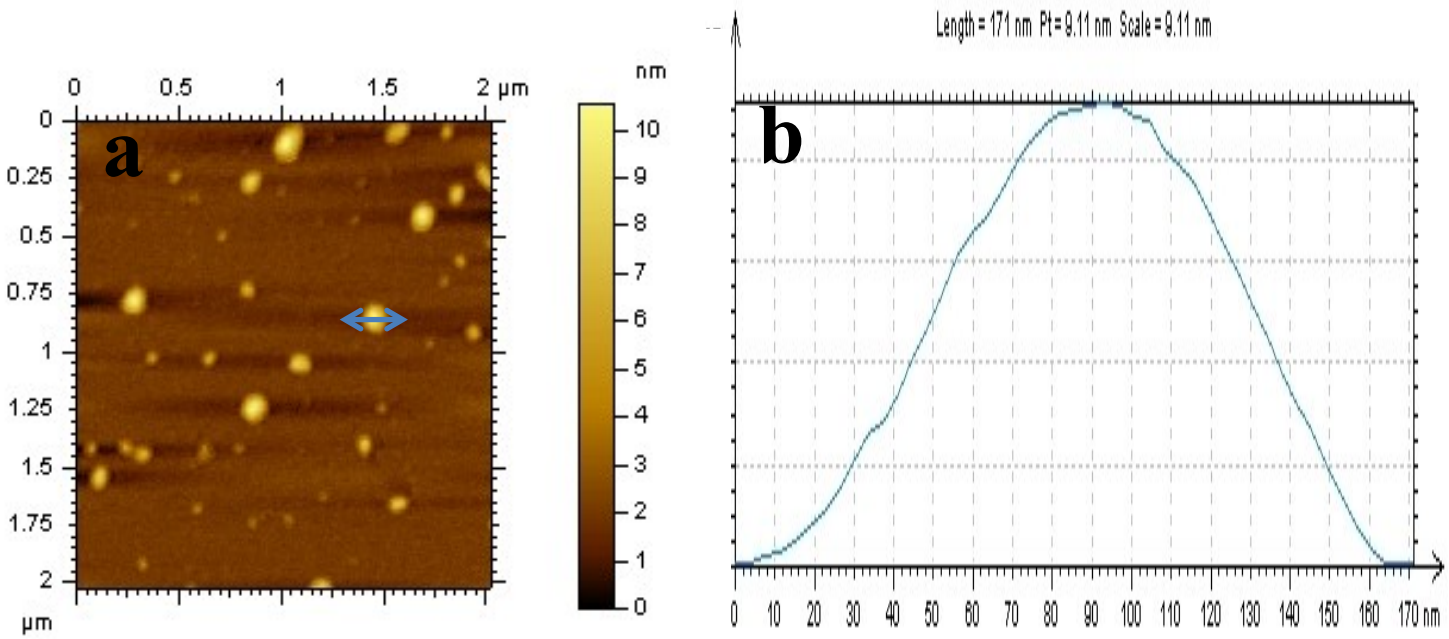


Figure S3. (a) The surface morphology of liposomes (b) Surface profile analysis of individual liposomes in the scanning area of 2 x 2 µm.

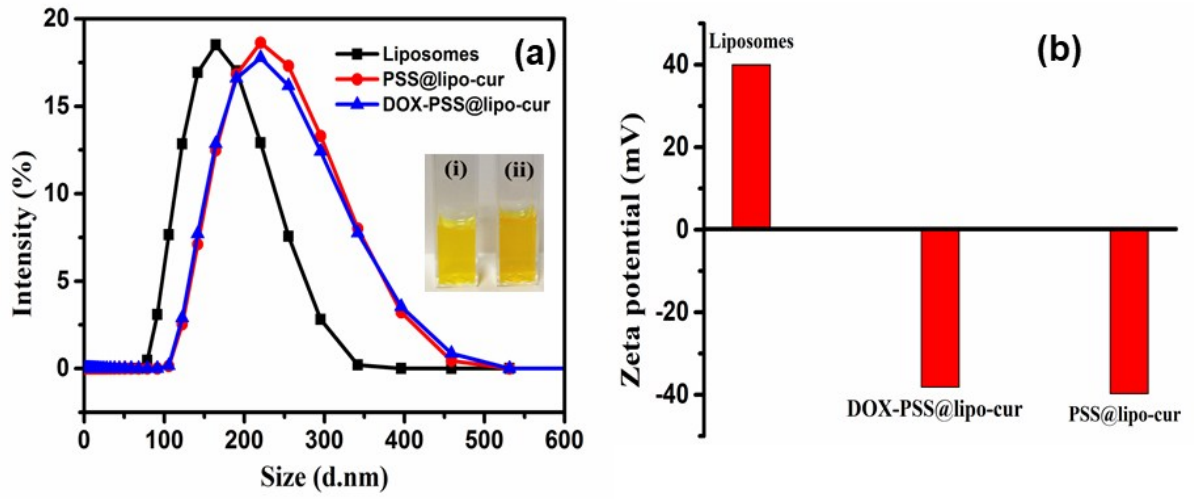


Figure S4. (a) DLS measurements reveal the hydrodynamic size distribution of liposomes, PSS@lipo-cur, and DOX-PSS@lipo-cur. The inset photograph depicts colloidal form of nanoformulations (i) PSS@lipo-cur (ii) DOX-PSS@lipo-cur (b) Zetapotential values of nanoformulations.

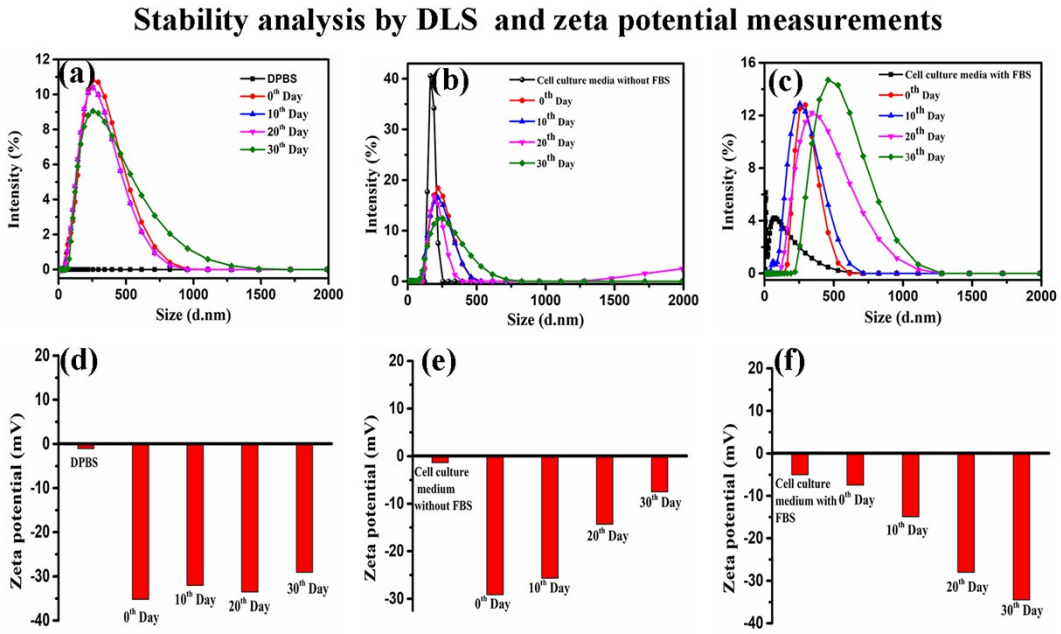


Figure S5. Stability of DOX-PSS@lipo-cur nanoformualtion by hydrodynamic size distribution in different condition such as (a) DPBS (b) cell culture medium without FBS (c) cell culture medium with FBS. Zetapotential analysis of nanoformulation in different condition such us (d) DPBS (e) cell culture medium without FBS (f) cell culture medium with FBS

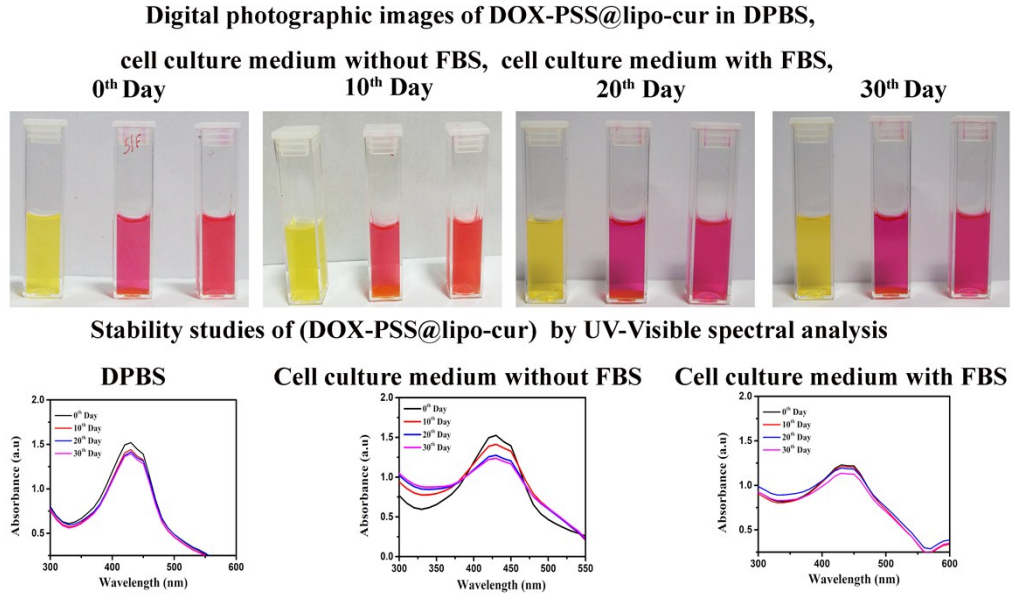


Figure S6. Digital photographic images of DOX-PSS@lipo-cur nanoformualtion in DPBS, cell culture medium without FBS and cell culture medium with FBS. Stability analysis by UV-visible spectral analysis in different condition such as DPBS, cell culture medium without FBS, cell culture medium with FBS.

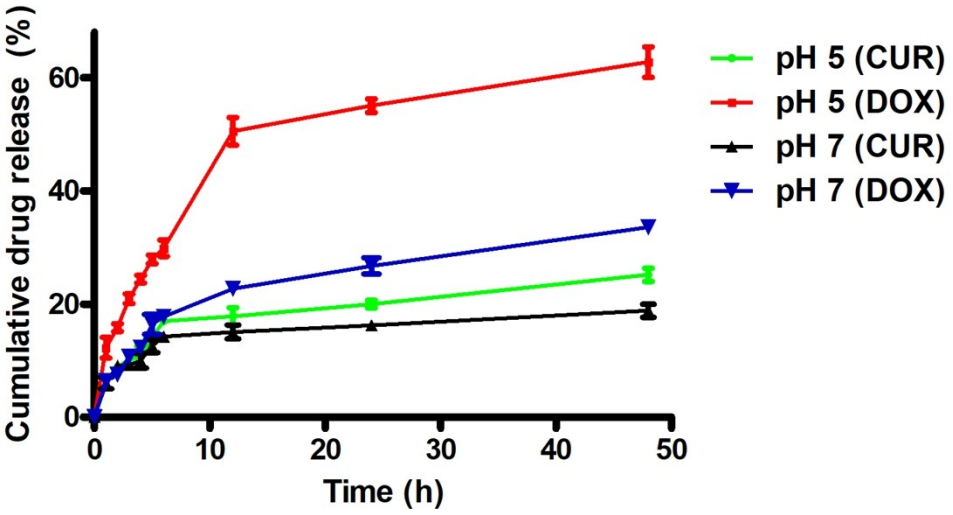


Figure S7. The cumulative drug release experiments of the nanoformulation (CUR and DOX) in acidic pH :5, and physiological pH :7.4.

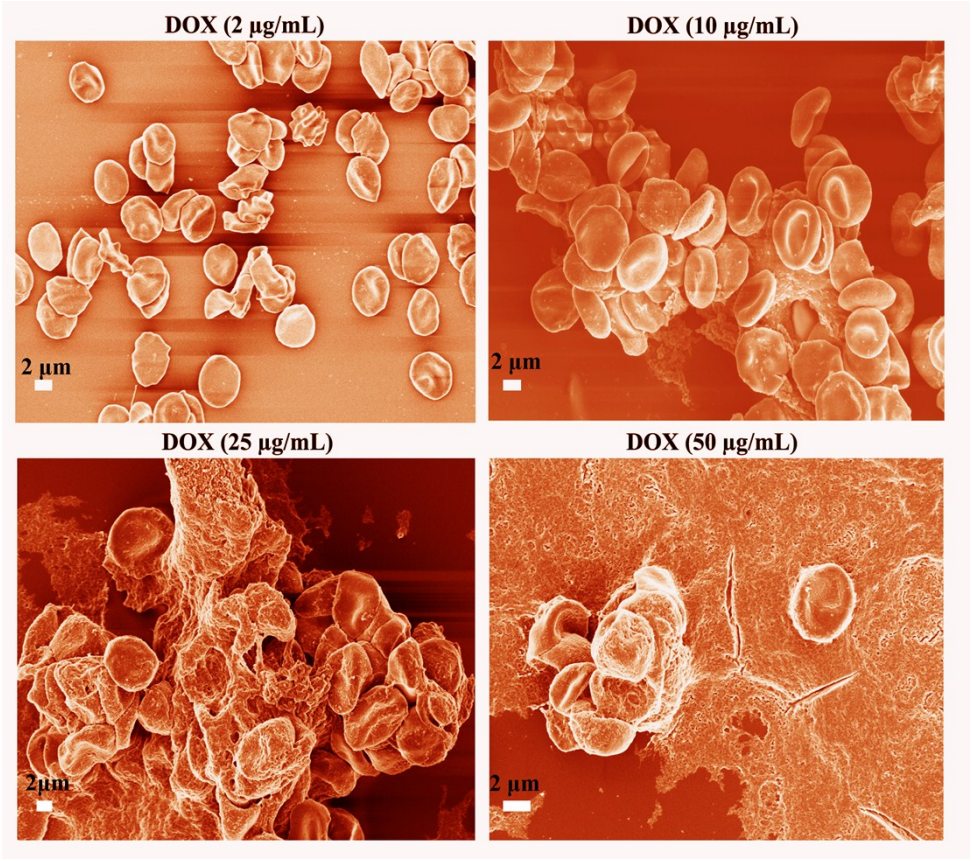


Figure S8. SEM micrograph images of RBCs incubated with various concentrations of DOX.

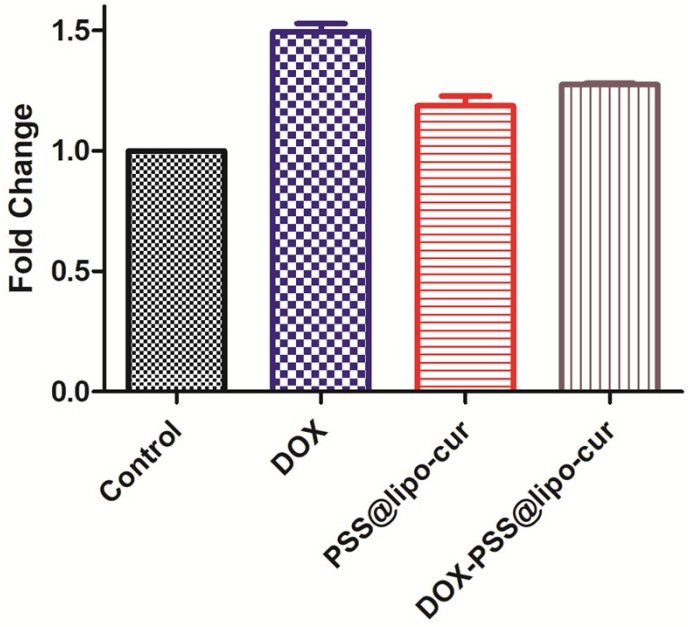


Figure S9. Effect of ROS generation in naoformulation. The perspective nanoformulation is noticed in the graph.

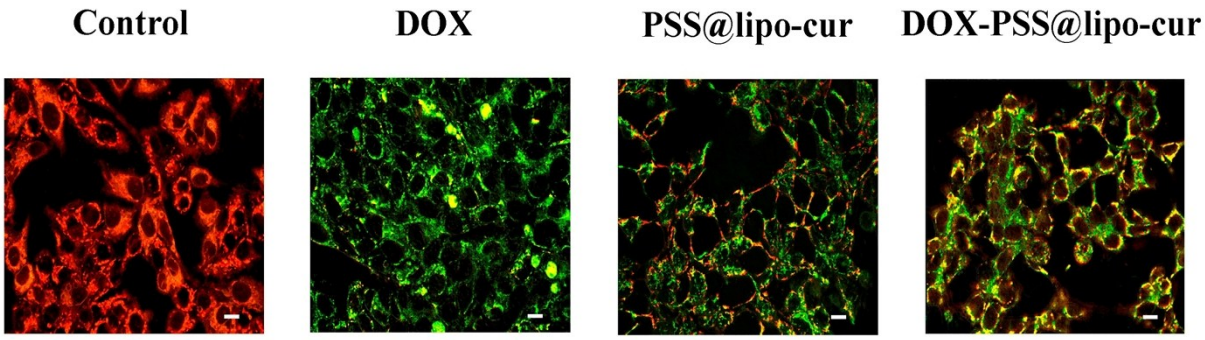


Figure S10. Confocal micrograph of transmembrance JC-1 staining of mitochondria in control and treated DOX, PSS@lipo-cur and DOX-PSS@lipo-cur. Scale bar 10 μm.

Table 1: Time resolved decay profile for the DOX with gradual addition of PSS@lipo-cur.

Concentration of DOX-PSS@lipo-cur (μM)	a_1	τ_1 (ns)	$<\tau>$(ns)	$\chi 2$
0	1	0.0668	1.003	1.268
0.13	1	0.0670	0.997	1.263
0.26	1	0.0651	0.995	1.261
0.4	1	0.0671	1.002	1.216
0.53	1	0.0663	1.001	1.193
0.66	1	0.0667	1.004	1.255
0.79	1	0.0657	1.000	1.225
0.92	1	0.0676	0.999	1.022
1.05	1	0.0670	0.995	1.139
1.18	1	0.0670	0.995	1.139
1.31	1	0.0663	1.003	1.230

1.1: Interaction of DOX-PSS@lipo-cur nanoformulation with different conditions DPBS, cell culture media without and with FBS

The interaction of nanoformulation with different cell culture media is useful to understand the physical and chemical properties of nanocarrier systems, when it's prior contact to the cellular surface [1]. In DPBS the surface charge has phosphate ions and different chemical constituent's which is giving a stable chemical stability. Thus the stable nanoformulation, is monitored through dynamic light scattering and UV-visible spectral studies, the results are shown in figure S5 (a & d) and figure S6. The nanoformulation which was treated with cell culture medium without FBS is found to be moderately stable. The perceptible changes were also observed in the hydrodynamic response as shown in figure S5 (b & e). After the 20^{th} day we have observed complete reduction in surface potential which is due to the aggregates of particles without FBS in the cell culture medium. This is directly evidenced through reduction in absorption intensity as shown in Figure S6 with the corresponding digital images. In the case of culture medium with FBS, which has a number of protein biomolecules, these constituents also have a role in hydrodynamic and zeta potential values. After 30 days observation we found successive increase of surface potential from to -

7.45 mV to -34.5 mV, and the hydrodynamic responses of spectra were changed (figure S5 (c) & f). Thus, one can speculate that the nanoformulation with FBS can be highly stable for a long time. However, the biomolecules that are added may be adsorbed on the surface which could be the reason for reduction in surface potential and increase in hydrodynamic size distribution. These stability measurements consequently strengthen the platform which would improve to understand the dynamics of nanoformulation in the cell culture medium.

1.2. Intracellular Reactive Oxygen Species (ROS) Measurements

ROS is an important factor to understand the apoptosis process during cell death. Here we carried out the experiments on measurement of ROS by 2′,7′ –dichloroflurescein diacetate (DCFH-DA) assay in *in vitro* level [2]. The production of intracellular ROS was measured using the fluorescent probe 2′,7′ –dichloroflurescein diacetate (DCFH-DA). DCFH-DA diffuses through the cell memberane and is enzymatically hydrolyzed by intracellular esterases to form the nonfluorescent compound probe 2′,7′ –dichloroflurescein (DCFH), which is then rapidly oxidized to form the highly fluorescent 2′,7′ –dichloroflurescein (DCF) in the presence of ROS (2).The DCF fluorescence intensity is believed to parallel the amount of ROS formed intracellularly. The cultured MCF-7 cells were treated with the IC_{50} concentration of DOX, PSS@lipo-cur, DOX-PSS@lipo-cur and the untreated cells were maintained as control. After 24 h incubation, the culture medium was replaced with PBS and the cells were treated with 1 µM CM-H2DCFDA (sigma-Aldrich, St.Louis,MO, USA) for 30 min at 37˚C in dark. After washing out the excess probe, the florescence was recorded at 495 nm excitation and 530 nm emission in a fluroskan ascent FL spectrofluorometer.

1.3. Evaluation of Mitochondrial Transmembrane Potential (Δψ)

Mitochondrial membrane potential (Δψ) was evaluated in live cells with the use of lipophilic cationic fluorescent probe JC-1 (5,5’,6,6’-tetrachlo-1,1’,3,3’tetraethylbenzimidazolcarbocyanine.[3] The cells were grown in glass cover slips and treated with the IC_{50} concentration of DOX, PSS@lipo-cur, DOX-PSS@lipo-cur. After 24 h treatment, the integrity of mitochondrial transmembrane was examined by incubating the slides with JC-1 for 30 min in the culture medium. The cover slips were washed with PBS and then mitochondrial depolarization patterns of the cells were examined under the laser scanning confocal microscope (Zeiss LSM710, 100x oil immersion objective). JC-1 is a lipophilic dye that can selectively enter the mitochondria and reversibly changes the color from green to red as the membrane potential increases. Due to the higher mitochondrial

potential in healthy cells, JC-1 form complexes as J-aggregates with intense red color which is matched with control. Also, the cells with apoptotic or unhealthy cells with low mitochondrial potential shows only green fluorescence which is in the monomeric form. The DOX, PSS@lipo-cur, DOX-PSS@lipo-cur treated samples shows depolarization of the mitochondrial membrane, however, DOX has been shown the loss of red fluorescence predominately as shown in figure S10. The loss of mitochondrial membrane potential followed by the release of cytochrome C from mitochondria to the cytosol which leads to subsequent activation of caspases, resulting the apoptosis process.[4] With this understanding we suspect that, disruption of mitochondrial membrane potential in DOX-PSS@lipo-cur nanoformulation triggered apoptosis mediated cell death.

References

1. T. L. Moore, L. Rodriguez-Lorenzo, V. Hirsch, S. Balog, D. Urban, C. Jud, B. Rothen-Rutishauser, M. Lattuada and A. Petri-Fink, *Chemical Society Reviews*, 2015, **44**, 6287-6305.
2. Z.-B. Wang, Y.-Q. Liu and Y.-F. Cui, *Cell biology international*, 2005, **29**, 489-496.
3. M. Mancini, B. O. Anderson, E. Caldwell, M. Sedghinasab, P. B. Paty and D. M. Hockenbery, The Journal of cell biology, 1997, 138, 449-469.
4. M. Bras, B. Queenan and S. Susin, *Biochemistry (Moscow)*, 2005, **70**, 231-239.