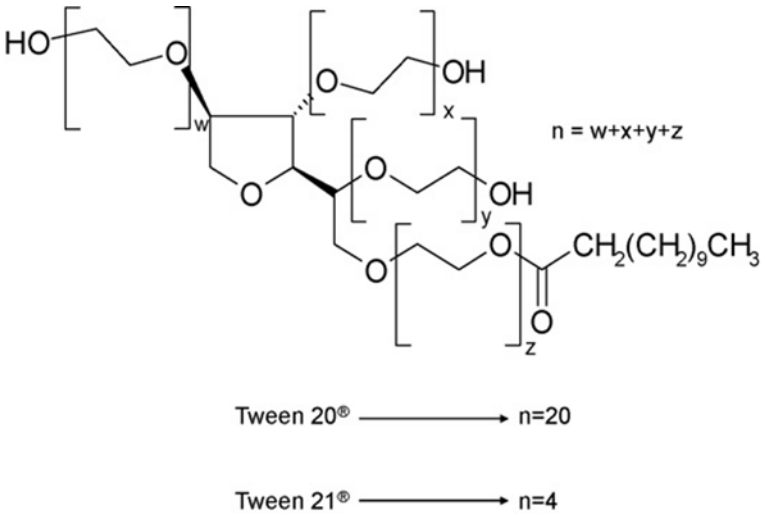
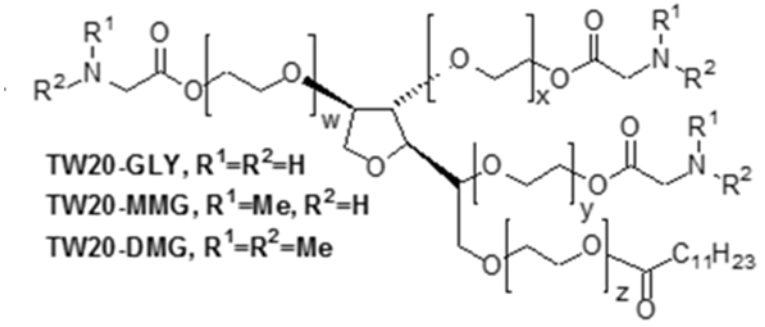
Supporting Information

Niosomes as Drug Nanovectors: Multiscale pH-dependent Structural Response

C. Marianecci[1,#], L. Di Marzio[2,#], E. Del Favero[3], L. Cantù[3], P. Brocca[3], F. Rinaldi[4], L. Dini[5], A. Serra[6], P. Decuzzi[7,8,9], C. Celia[2,10], D. Paolino[11,12], M Fresta[11,12], M. Carafa[1,*]



a



b

Figure S1. Chemical structures of Tweens: (a) chemical structure of Tween20 and Tween21; (b) chemical structure of Tween20 derivatives. Surfactants were synthesized by conjugating glycine derivatives to Tween20 as reported in Materials and Methods section of main text.

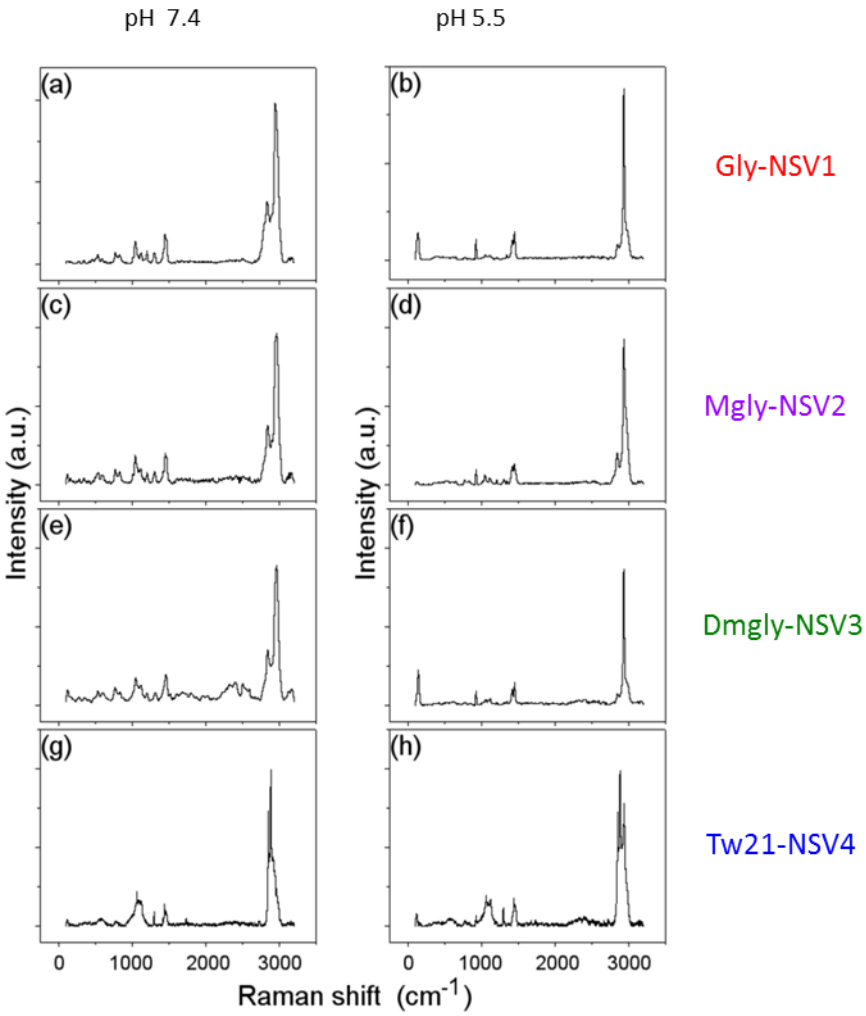


Figure S2. Survey Raman spectra of niosomes: (a) Gly-NSV1, (c) Mgly-NSV2, (e) Dmgly-NSV3 and (g) Tw21-NSV4 at pH 7.4; while picture b, d, f, and h are obtained from samples (b) Gly-NSV1, (d) Mgly-NSV2, (f) Dmgly-NSV3 and (h) Tw21-NSV4 at pH 5.5.

Table S1. Intensity Raman ratios I_{2890}/I_{2850} for the C-H stretching vibrational bands and I_{1125}/I_{1050} for the C-C stretching vibrational bands of niosomes obtained at different pHs. The analysis was carried out at room temperature.

	C-H stretching - I_{2890}/I_{2850}		**C-C stretching - I_{1125}/I_{1050}**	
Sample	**pH 7.4**	**pH 5.5**	**pH 7.4**	**pH 5.5**
Gly-NSV1	0.79	1.11	0.45	0.82
Mgly-NSV2	0.75	0.87	0.55	0.77
Dmgly-NSV3	0.74	1.11	0.77	1.13
Tw21-NSV4	1.33	1.35	0.82	0.76

Table S2. Diffraction peaks positions and corresponding distances for cholesterol crystallites obtained from SAXS and WAXS experiments for niosomes. Results represent data for formulations #1, #4, #5, #6).

Peak position (nm^{-1})	Distance (nm)
1.84	3.41
3.7	1.70
4.42	1.42
4.63	1.35
5.02	1.25
5.45	1.15
9.96	0.63
10.85	0.58
12	0.52
12.29	0.51
12.82	0.49

The diffraction peak at 1.84 nm^{-1} corresponds to a specific distance of 3.41 nm and represents the cholesterol crystallites dispersed in surfactants/cholesterol mixtures. This result allows to resolve cholesterol mixed into noisome bilayer as crystallites both at anhydrous or monohydrate forms. A better resolution of both anhydrous and monohydrate cholesterol can be carried out based on packing of surfactants and distances between lipids and cholesterol. The resultant data shows several peaks corresponding to different formulations. The data provided similar results for wide-angle region of the spectrum at $q \leq 30$ nm^{-1}. Results demonstrate that crystallites are made from cholesterol at anhydrous form with the following parameters: a = 1.05 nm, b = 1.42 nm, c = 3.41 nm.[1]

REFERENCES

(1) Shieh, H. S.; Hoard, L. G.; Nordman, C. E. Crystal structure of anhydrous cholesterol. *Nature* **1977**, *267*, 287-289.