Electronic Supplementary Material (ESI) for Soft Matter.

SUPPORTING INFORMATION

Controlled formation of chitosan particles by a clock reaction

Guido Panzarasa[1*], Alina Osypova[2], Alba Sicher[1,3], Arie Bruinink[4] and Eric R. Dufresne[1]

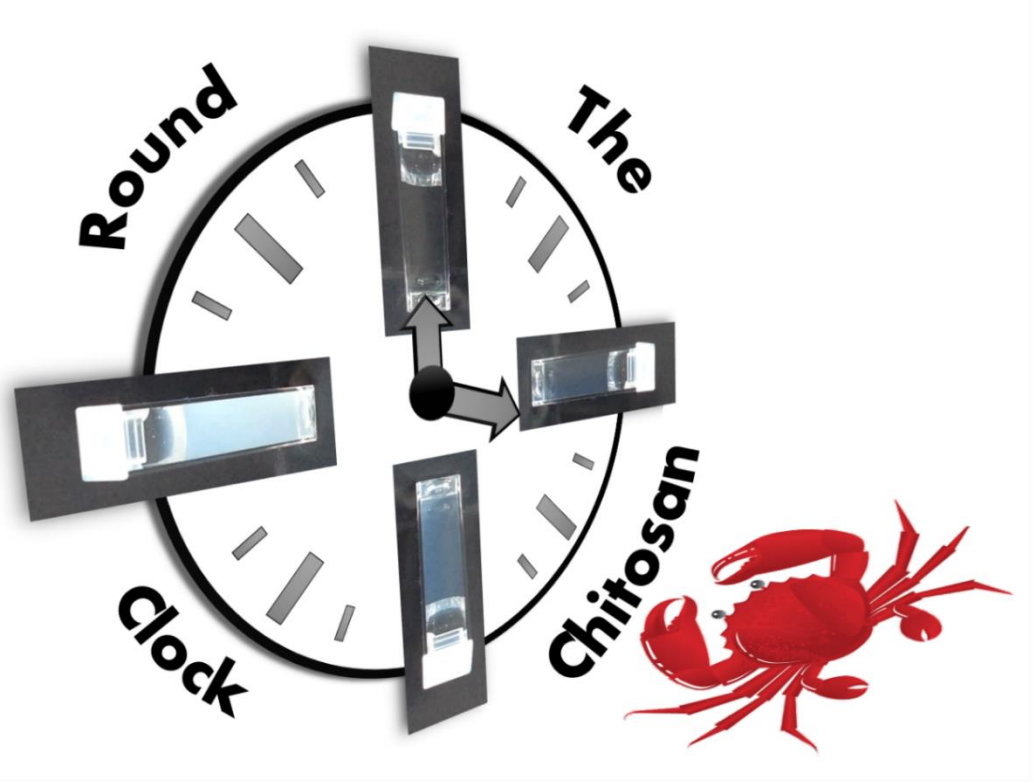
[1]Laboratory for Soft and Living Materials, Department of Materials, ETH Zürich, Vladimir-Prelog-Weg 5, 8093 Zürich, Switzerland.

[2]Innovative Sensor Technology, IST AG Stegrütistrasse 14, 9462 Ebnat-Kappel, Switzerland.

[3]Empa, Swiss Federal Laboratories for Materials Science and Technology, Laboratory for Biomimetic Membranes and Textiles, CH-9014 St. Gallen, Switzerland

[4]Empa, Swiss Federal Laboratories for Materials Science and Technology, Laboratory for Joining Technologies and Corrosion, CH-8600 Dübendorf, Switzerland.

*Corresponding author. E-mail: gp4779@gmail.com ; guidop@ethz.ch



EXPERIMENTAL

Materials and methods

Chitosan with different degrees of deacetylation (DDA) and nominal viscosity values were purchased from Heppe Medical Chitosan GmbH (Halle, Germany) and used as received. The complete description of the chitosan types employed are summarized in Table S1. Sodium sulfite (anhydrous) and sodium bisulfite (anhydrous) were purchased from Merck (Switzerland). Formaldehyde solution (for molecular biology, 36.5-38% in water, ca. 13 M in CH_2O, with 10-15% methanol as stabilizer), sodium hydroxide, glacial acetic acid and D-(+)-glucosamine hydrochloride were purchased from Sigma-Aldrich (Switzerland). Unless otherwise stated, all chemicals were of analytical or reagent grade purity and used as received. Purified water obtained from a MilliQ purification system (resistivity ≥ 18 MΩ) was thoroughly used. All the experiments were carried out at room temperature (22°C).

Stock solutions of 0.2% m/v chitosan were prepared by dissolving 0.1 g of solid in 5 mL of 1% v/v acetic acid under stirring. The solution was diluted with 40 mL of water and the pH was adjusted, with the aid of a pH-meter, by small additions of NaOH 0.5 M until a stable pH = 5.10 ± 0.05 was obtained. The volume was adjusted with water to a final volume 50 mL. The resulting solution could be stored at room temperature for at least one week. A sulfite-bisulfite stock solution was prepared by dissolving 0.126 g of anhydrous sodium sulfite Na_2SO_3 and 1.04 g of anhydrous sodium bisulfite $NaHSO_3$ in 100 mL of water, previously degassed by bubbling nitrogen for at least 15 min to avoid oxidation. This solution contains 0.01 M sulfite and 0.1 M bisulfite. To ensure reproducibility of the results, this solution should be used within two hours from its preparation.

Measurement of pH

For routine pH measurements, a glass electrode-equipped pH meter (Orion Star A111, Thermo Scientific) was used, while for the determination of pH kinetics, a glass electrode (Vuille pH Elektroden AG, Switzerland) interfaced with a home-built Arduino datalogger system was used, allowing to perform measurements with a <0.01 pH unit precision. Both electrodes were calibrated with standard buffer solutions (pH 4.01, 7.01 and 10.01) just before use and thoroughly washed with water before and after each set of measurements.

Turbidimetry

Turbidimetry measurements were performed in standard single-use, 1 cm-optical path, 5 mL-volume poly(styrene) cuvettes (dimensions: 10 x 10 x 45 mm; Sarstedt AG & Co., Germany) by

means of a UV-vis spectrophotometer (Agilent). The extinction at 600 nm was measured each 0.05 min over a selected timeframe (usually between 5 and 15 min). The background was recorded before each analysis by measuring the absorbance of the solution at 600 nm before the start of the reaction. For a typical turbidimetry experiment: 1.5 mL of chitosan solution and 1.35 mL of sulfite-bisulfite solution were mixed in a cuvette. Water was then added in the required amount to have a final volume of 3 mL (see Table S2 for details). The closed cuvette containing the solution was inverted several times to mix the contents and the background was recorded. A selected amount of formaldehyde (Table S2) was then added to the cuvette. The cuvette was immediately closed, quickly inverted for a few times to ensure proper mixing and the measurement was started. The time required for this operation was estimated to be around 4 s.

Dynamic light scattering and zeta potential analysis

The samples obtained from the turbidimetry experiments were analyzed immediately after, by means of dynamic light scattering (DLS). Prior to the analysis, the samples were diluted 20-fold with water. Dynamic light scattering and zeta potential analyses were performed using a Malvern Zetasizer Nano ZS-90 (Malvern Instruments Ltd., UK). DLS measurements were performed in single-use poly(styrene) cuvettes (dimensions: 12.5 x 12.5 x 45 mm; Sarstedt AG & Co., Germany) while, for zeta potential, disposable folded capillary cells (Malvern Instruments Ltd., UK) were used. Five sets of measurements were performed and the results averaged. No additional electrolytes were required to perform zeta potential analyses. Data were fitted automatically by the instrument using the Smoluchowsky model, with refractive index RI = 1.45 and absorption A = 0.001.

Attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR)

To prepare the samples for ATR-FTIR measurements, the suspensions were centrifuged (8000 rpm, 30 min) then the pellets were washed two times with water and lyophilized. The analyses were performed using a Nicolet iN10 (Thermo Fisher Scientific) with a diamond optical window, scanning the spectral range 4000–600 cm^{-1} with a resolution of 4 cm^{-1}. Background (100 scans) was collected before each analysis (200 scans).

Scanning electron microscopy (SEM)

Scanning electron microscopy was performed with a Hitachi SU5000 FE-SEM. To prepare the samples, a thin layer of concentrated aqueous particle suspension was pipetted on acid piranha-cleaned, superhydrophilic silicon substrates followed by rapid drying with compressed air. The samples were mounted on carbon tape and sputtered-coated with 4 nm Au-Pd alloy to make them conductive.

Particle isolation and purification

The obtained particles could be isolated by centrifugation and redispersion in water. All suspensions were diluted five-fold with water before centrifugation. Particles obtained with 0.5 M and 0.25 M CH_2O were harvested by centrifugation at 13000 rpm for 10 min, while for those obtained with 0.125 M CH_2O centrifugation at 6000 rpm for 5 min was enough. The particles obtained with 0.0625 M CH_2O could be centrifuged at 4200 rpm for 10 min. Redispersion in water was aided by means of gentle sonication and vortexing.

Table S1. Description of the different chitosan types selected for the experiments.

Chitosan	*Nominal DDA*[a] *(%)*	*DDA range*[a] *(%)*	*Nominal viscosity*[a] *(mPa·s)*	*Viscosity range (mPa·s)*
75/100	75	72.6 - 77.5	100	71 - 150
95/100	95	≥ 92.6	100	71 - 150
85/50	85	82.6 - 87.5	50	31 - 70
85/100			100	71 - 150
85/200			200	151 - 350
85/1000			1000	751 - 1250

[a]Values according to the producer.

Table S2. Conditions used for a typical turbidimetry experiment.

Final formaldehyde concentration (M)	*Chitosan solution (mL)*	*Sulfite-bisulfite solution (mL)*	*Formaldehyde solution (mL)*	*Water (mL)*	*Final volume (mL)*
0.5	1.5	1.35	0.115	0.035	3
0.25			0.058	0.092	
0.125			0.030	0.120	
0.0625			0.014	0.136	

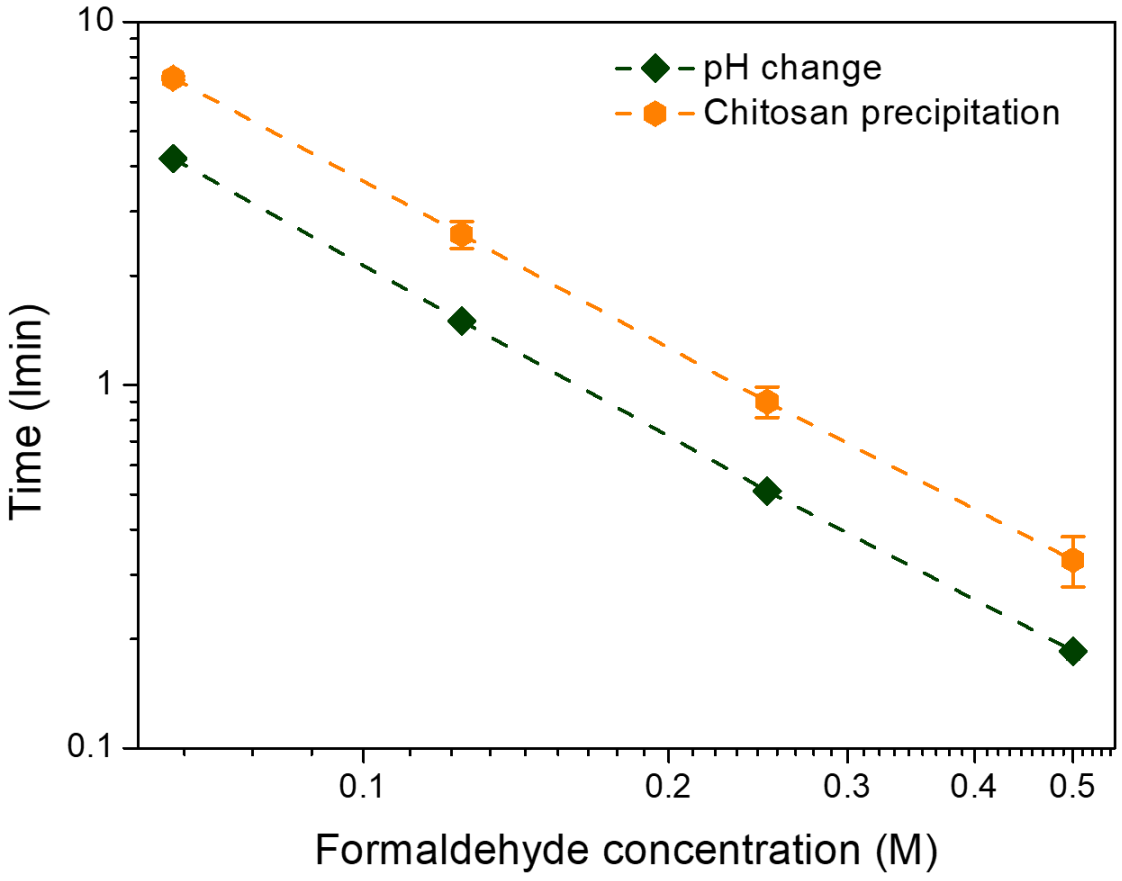


Figure S1. Correlation between the time of pH change (for the formaldehyde clock) and the time of chitosan precipitation (for the formaldehyde clock in presence of 0.1% m/v chitosan). Data points and error bars represent the average and the standard deviation values obtained from three independent sets of experiments. The error bars for the time of pH change data points are smaller than the symbols.

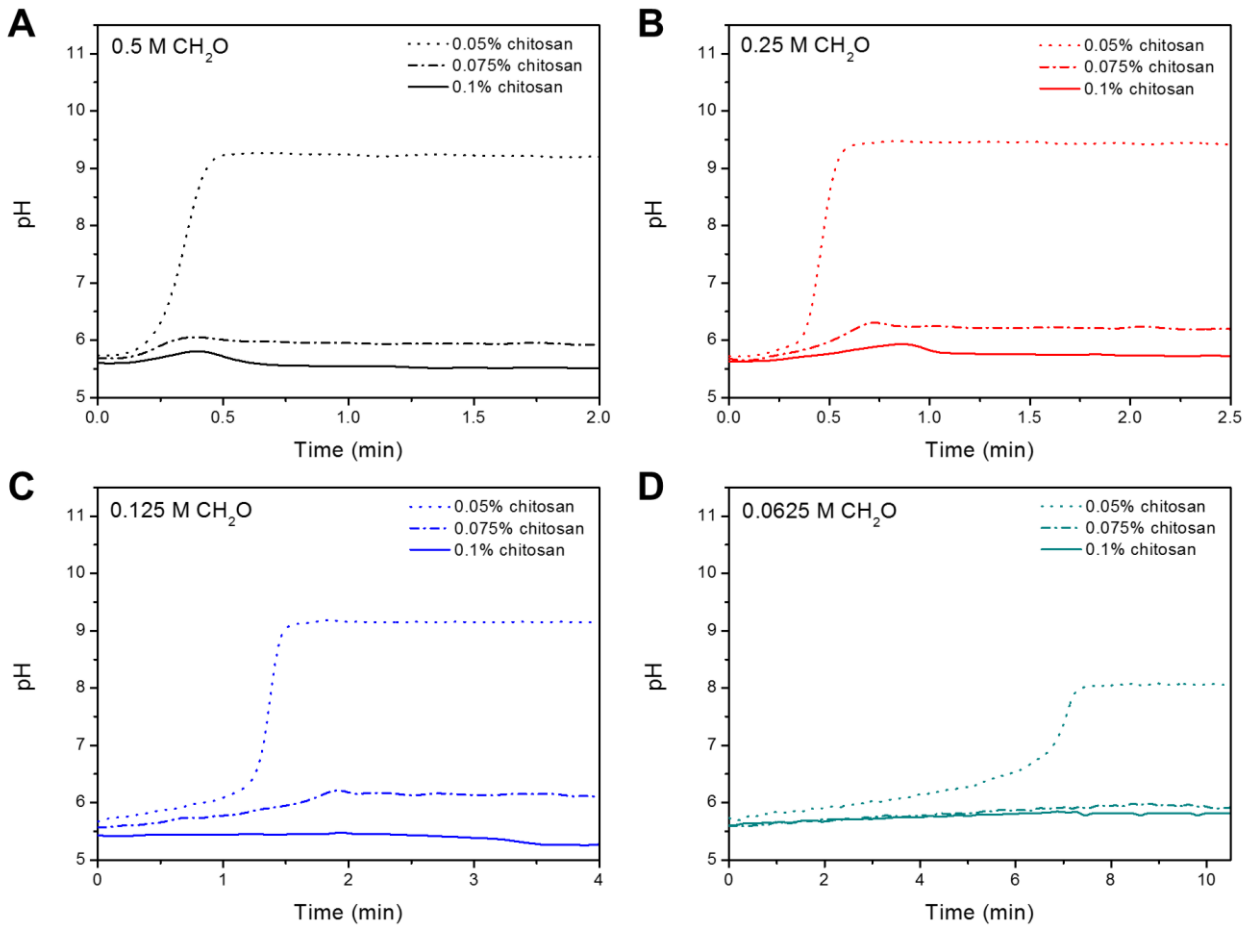


Figure S2. Effect of different chitosan concentrations on the pH evolution of the formaldehyde clock. For chitosan concentrations ≥ 0.075%, a transient increase in pH followed by a decrease can be observed, especially for the higher formaldehyde concentrations *i.e.* for the lower induction times. This behavior could be explained by an inability of chitosan to scavenge hydroxyl ions at a rate comparable to their production.

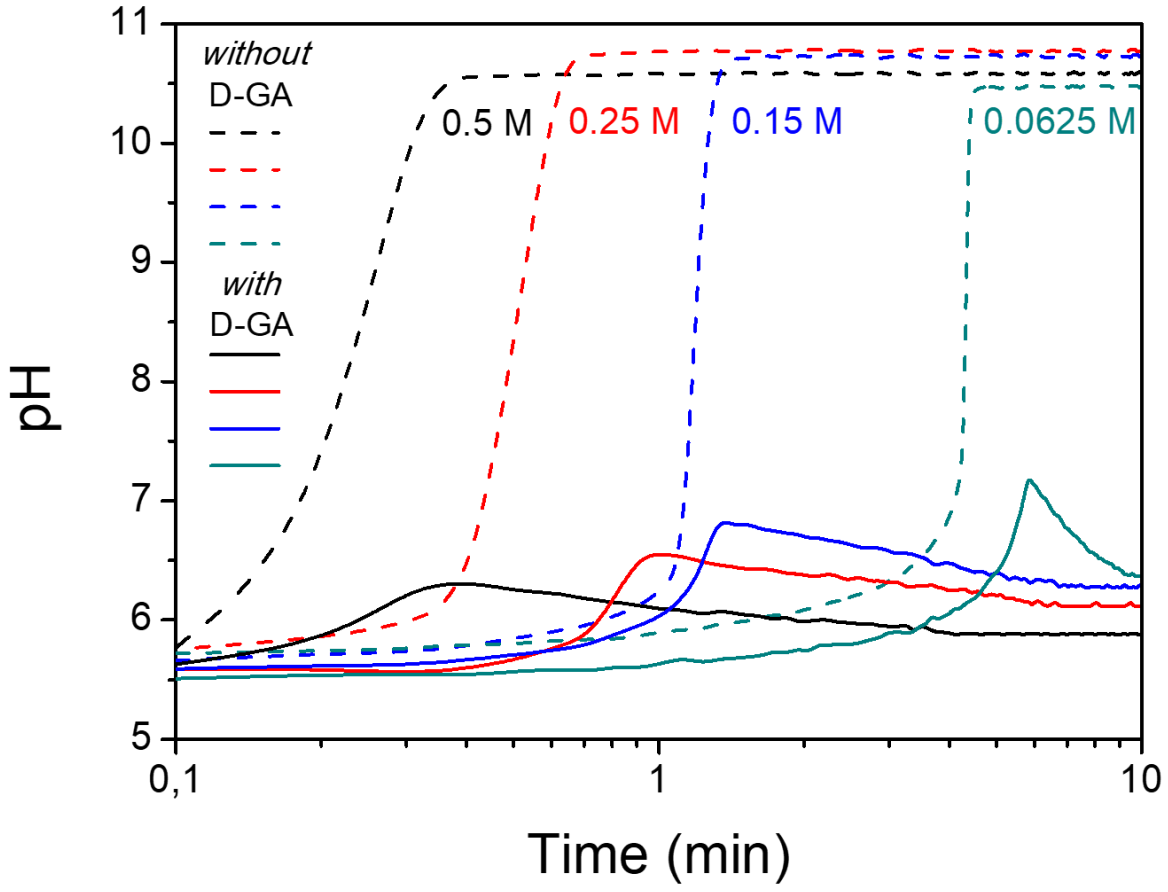


Figure S3. Effect of 0.1% m/v D-glucosamine (D-GA) on the pH evolution of the formaldehyde clock: the pH-buffering behavior of D-glucosamine is evident.

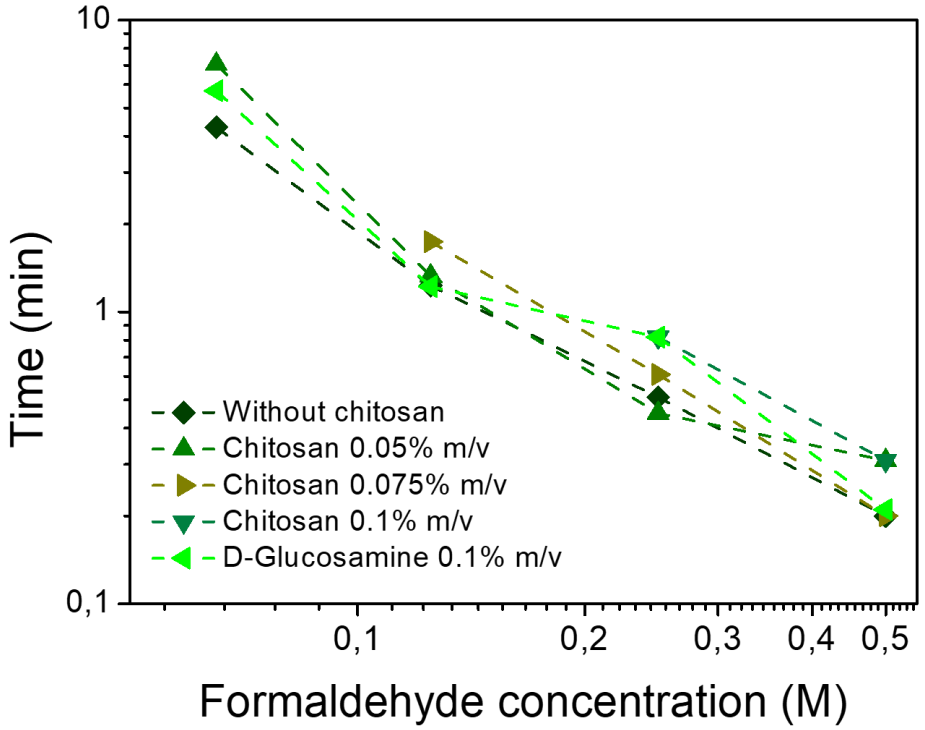


Figure S4. Demonstration that chitosan does not inhibit the formaldehyde clock reaction: effect of different amounts of chitosan and D-glucosamine on the time of sudden pH change. For the experiments with chitosan 0.1% m/v, no pH change peaks were detected with 0.125 M and 0.0625 M CH_2O.

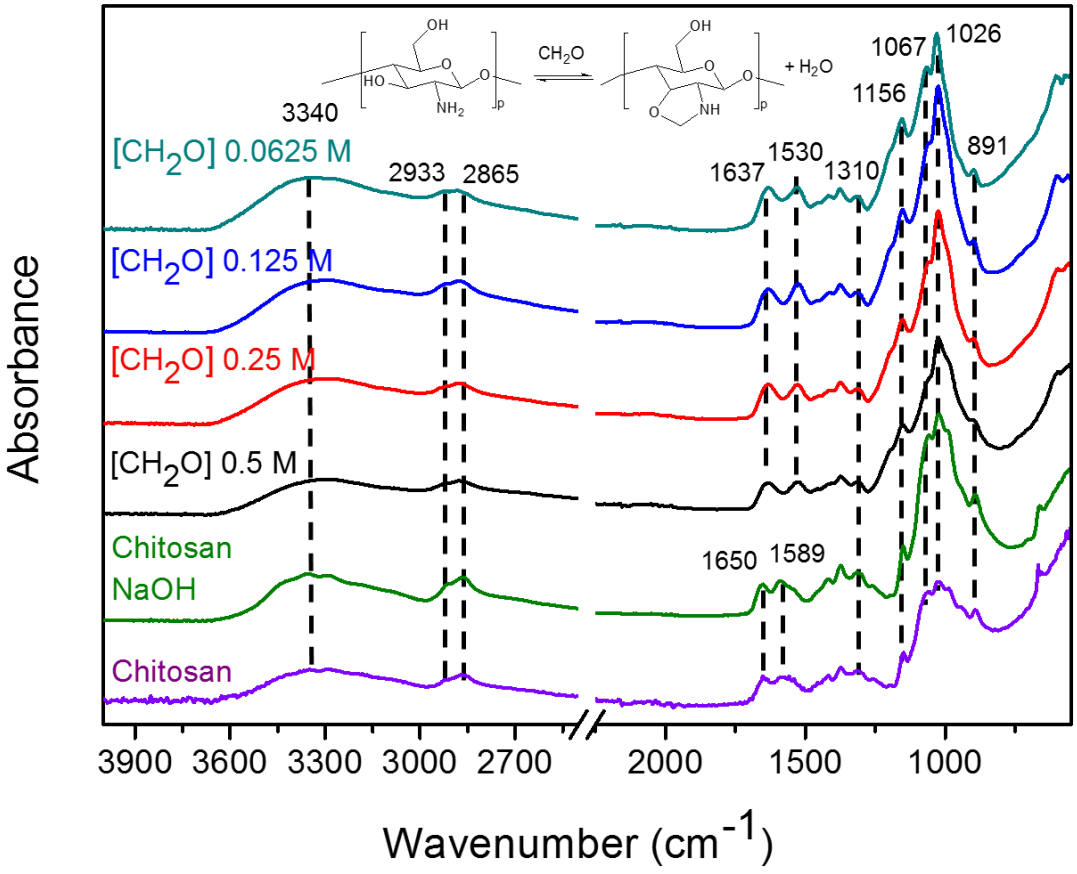


Figure S5. Normalized FTIR spectra for different chitosan samples (pristine, precipitated by NaOH and precipitated by the formaldehyde clock with different formaldehyde concentrations). The structure of the carbinolamine formed by the reaction between chitosan and formaldehyde is also shown.

The strong band, centered at 3340 cm^{-1}, is related to the stretching of O–H and N–H as well as to intermolecular hydrogen bonding. The peaks at 2933 cm^{-1} and 2865 cm^{-1} are due respectively to the asymmetric and symmetric stretching of aliphatic $–CH_2$ groups. The characteristic antisymmetric and symmetric stretching of amine groups $–NH_2$ are visible at 1650 cm^{-1} and 1589 cm^{-1} for both the pristine chitosan and the chitosan precipitated with NaOH, while they are shifted to 1627 cm^{-1} and 1530 cm^{-1} for the chitosan precipitated by the formaldehyde clock. The antisymmetric and symmetric stretching of the C–O–C bridge are visible at 1156 cm^{-1} and 891 cm^{-1}, respectively. The peak at 1067 cm^{-1} may be assigned to skeletal C–O stretching while that at 1026 cm^{-1} could be due to the aliphatic C–N stretching.

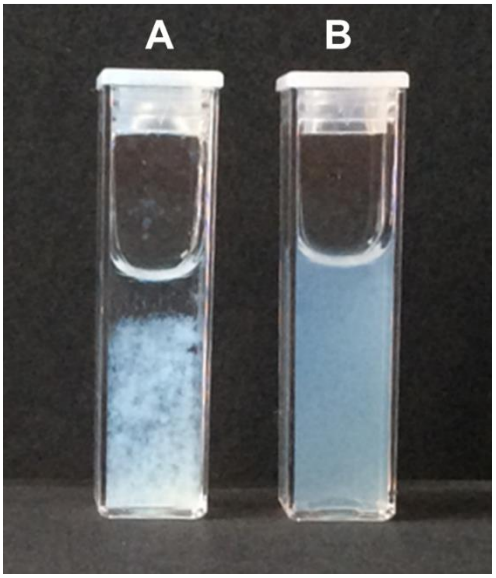


Figure S6. Photograph showing the different results obtained, all other conditions being the same, for the precipitation of chitosan with a) dropwise addition of 0.5 M NaOH; b) formaldehyde clock.

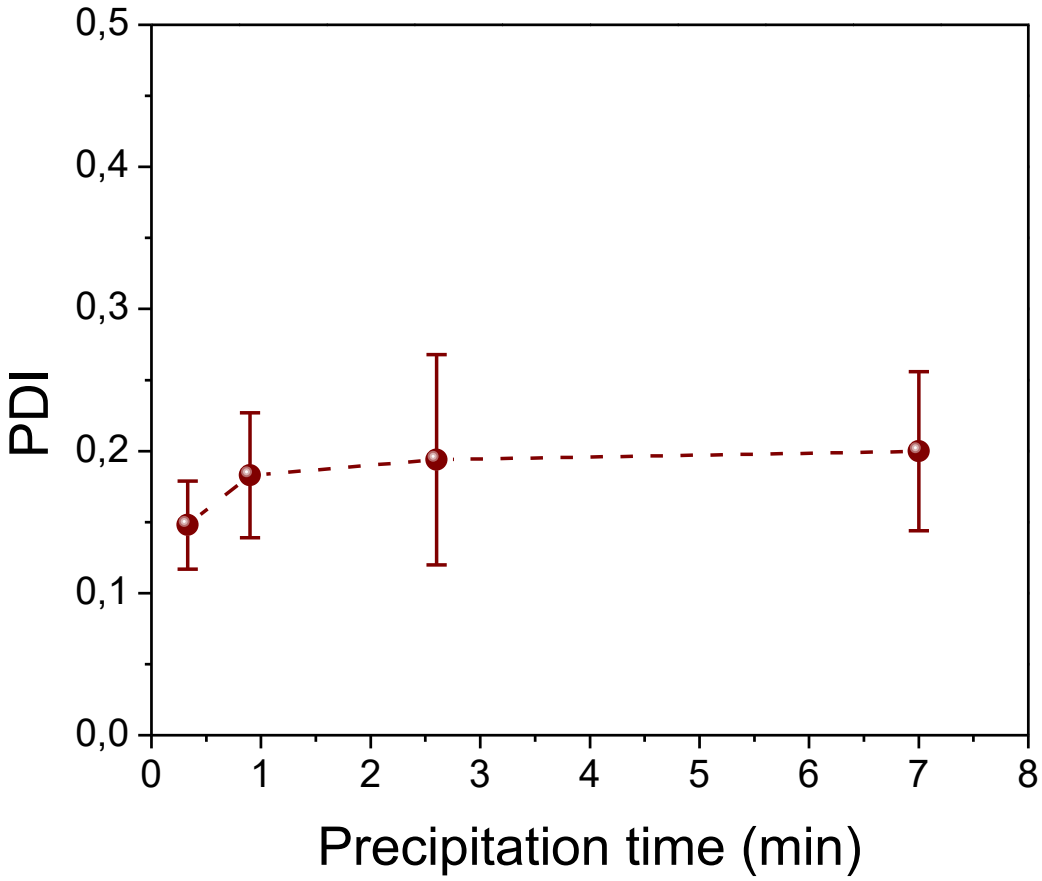


Figure S7. Polydispersity index (PDI) values, obtained by DLS, for the chitosan (DDA 85%, nominal viscosity 200 mPa·s) particles obtained by precipitation *via* the formaldehyde clock reaction. See also **Figure 3b**.

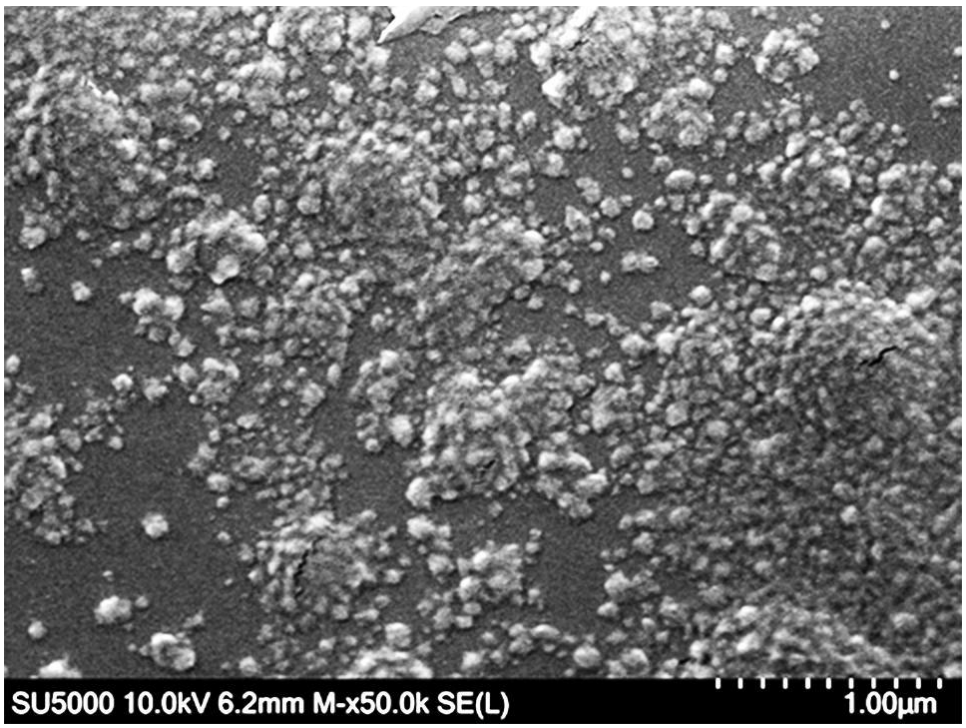


Figure S8. Representative SEM image of chitosan nanoparticles obtained by precipitation with the formaldehyde clock (0.5 M CH_2O). The effects of sample preparation, including drying, have not been systematically explored.

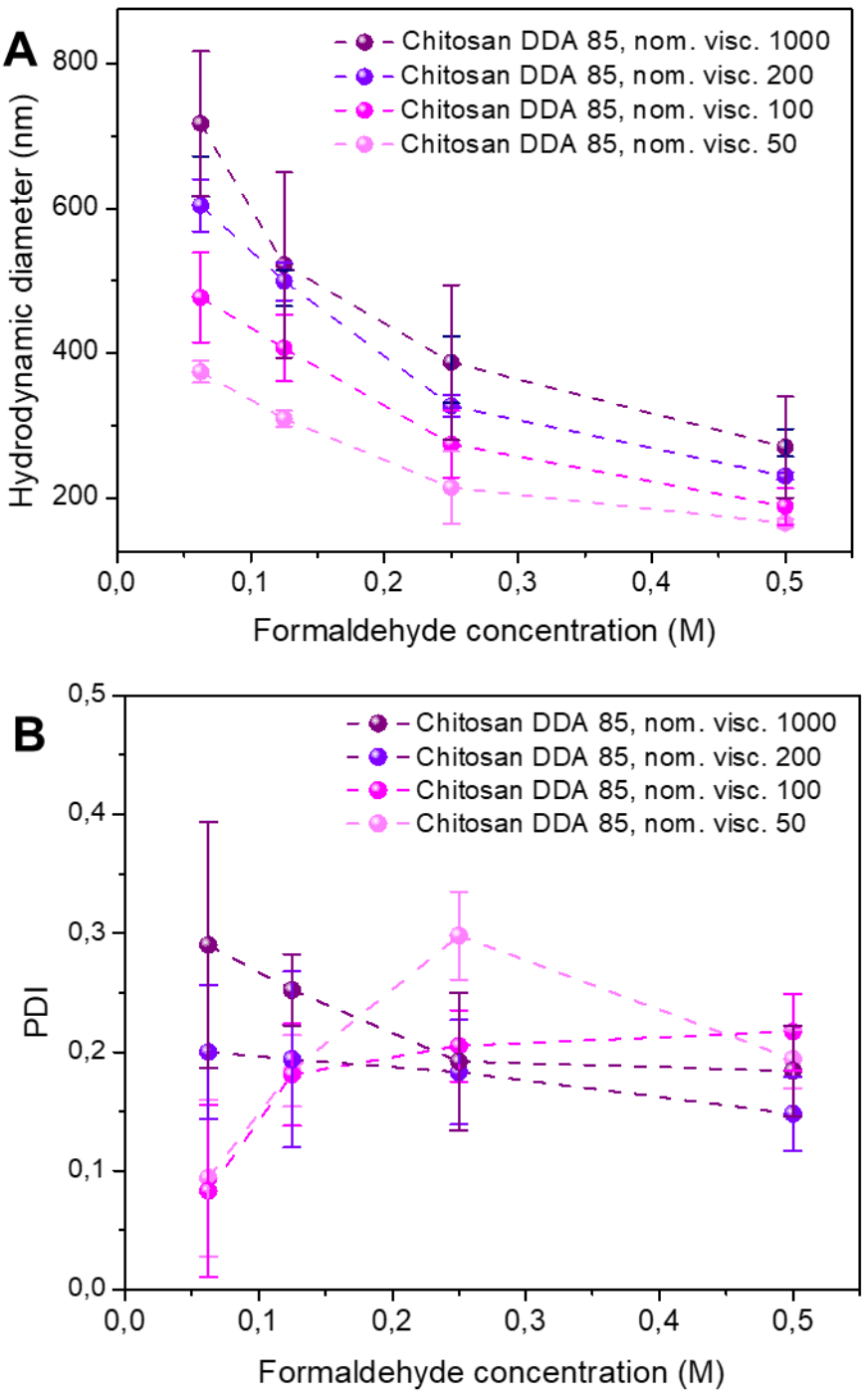


Figure S9. Effect of the nominal viscosity of chitosan on a) the hydrodynamic diameter and b) the polydispersity index (PDI) of the particles obtained by precipitation *via* the formaldehyde clock.