polymers

Supplementary materials

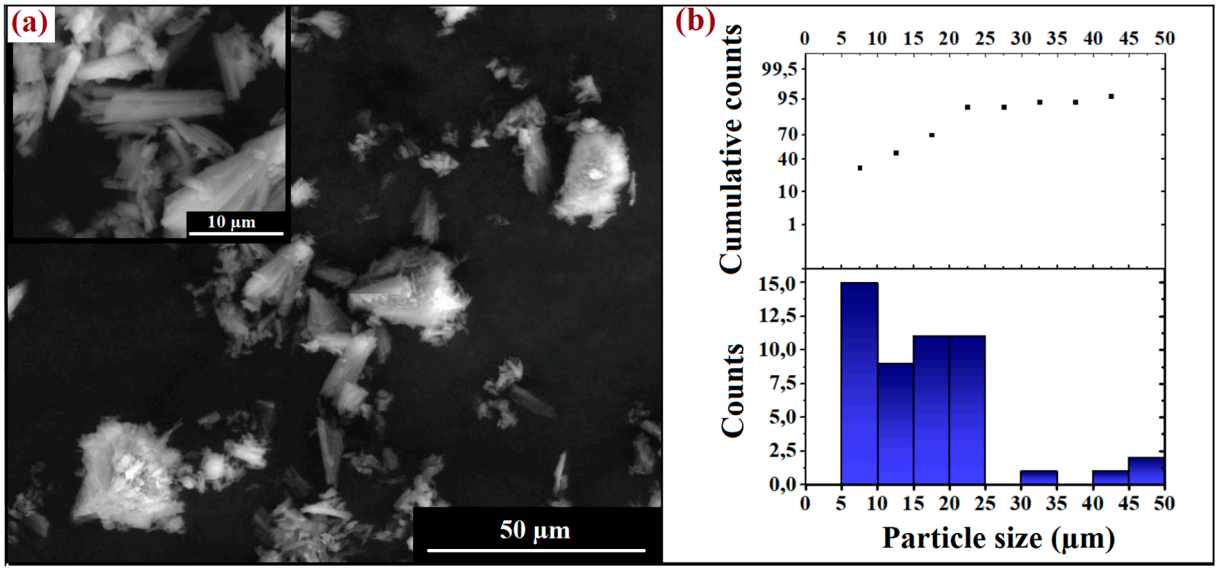

Figure S1. Nystatin powder: (**a**) Scanning electron microscopy images for nystatin powder and (**b**) particle size distribution histogram (particle sizes were determined using NIH Image J software).

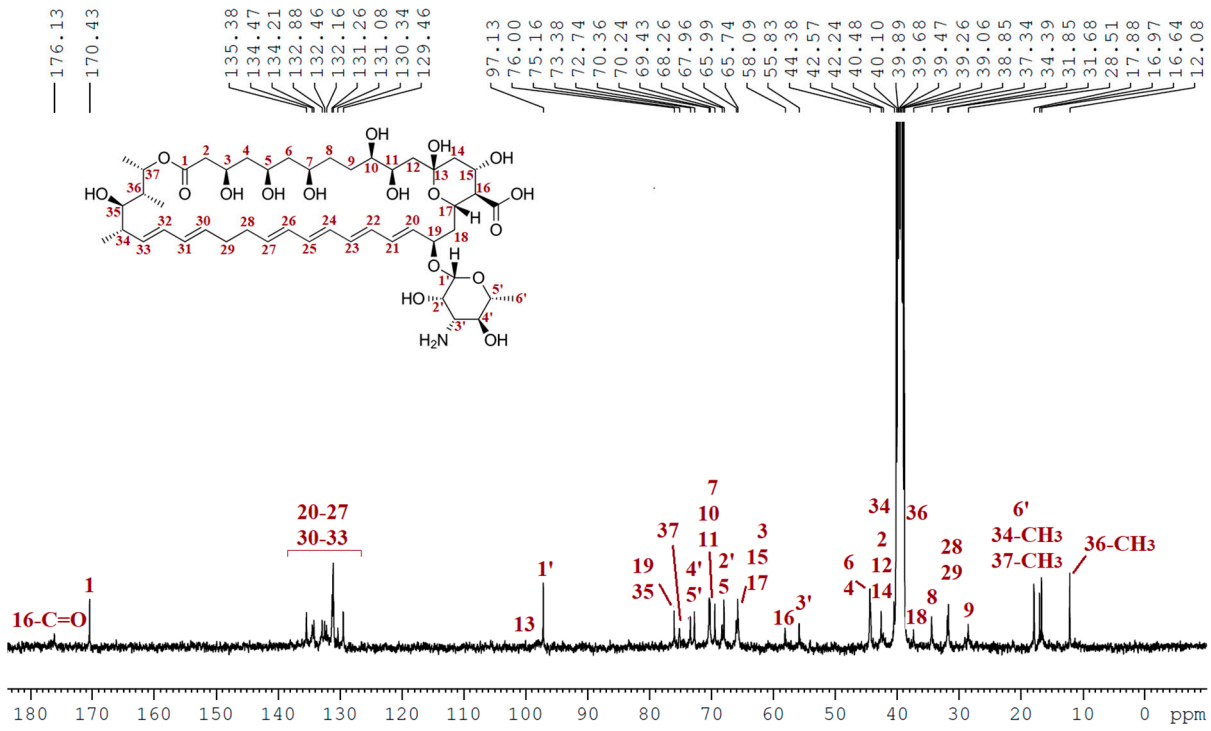

Figure S2. ^{13}C-RMN spectrum of nystatin; IUPAC Name:(1S,3R,4R,7R,9R,11R,15S,16R,17R,18S,19E, 21E,25E, 27E, 29E,31E,33R,35S,36R,37S)-33-[(2R,3S,4S,5S,6R)-4-amino-3,5-dihydroxy-6-methyloxan-2-yl]oxy-1,3,4,7,9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo-14,39-dioxabicyclo[33.3.1]nonatriaconta-19,21,25,27,29,31-hexaene-36-carboxylic acid); signal attributions made according to ref. [44-46].

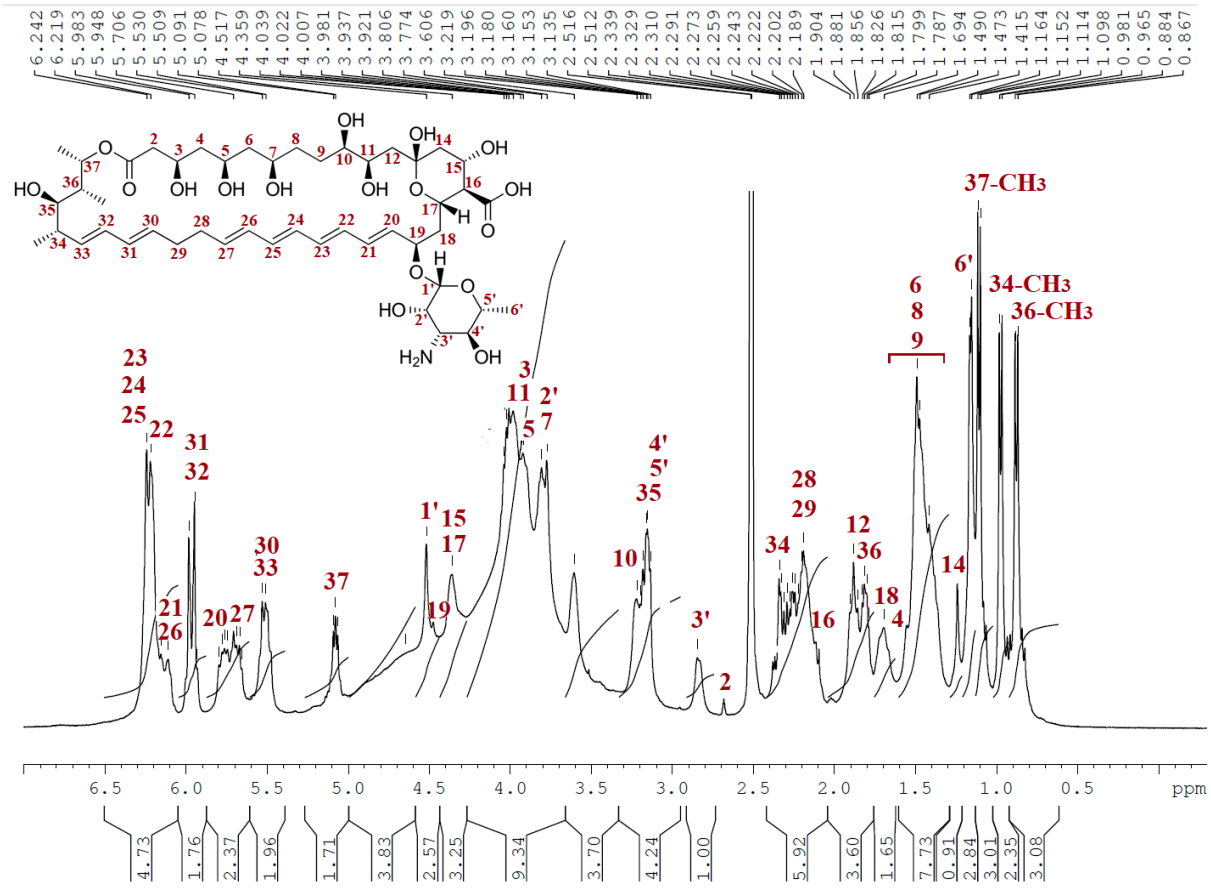

Figure S3. ^{1}H-RMN spectrum of nystatin. IUPAC Name:(1S,3R,4R,7R,9R,11R,15S,16R,17R,18S,19E, 21E,25E, 27E,29E,31E,33R,35S,36R,37S)-33-[(2R,3S,4S,5S,6R)-4-amino-3,5-dihydroxy-6-methyloxan-2-yl]oxy-1,3,4,7,9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo-14,39-dioxabicyclo[33.3.1]nonatriaconta-19,21,25,27,29,31-hexaene-36-carboxylic acid); signal attributions made according to ref. [44-46].

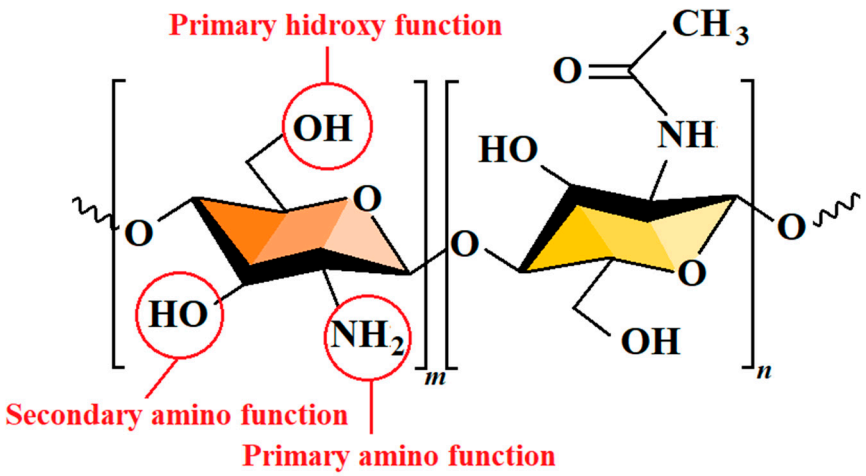

Figure S4. Chemical formula of chitosan with highlighting the functional groups: free primary amino groups (NH_2 to C2), primary hydroxyl group (OH to C6) and secondary hydroxyl groups (OH to C3); *m* and *n* represent the numbers of deacetylated (d-glucosamine) and N-acetyl-d-glucosamine repeating units, linked by (1-4)-β-glycosidic linkages, respectively.

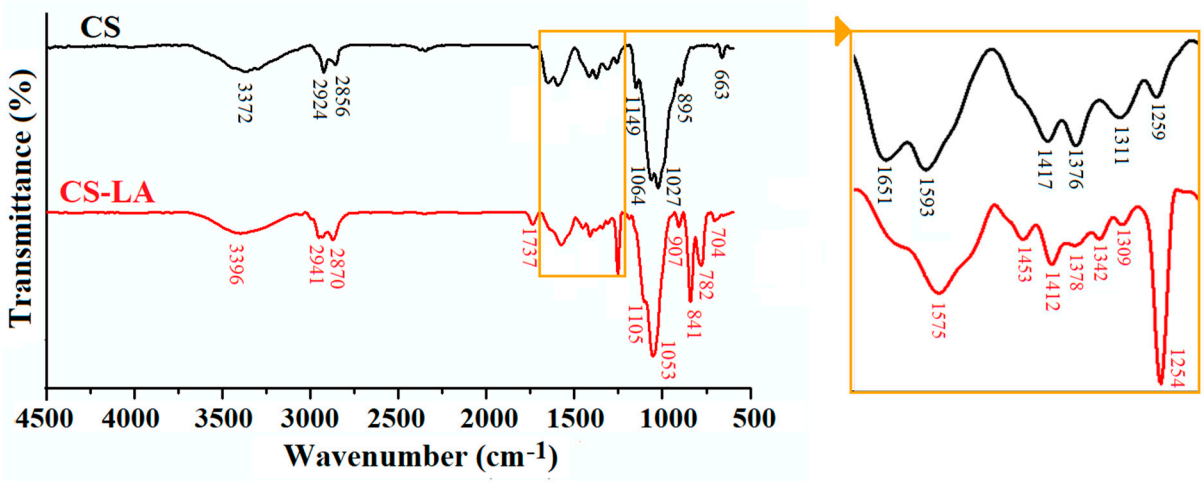

Figure S5. FTIR spectra of chitosan powder (CS) and chitosan gel (3%) after dispersion in 2% lactic acid solution (CS-LA).

The spectrum of chitosan shows polysaccharide characteristic absorption bands found in the range 4000-600 cm^{-1}: the broad band located between 3500-3100 cm^{-1} results from the stretching vibrations of superimposed O–H and N–H bonds; the absorption bands corresponding to the asymmetric and symmetrical stretching vibrations of the C–H are observed at 2924 cm^{-1}, and 2856 cm^{-1} respectively; the stretching vibration of the C=O bond of amide I (O=C–NHR) is distinguished at 1651 cm^{-1}; the band at 1593 cm^{-1} is caused by the in-plane deformation vibration of the primary amine group NH_2 coupled with the stretching vibrations of amide II, and that at 1311 cm^{-1} by the tensile vibrations of the C–N bond (amide III) ; the absorption bands given by the deformation vibrations of the CH_2 and CH_3 group appear at 1417 cm^{-1}, respectively 1376 cm^{-1}; the absorption band from 1149 cm^{-1} is attributed to the asymmetric stretching vibrations of the C–O–C glycosidic bonds, and the bands from 1064 and 1027 cm^{-1} are due to the C–O stretching vibrations, characteristic of the saccharide structure [19].

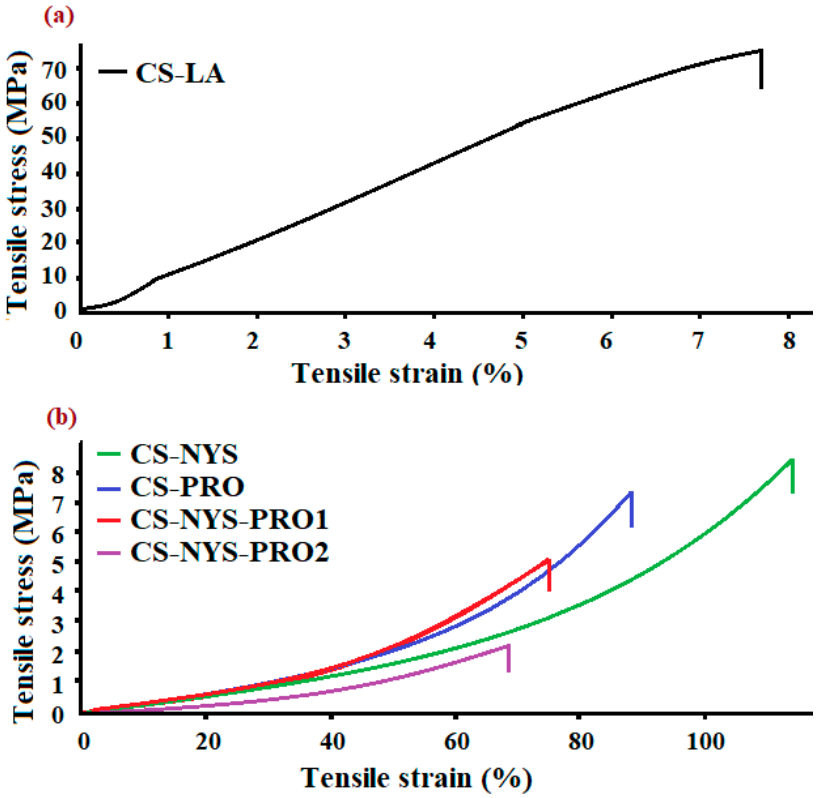

Figure S6. Characteristic stress-strain curves of the films: (**a**) without biological active compounds (CS-LA); (**b**) nystatin, propolis and nystatin/propolis loaded chitosan films (CS-NYS, CS-PRO, CS-NYS-PRO1, CS-NYS-PRO2).

Table S1. Parameters of pseudo-second order (PSO) and Korsmeyer-Peppas (K-P) kinetic models, where k_s is the constant of the swelling rate, S_e is the theoretical swelling capacity at equilibrium, k_p is a constant dependent on the polymeric network; n is the diffusion parameter of aqueous PBS in the formulation film and SD represents the standard deviation of n=11); swelling capacity in PBS of chitosan charged films after 5 and 24 h.

Film code	PSO model parameters	K-P model parameters	Swelling capacity after 5 h (%)	Swelling capacity after 24 h (%)
CS-NYS	$k_s = 1.86 \cdot 10^{-3}$ $Se = 600.74$ SD = 6.10	$k_p = 450.17$ $n = 5.9 \cdot 10^{-2}$ SD = 8.03	618	751
CS-PRO	$k_s = 5.29 \cdot 10^{2}$ $Se = 121.92$ SD = 0.37	$k_p = 121.14$ $n = 4.87 \cdot 10^{-18}$ SD = 0.44	122	122
CS-NYS-PRO1	$k_s = 1.21 \cdot 10^{-1}$ $Se = 130.84$ SD = 0.56	$k_p = 129.14$ $n = 1.40 \cdot 10^{-3}$ SD = 0.71	129	129
CS-NYS-PRO2	$k_s = 6.54 \cdot 10^{3}$ $Se = 119.35$ SD = 0.56	$k_p = 117.93$ $n = 5.42 \cdot 10^{-18}$ SD = 0.71	118	118

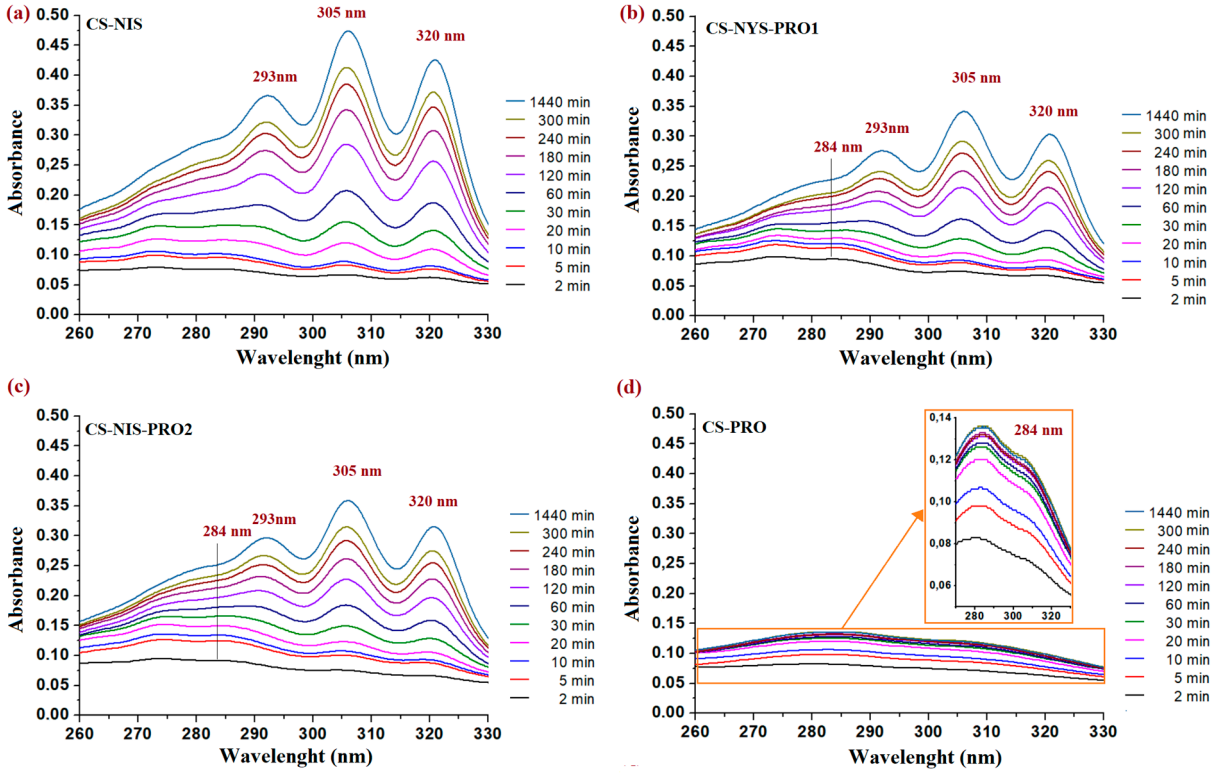

Figure S7. Evolution in time of UV-VIS spectra of: nystatin released from CS-NYS (**a**), CS-NYS-PRO1 (**b**) and CS-NYS-PRO2 (**c**); propolis released from CS-PRO (**d**).

Table S2. NYS and PRO release from chitosan films: Korsmeyer-Peppas kinetics parameters (k represents the transport constant, n is the diffusion exponent and SD is standard deviation of n=11), and release efficiency after 5 and 24 hours.

Film code	Drug released	Korsmeyer-Peppas model parameters	Drug release efficiency after 5 hours (%)	Drug release efficiency after 1 day (%)
CS-NYS	NYS	n = 0.4189821 k = 0.045 SD = 0.37	48.6	55.2
CS-NYS-PRO1	NYS	n = 0.3209252 k =0.072 SD = 0.50	45.9	53.3
CS-NYS-PRO2	NYS	n = 0.3098546 k =0.071 SD = 0.34	42.8	48.7
CS-PRO	PRO	n = 0.0806324 k = 0.157 SD = 0.35	24.2	24.2

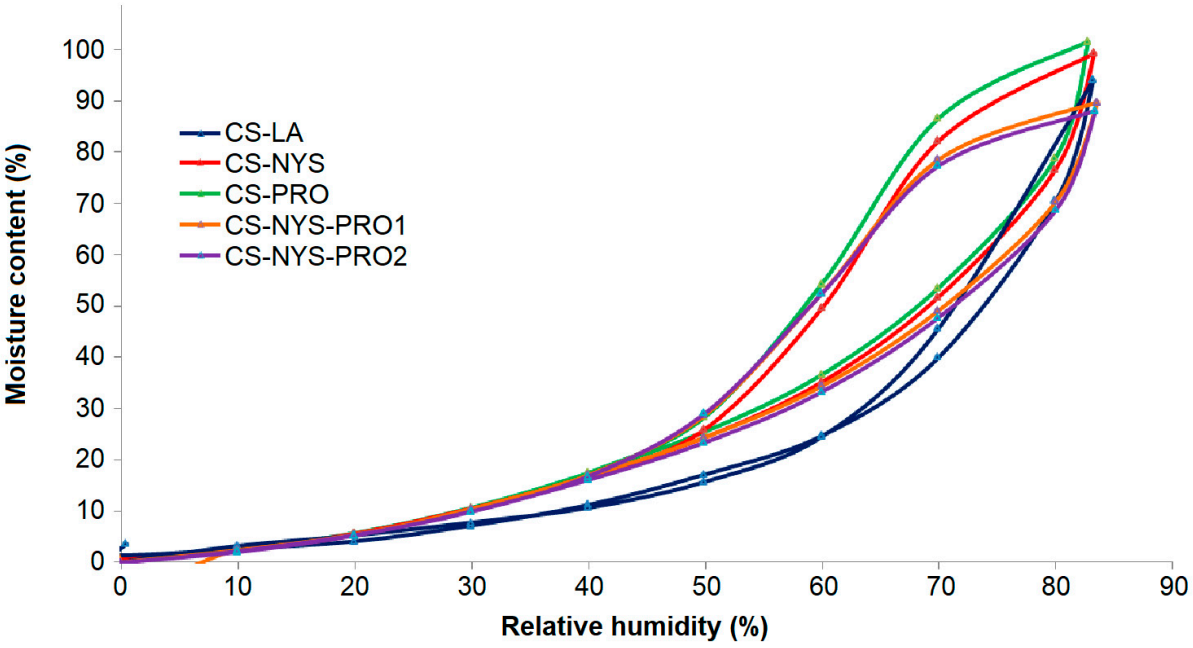

Figure S8. Sorption/desorption isotherms for the hydrogel formulations

Table S3. Surface parameters of the hydrogels evaluated based on adsorption/desorption isotherms: water vapor sorption capacity, final weight (W); average pore size (r_{pm}) and BET data (surface area and monolayer weight).

Hydrogel Surface	W (%)	r_{pm} (BJH model) (nm)	BET data *	
			Area (m^2/g)	Monolayer (mg/g)
CS-LA	94.1	6.8	275	788
CS-NYS	99.3	1.4	1379	399
CS-PRO	101.6	2.0	1035	294
CS-NYS-PRO1	89.8	1.2	1444	412
CS-NYS-PRO2	88.1	1.0	1727	492

Determined based on desorption branch of the isotherm (registered up to a relative humidity of 40 %)

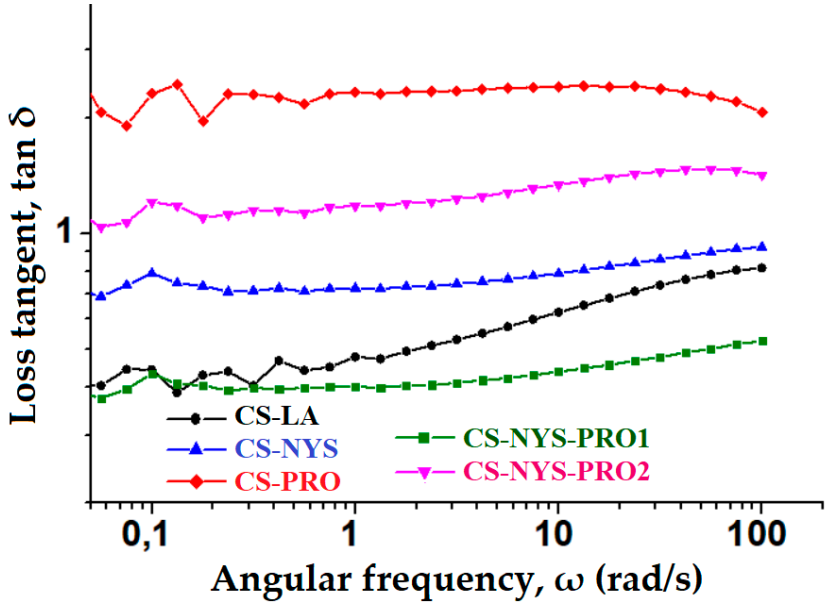

Figure S9. The loss tangent (tan δ = G'' / G') as a function of angular frequency.

Table S4. Viscoelastic parameters (G′, G′′ and tan δ) at a frequency of 1 Hz and viscous flow activation energy (E_η) calculated by fitting the temperature-dependence curve of the viscosity by Arrhenius equation.

Gel formulation	G′ (Pa)	G′′ (Pa)	tan δ	E_η (kcal/mol)
CS-LA	42.64	20.30	0.47	5.18
CS-NYS	20.70	14.70	0.73	5.51
CS-PRO	0.94	2.22	2.31	5.67
CS-NYS-PRO1	107.05	43.96	0.40	4.81
CS-NYS-PRO2	4.43	5.45	1.18	5.28

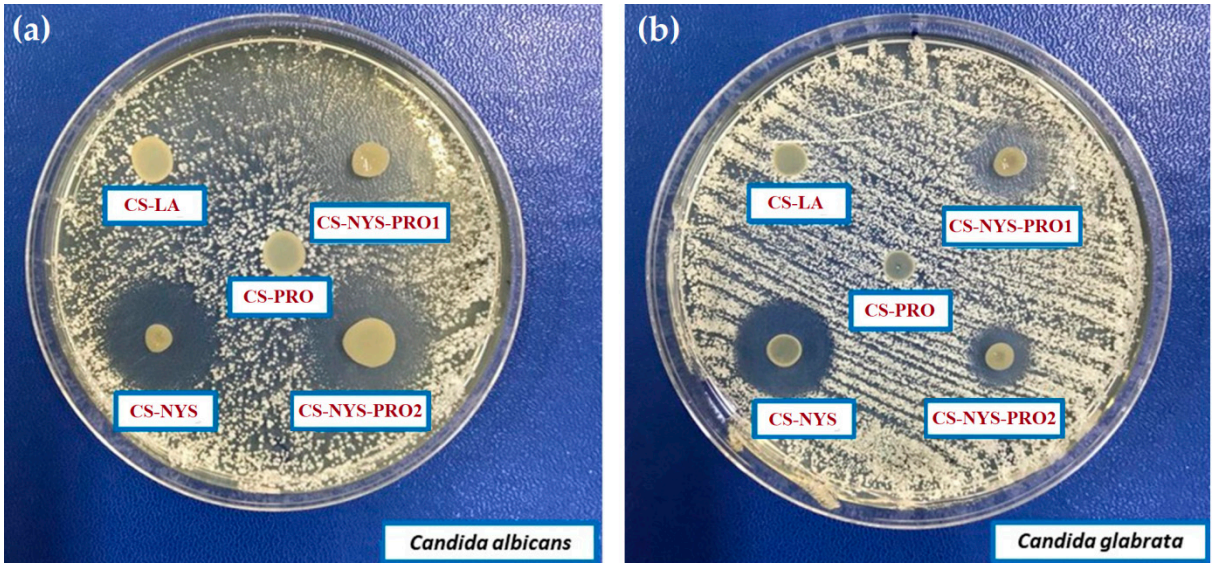

Figure S10. Antifungal activity of chitosan hydrogels against: (**a**) *C. albicans* and (**b**) *C. glabrata*

Table S5. Killing efficiency as a function of time for CS-NYS, CS-PRO and CS-NYS-PRO1 hydrogels, against *Candida albicans* in 24 h, as compared to the control test.

Time (hours)	***Candida albicans* (CFU/mL)**			
	Control	**CS-NYS**	**CS-PRO**	**CS-NYS-PRO1**
0	$1.24x10^7$	$1.24x10^7$	$1.24x10^7$	$1.24x10^7$
6	$1.64x10^7$	$2.40x10^2$	$7.60x10^6$	$<10^1$
12	$1.96x10^7$	$<10^1$	$3.00x10^6$	$<10^1$
24	$4.32x10^7$	$<10^1$	$2.28x10^6$	$<10^1$