Supporting Information

Squalenoylation of Chitosan: a Platform for Drug-Delivery?

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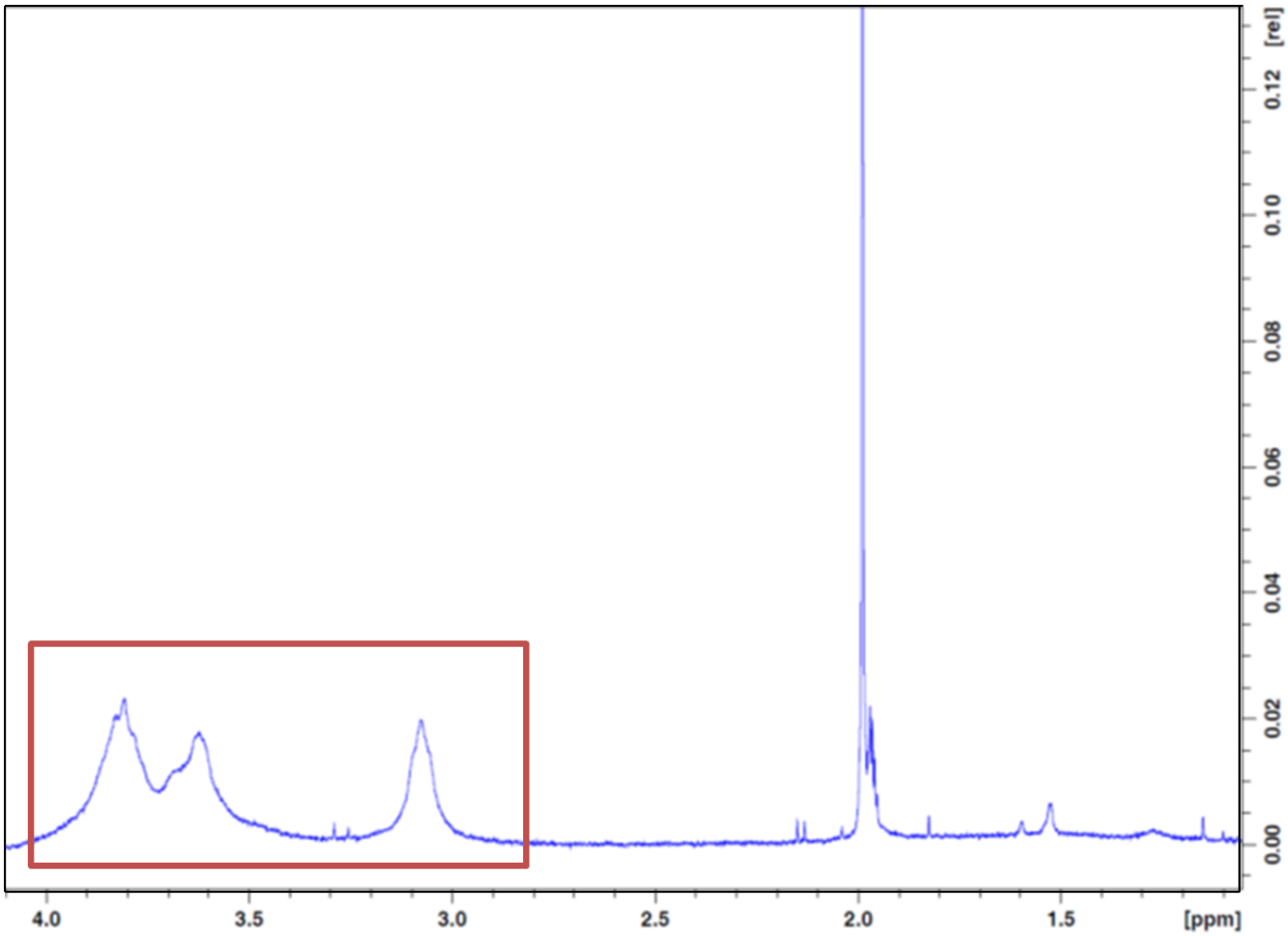

Figure S1. ^{1}H NMR spectra of chitosan-SQ 13:1 in D_2O with 5% (v/v) of acetic acid-d_4: opalescence colloidal suspension was obtained and only the protons from the chitosan can be observed (red square).

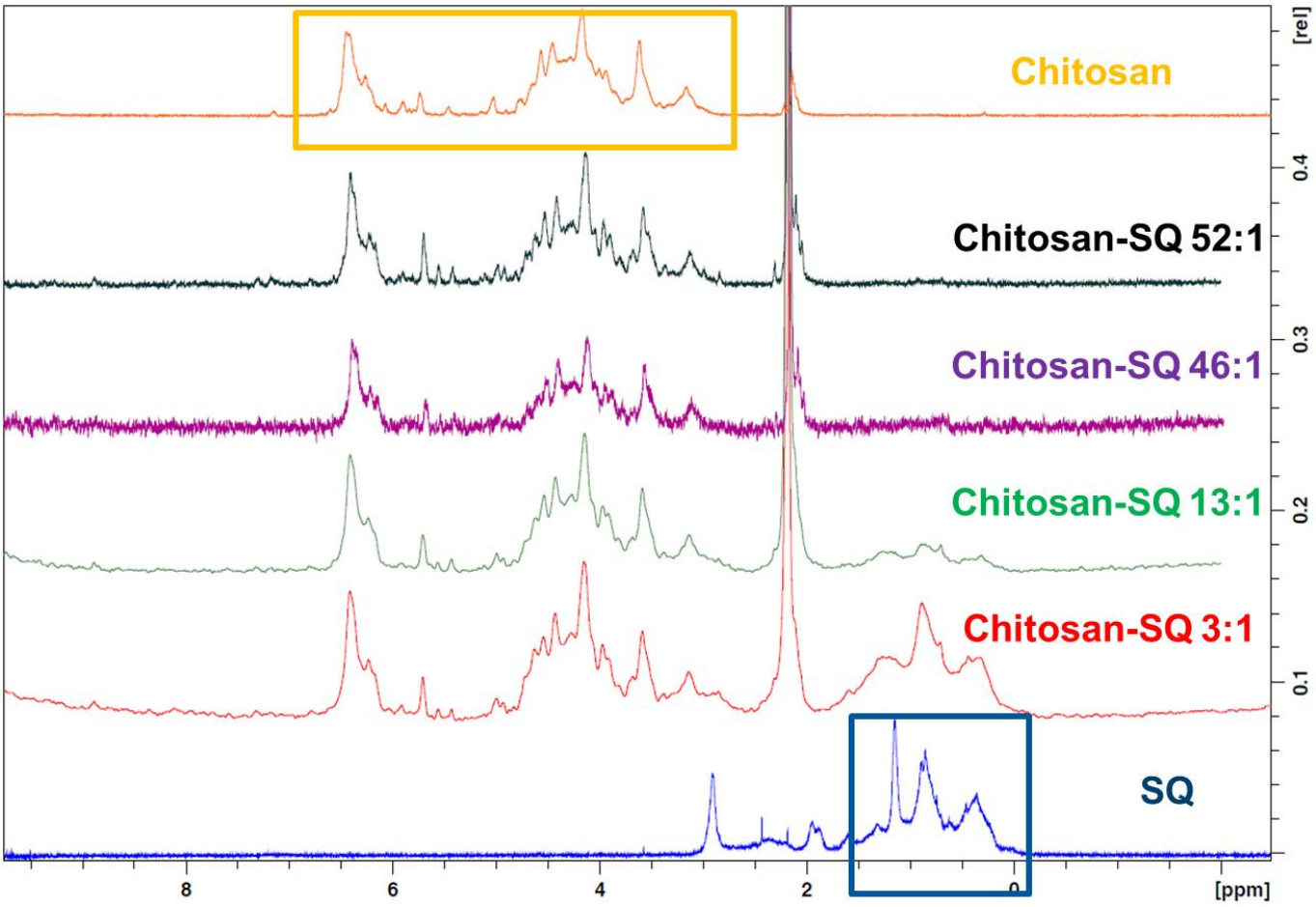

Figure S2. ^{1}H NMR spectra of the different chitosan-SQ derivatives in sulfuric acid-d_2: the compounds are degraded but the protons from the SQ molecule (blue square) and the chitosan (yellow square) can be clearly observed on the chitosan-SQ 3:1 and 13:1 spectra.

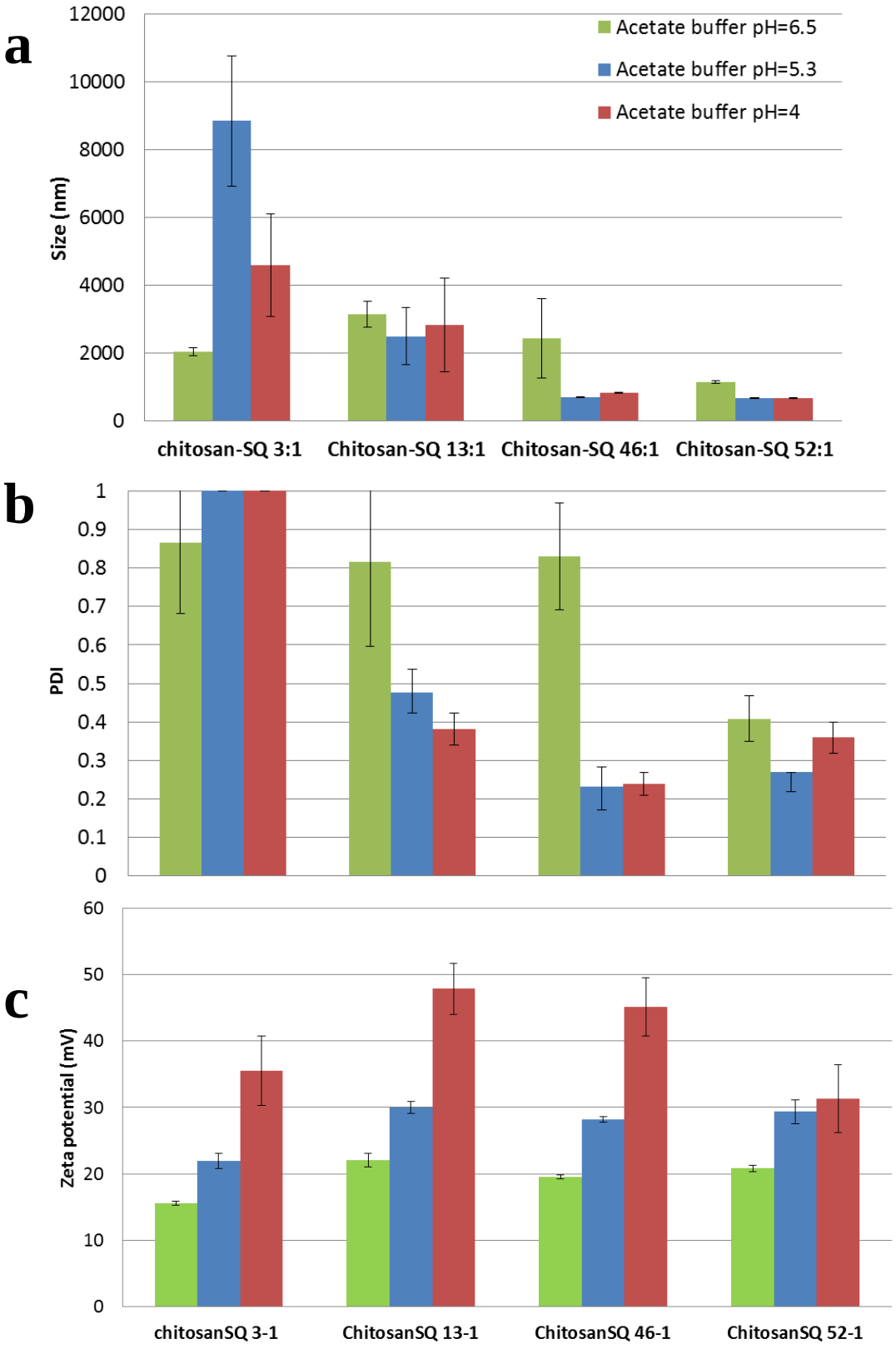

Figure S3. Characteristics of the chitosan-SQ nanoparticles according to the molar ratio of SQ and the buffer used: size (a), PDI (b) and zeta potential (c) of the particles.

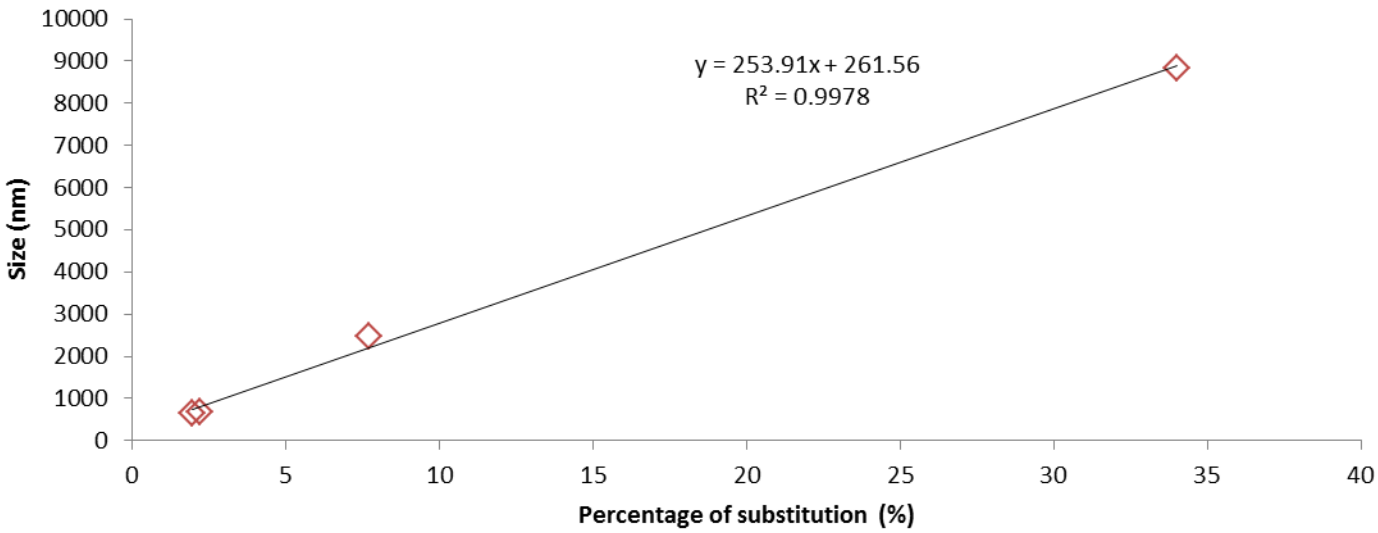

Figure S4. Linear regression analysis showing that the size of the particles is proportional to the degree of substitution with an R^2 value of 0.99.

a

b

c

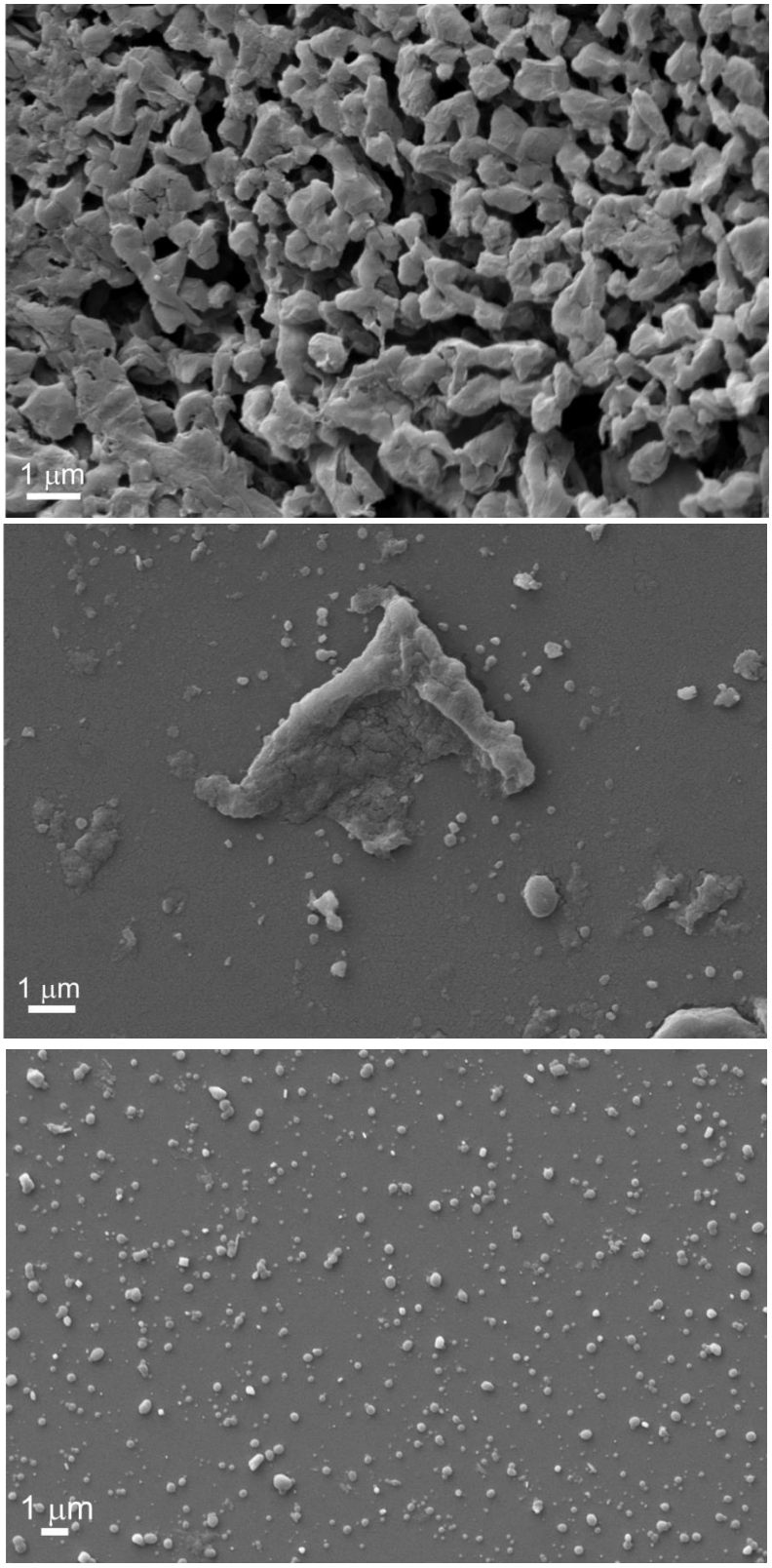

Figure S5. Representative SEM pictures of Chitosan (a), Chitosan-SQ 3:1 (b) and Chitosan-SQ 46:1 in pH = 5.3 acetate buffer (c).

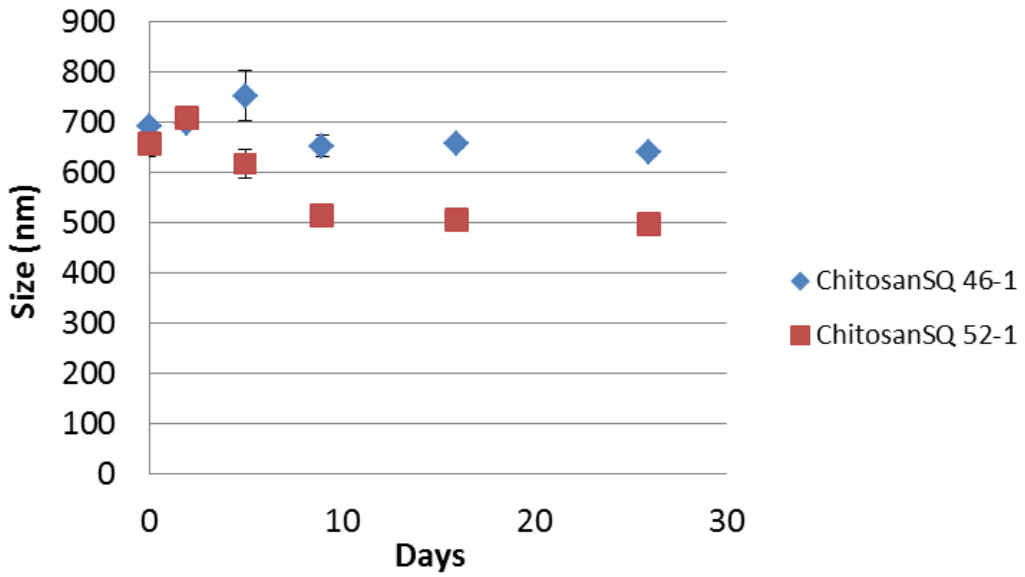

Figure S6. Stability of nanoparticles (~1 × 10^{-3} mol/L) in pH = 5.3 acetate buffer and analyzed at room temperature. The mean diameter of nanoparticles, determined by DLS, is the average of three measurements.

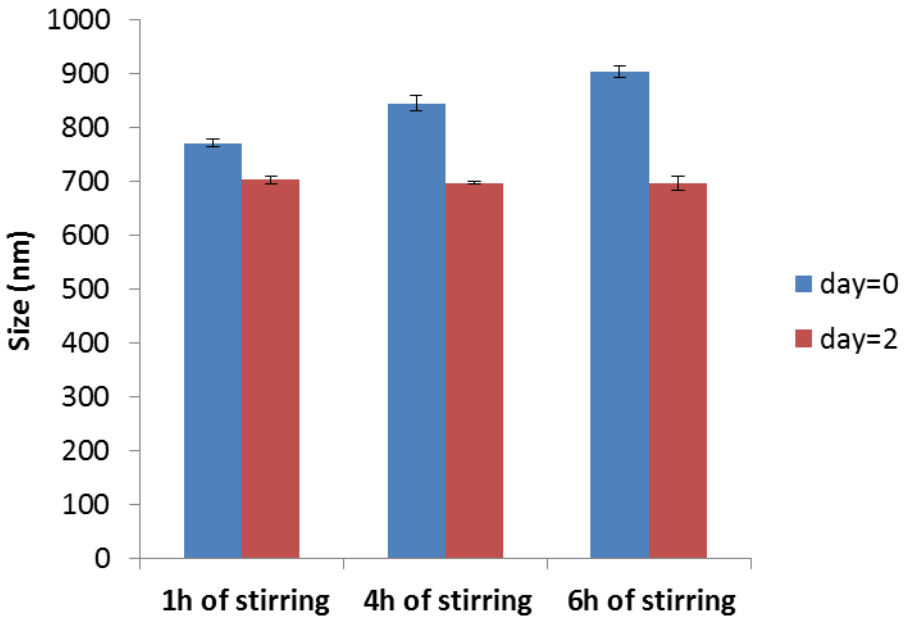

Figure S7. Influence of the stirring time during the preparation of the chitosan-SQ 46:1 particles on the size: after 2 days, whatever the stirring time, the same size is obtained.

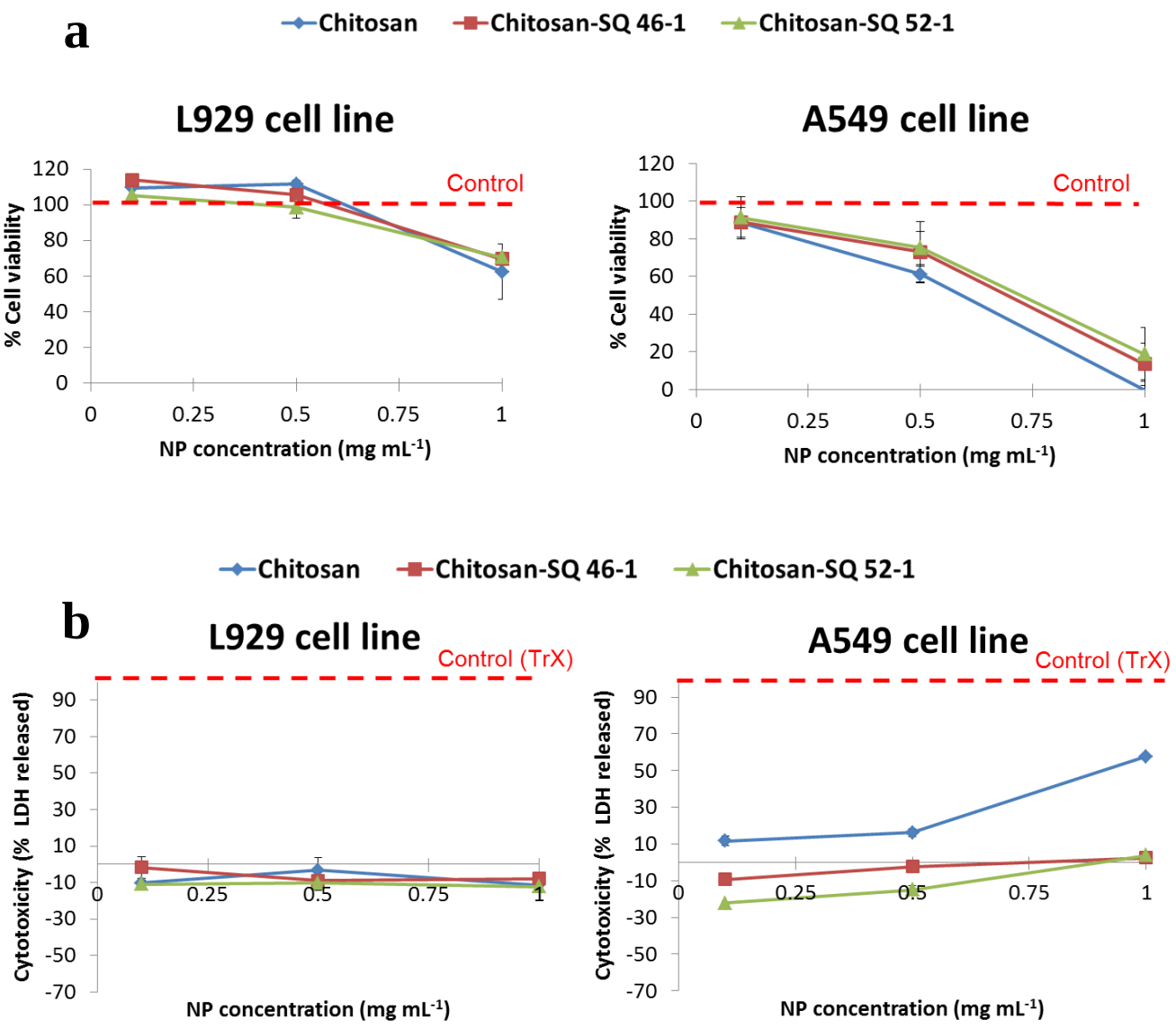

Figure S8. MTT (a) and LDH (b) assays on L929 and A549 cell lines with different concentrations of chitosan, chitosan-SQ 46:1 and 52:1 nanoparticles in pH = 5.3 acetate buffer: the nanoparticles of chitosan-SQ show no severe cytotoxicity compared with the chitosan in solution. The pH = 5.3 acetate buffer was considered as the 100 % of cell viability (same values as for HBSS buffer) and the Triton X-100 (TrX) solution as the 100 % of cytotoxicity.

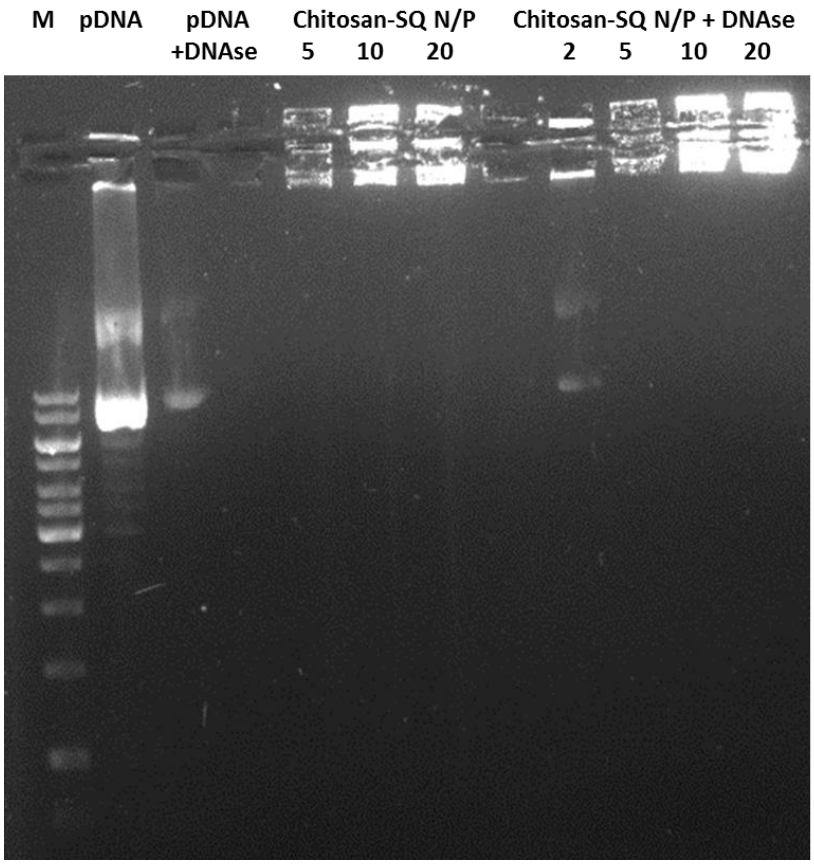

Figure S9. Gel electrophoresis of chitosan-SQ 52:1/pDNA polyplexes: N/P = 2, 5, 10 and 20. In presence of DNAse, no degradation can be observed with N/P = 10 and 20, compared with the control, pDNA+DNAse. M: molecular weight markers.