

Antigenic and physicochemical characterization of Hepatitis B surface protein under extreme temperature and pH conditions

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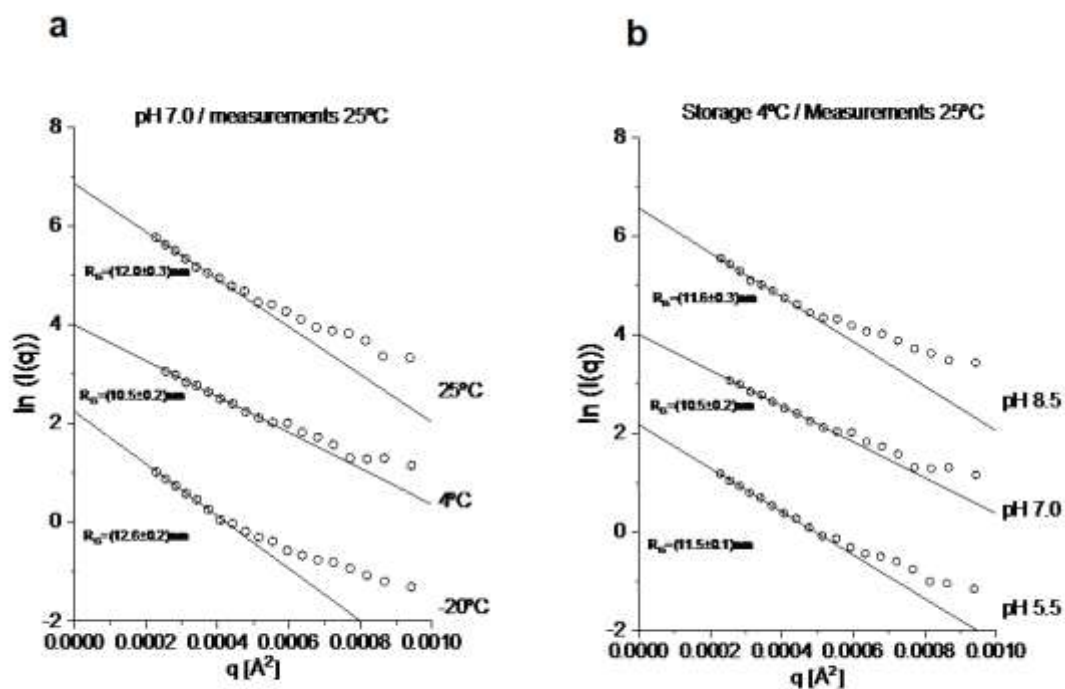
SUPPLEMENTARY MATERIAL

Supplementary Table 1: Volume fractions and number fractions calculated from the size distributions obtained from the fitting of DLS data.

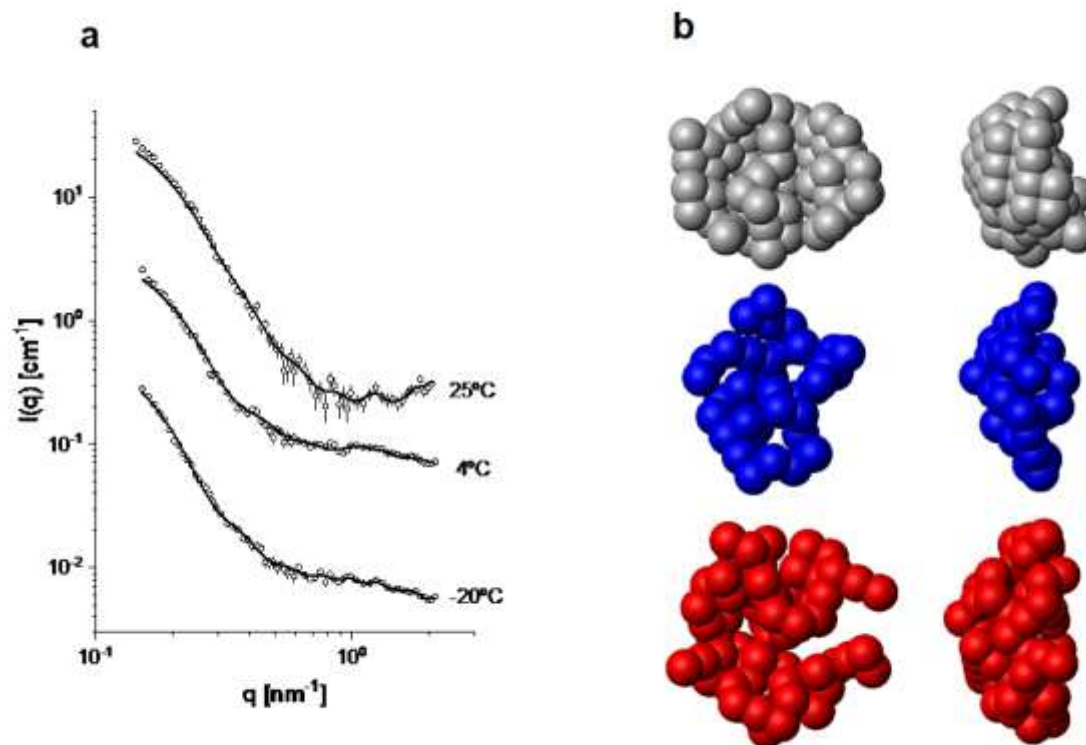
pH	Storage [°C]	Volume		Number*	
		Mon. VLP [%]	Aggreg. VLP [%]	Mon. VLP [%]	Aggreg. VLP [%]
5.5	-20.0	69.6	30.4	> 99.9	< 0.1
	4.0	91.5	8.5	> 99.9	< 0.1
	20.0	96.7	3.3	> 99.9	< 0.1
7.0	-20.0	76.3	23.7	> 99.8	< 0.2
	4.0	90.2	9.8	> 99.9	< 0.1
	20.0	92.5	7.5	> 99.9	< 0.1
8.5	-20.0	72.1	27.9	> 99.8	< 0.2
	4.0	92.8	7.2	> 99.9	< 0.1
	20.0	96.7	3.3	> 99.9	< 0.1

* The number fractions were estimated from the volume fractions values.

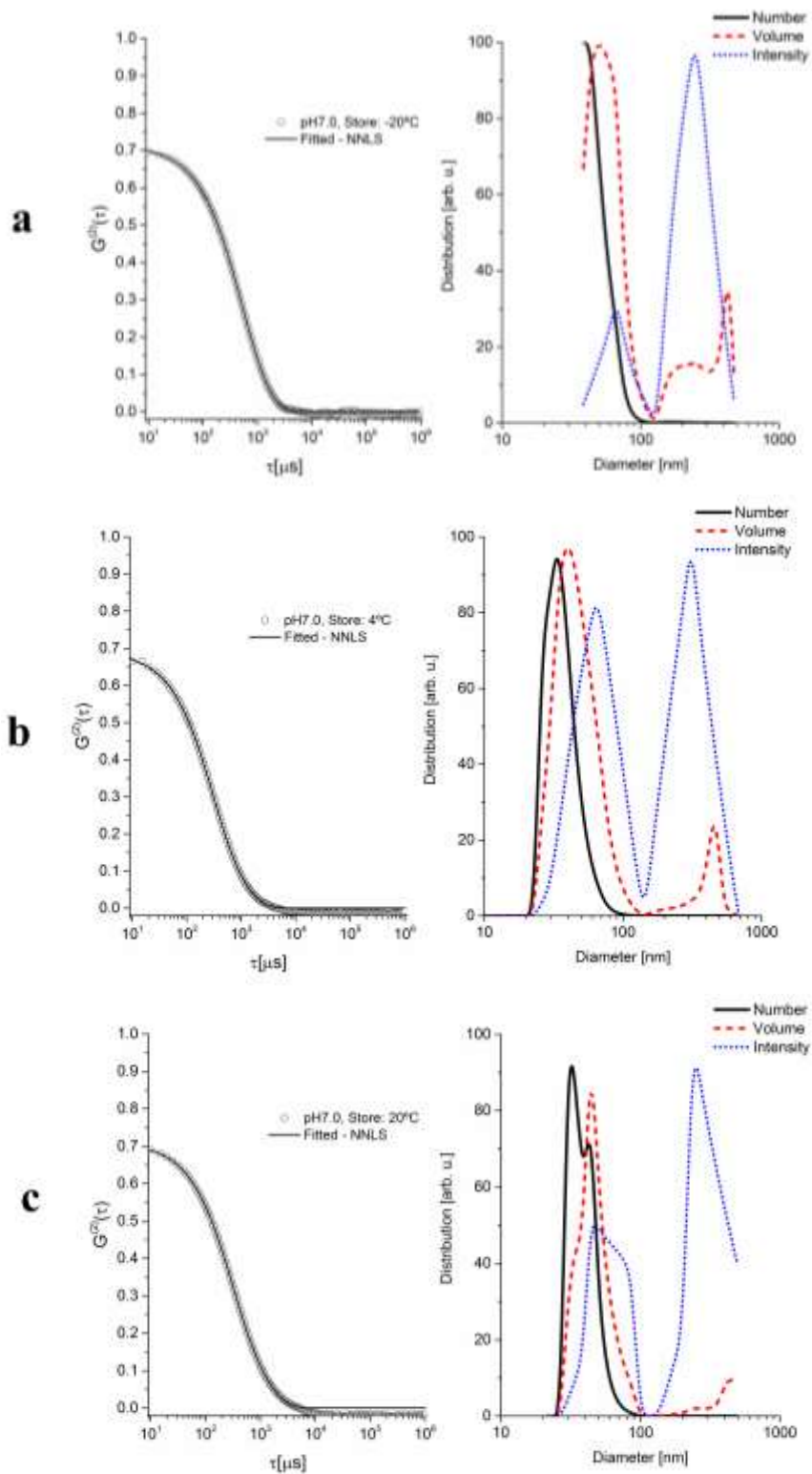
Supplementary Figure 1. Guinier plots for the investigated samples. The curves were shifted for an easier visualization.



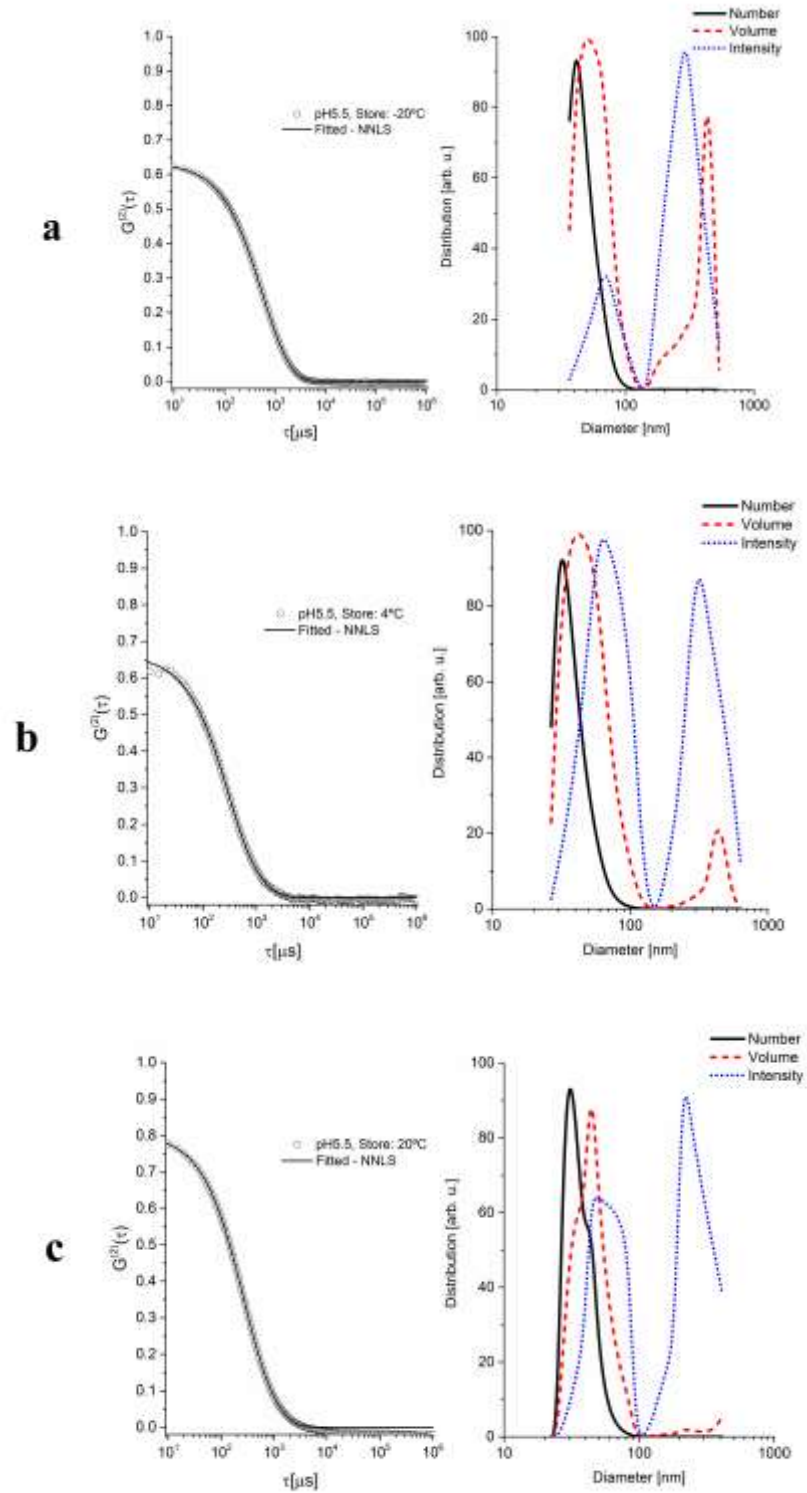
Supplementary Figure 2. *Ab initio* modeling for the samples at pH 7.0. (a) SAXS curves (symbols) and fitting from *ab initio* modeling (solid lines). (b) *Ab initio* models calculated from the SAXS data in two views, rotated by 90 degrees. The radius of the spherical subunit is 2 nm and represents the overall size of HBsAg protein monomer.



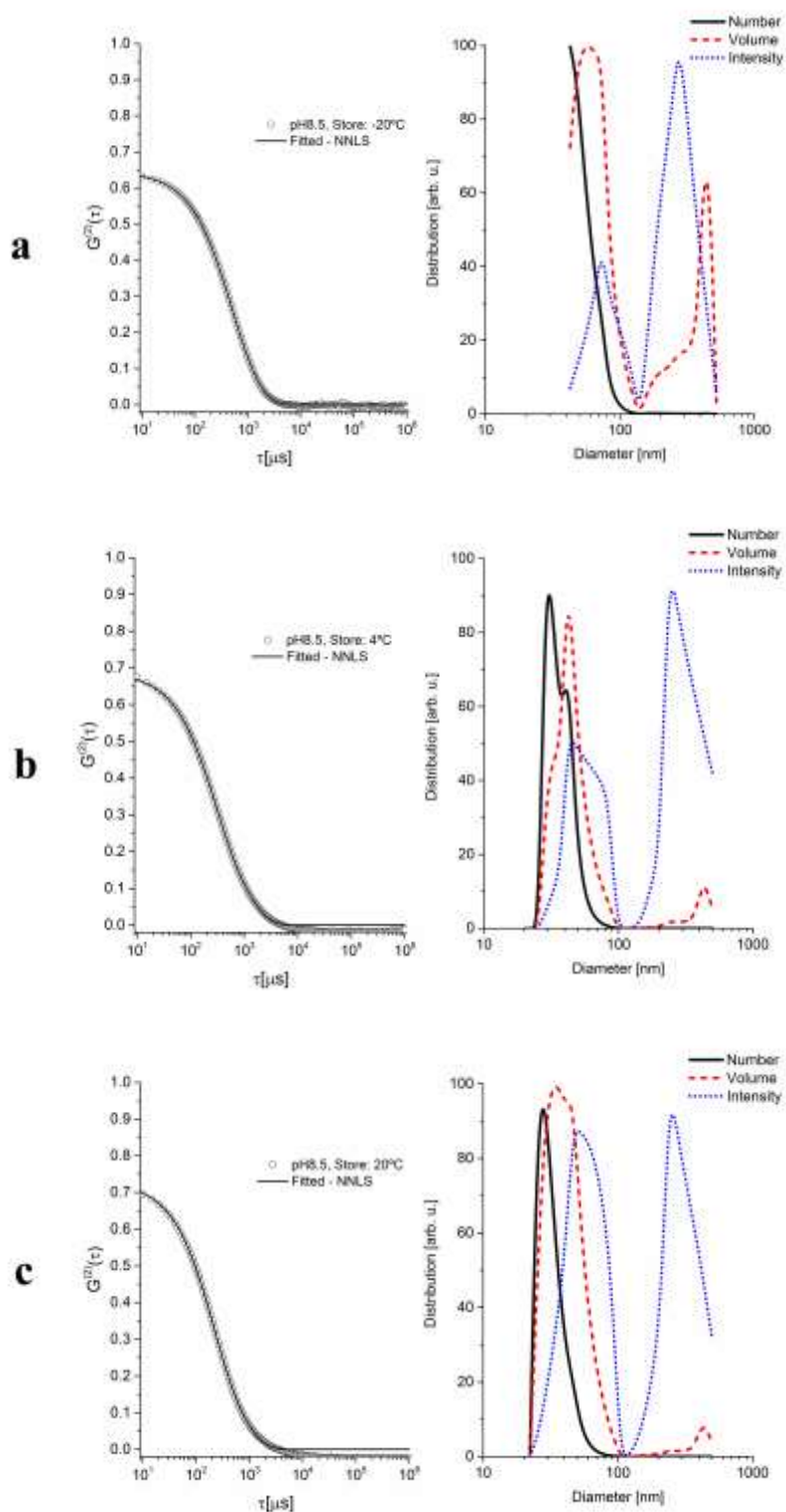
Supplementary Figure 3 - DLS results for HBsAg samples prepared at pH 7.0 and stored at a) -20°C, b) 4°C, and c) 20°C. In these plots on the left hand side it is shown the correlation curve (symbols) together with NNLS fit (lines). On the right hand side the obtained size distributions weighted by number, volume and intensity are shown.



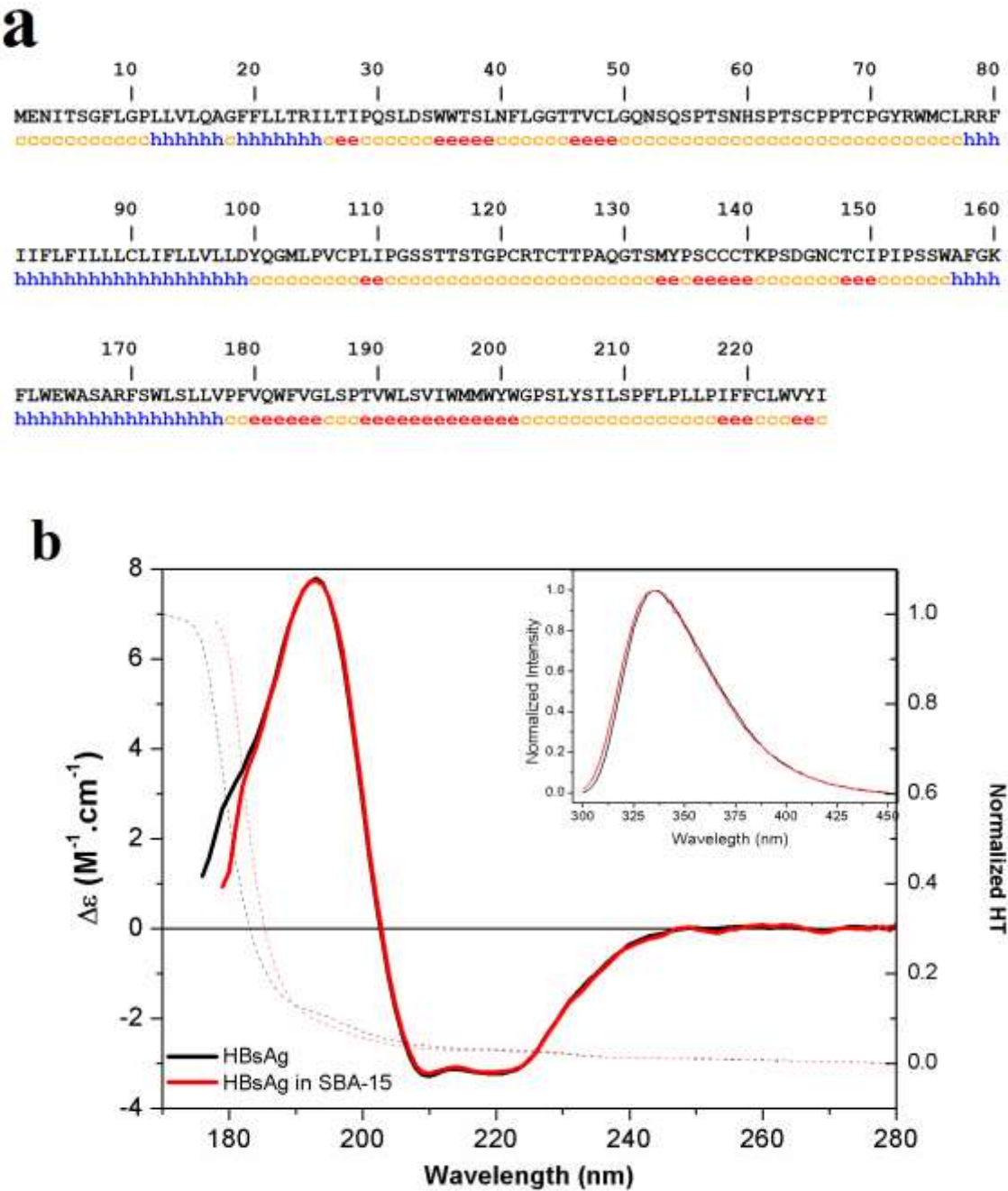
Supplementary Figure 4 - DLS results for HBsAg samples prepared at a) pH 5.5 and stored at -20°C, b) 4°C, and c) 20°C. In these plots on the left hand side it is shown the correlation curve (symbols) together with NNLS fit (lines). On the right hand side the obtained size distributions weighted by number, volume and intensity are shown.



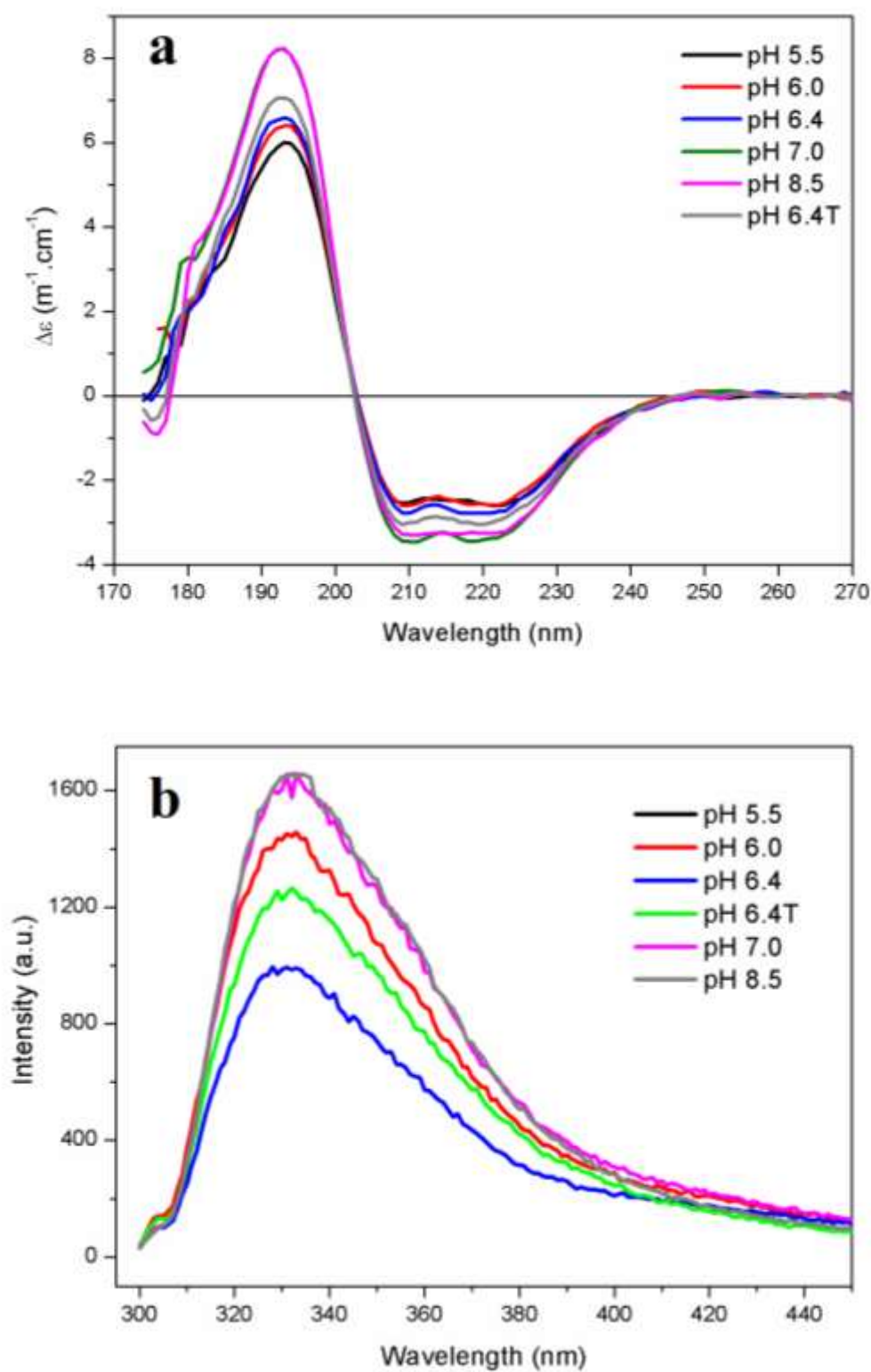
Supplementary Figure 5 - DLS results for HBsAg samples prepared at pH 8.5 and stored at a) -20°C, b) 4°C, and c) 20°C. In these plots on the left hand side it is shown the correlation curve (symbols) together with NNLS fit (lines). On the right hand side the obtained size distributions weighted by number, volume and intensity are shown.



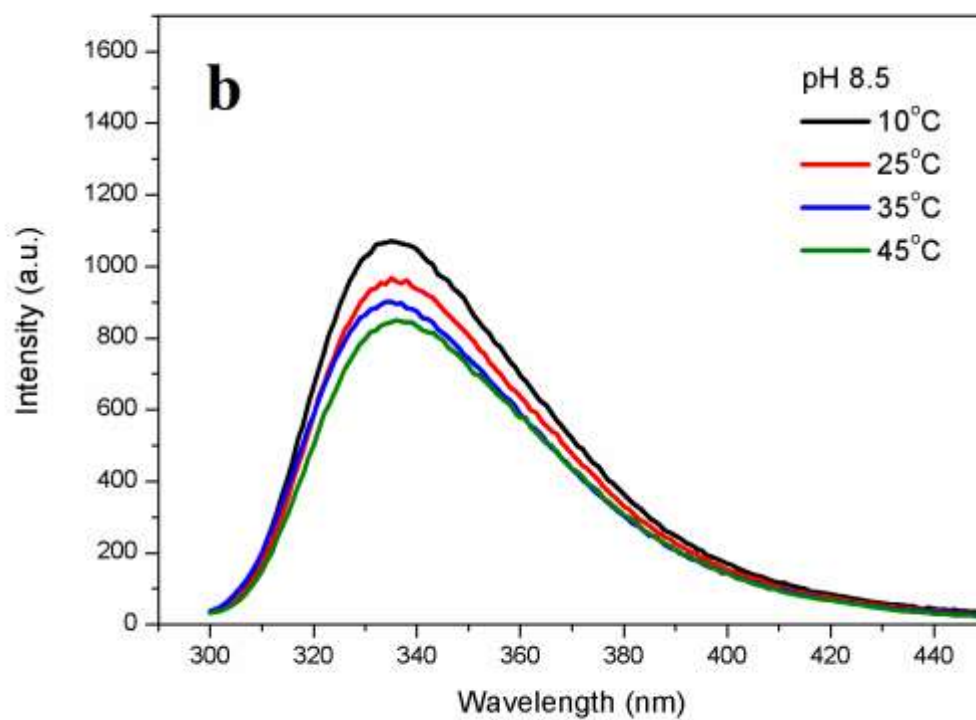
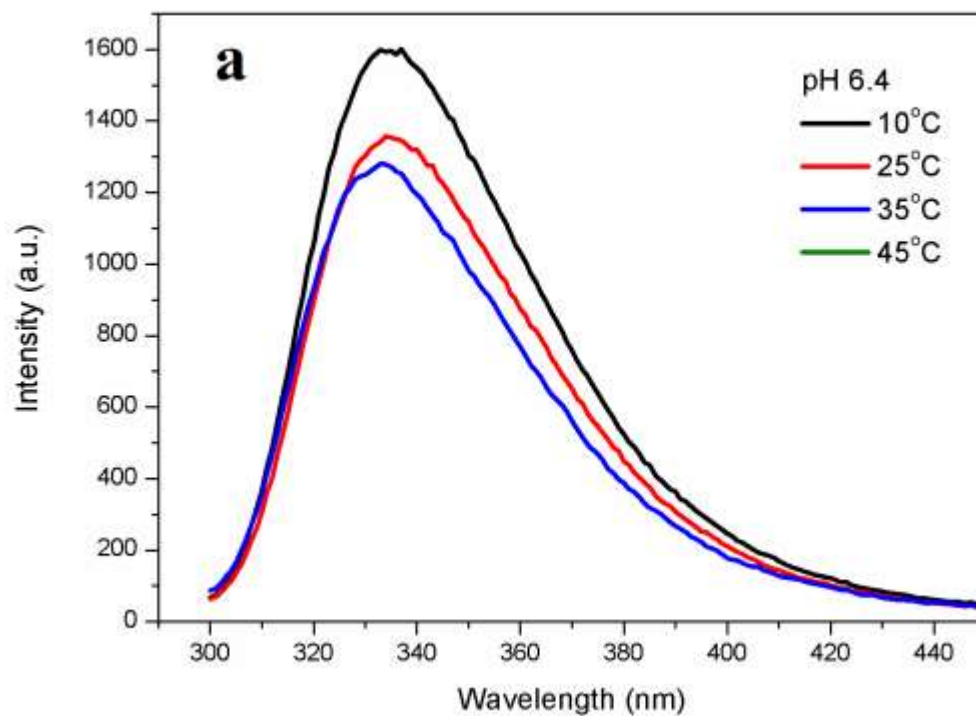
Supplementary Figure 6 – a) Secondary structure predictions of HBsAg (h to alpha-helix, c to coil, and e to extended beta-strand. b) SRCD spectra of HBsAg at pH 7.0 (black) and incorporated into SBA-15 particles (red). Inset: their respective Trp emission spectra. HT curves are shown in dot.



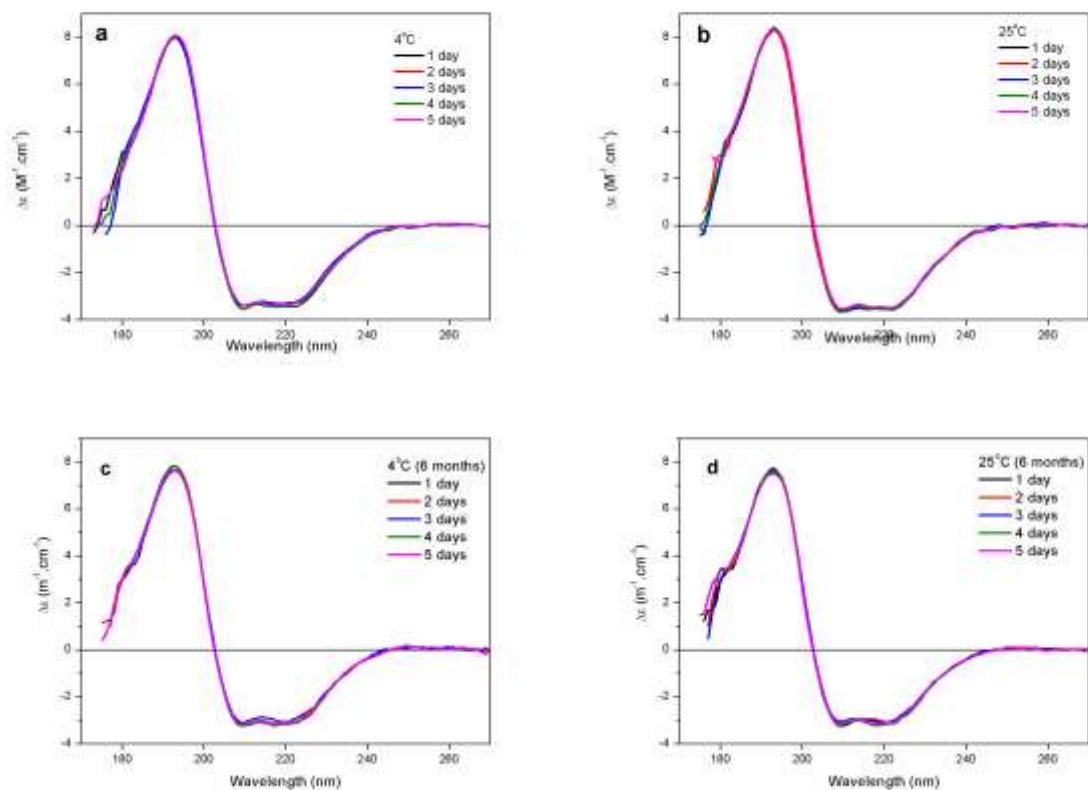
Supplementary Figure 7 – a) Effect of pH on the secondary structure of HBsAg after 6 months storage and b) their respective Trp emission spectra during pH assay.



Supplementary Figure 8 – a) Effect of temperature on the Trp emission spectra of HBsAg incubated at pH 6.4 or b) at pH 8.5.



Supplementary Figure 9 – a) SRCD spectra of HBsAg (pH 7.0) taken when sample was kept at 4°C or b) at 25°C for five successive days, and after 6 months storage (c,d), respectively.



Supplementary Figure 10 - SDS-PAGE of HBsAg sample in denaturant conditions (AgNO_3 stained) - lane 1; LMW- lane2; 2 μg of HBsAg (in PBS - pH 7.0) at 4°C – lane 3; 2 μg of HBsAg (in PBS - pH 7.0) heated at 100°C for 7 days – lane 4; 2 μg of HBsAg (in PBS - pH 7.0) heated at 60°C for 7 days.

