Preparation and characterization of ACE2 receptor inhibitor loaded chitosan hydrogels for nasal formulation to reduce the risk of COVID-19 viral infection

Barbara Vörös-Horváth[1], Pavo Živković[2], Krisztina Bánfai[3], Judit Bóvári-Biri[3], Judit Pongrácz[3], Gábor Bálint[1], Szilárd Pál[1], Aleksandar Széchenyi[1,2*1] Institute of Pharmaceutical Technology and Biopharmacy, University of Pécs, Faculty of Pharmacy, Rókus u. 2., H-7624 Pécs, Hungary, horvath.barbara@gytk.pte.hu, pal.szilard@gytk.pte.hu, szechenyi.aleksandar@gytk.pte.hu

[2] Department of Chemistry, Josip Juraj Strossmayer University of Osijek, Ulica cara Hadrijana 8/A, HR-31000 Osijek, Croatia; pzivkovic@kemija.unios.hr

[3] Department of Pharmaceutical Biotechnology, University of Pécs, Faculty of Pharmacy, Rókus u. 2. H-7624 Pécs, Hungary; banfai.krisztina@pte.hu, bovari.judit@pte.hu, pongracz.e.judit@pte.hu

*corresponding author. E-mail: szechenyi.aleksandar@gytk.pte.hu Phone: +36-70-3814462

Authors declare no conflict of interest.

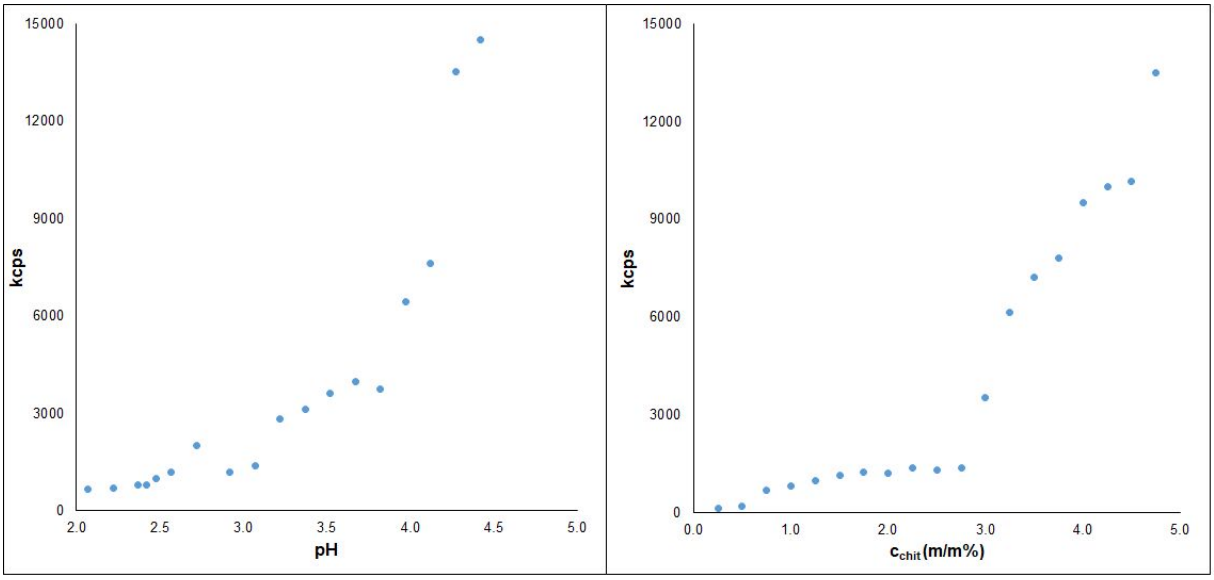
The Supporting information consist of 12 pages, and contains 6 chapters with 14 figures and 4 tables.

1. Determination of chitosan solubility

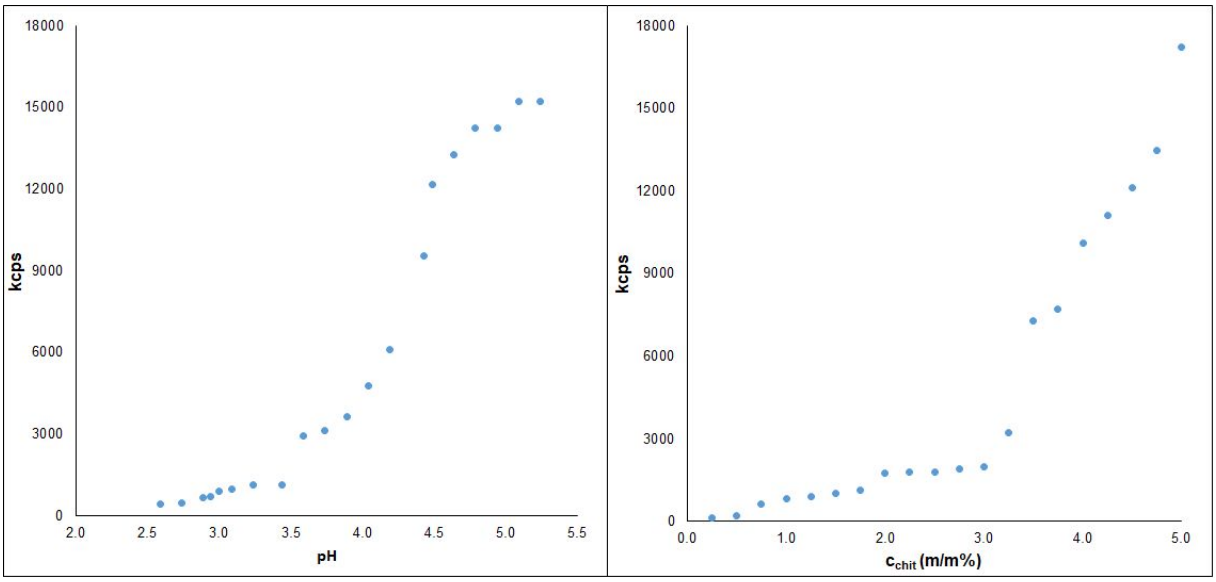
The influence of pH and chitosan concentration on chitosan solubility were examined in both malic acid and glutaric acid solutions. In case of examine the influence of pH on solubility, an initial solution was prepared by weight 1.0 m/m% chitosan in 5.0 m/V% dicarboxylic acid solutions, and diluted until the solution turned into a suspension. In case of examine the influence of chitosan concentration on chitosan solubility, an initial solution was prepared by weight 0.25 m/m% chitosan in 2.0 m/V% dicarboxylic acid solution, and added chitosan until the solution turned into a suspension.

The light scattering of solutions were determined visually and with instrumental measurement of scattered light intensity (Zetasizer NanoS, Malvern Panalytical Ltd. UK). The examinations were performed at ambient temperature (25°C). The measurements were carried out in autocorrelation mode, and the following parameters were constant: scattering angle 173°, attenuator 7 and its factor 0.0146, measurement position 0.46 mm.

The result shows, that in case of malic acid the chitosan can be dissolved under 3.22 pH value, which means more than 0.025 m/V% acid concentration. At 2.0 m/V% malic acid concentration (pH=2.27) the chitosan can be dissolved until 2.75 m/m% concentration. In glutaric acid the chitosan can be dissolved under 3.59 pH value (0.05 m/V% acid concentration). At 2.0 m/V% glutaric acid concentration (pH=2.79) the chitosan can be dissolved until 3.0 m/m% concentration.



S1. Figure: Determination of chitosan solubility in malic acid with light scattering measurements.



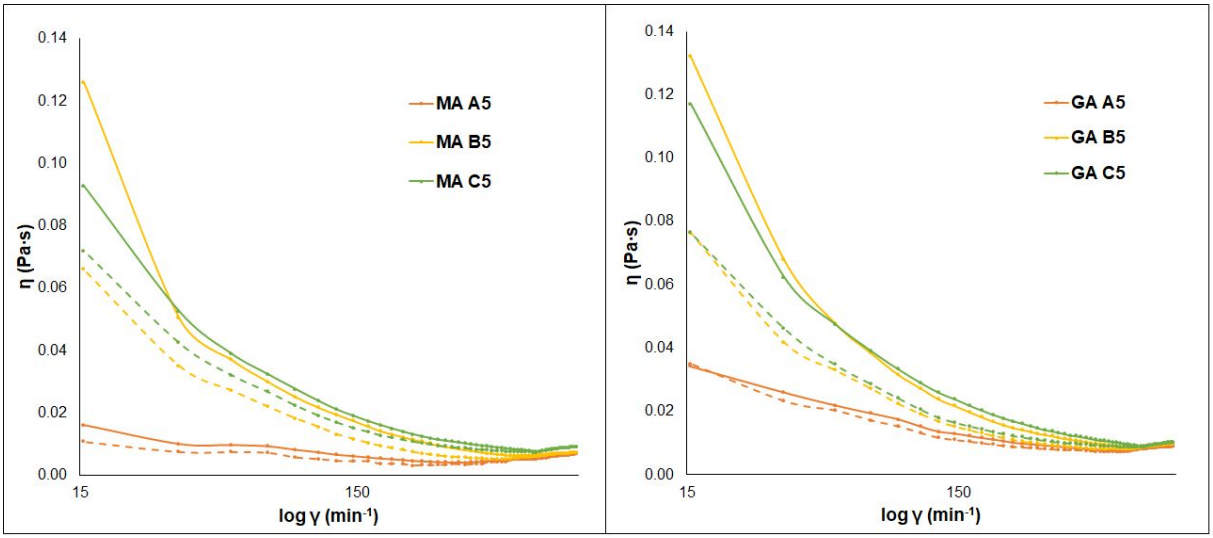
S2. Figure: Determination of chitosan solubility in glutaric acid with light scattering measurements.

2. Results of calculation of cross-linking degree (CR) and cross-linking efficacy (CE)

Sample	n_A (mol/g)	CR (%)	CE (%)	Sample	n_A (mol/g)	CR (%)	CE (%)
Chitosan	$5.25 \cdot 10^{-3}$	-	-	GA 253	$4.28 \cdot 10^{-3}$	18.57	3.22
MA 253	$4.23 \cdot 10^{-3}$	19.52	3.44	GA 258	$4.13 \cdot 10^{-3}$	21.43	3.72
MA 258	$4.13 \cdot 10^{-3}$	21.43	3.77	GA 2524	$4.00 \cdot 10^{-3}$	23.81	4.13
MA 2524	$4.03 \cdot 10^{-3}$	23.33	4.11	GA 403	$3.70 \cdot 10^{-3}$	29.52	5.12
MA 403	$4.08 \cdot 10^{-3}$	22.38	3.94	GA 408	$3.50 \cdot 10^{-3}$	33.33	5.78
MA 408	$3.88 \cdot 10^{-3}$	26.19	4.61	GA 4024	$3.43 \cdot 10^{-3}$	34.76	6.03
MA 4024	$3.83 \cdot 10^{-3}$	27.14	4.78	GA 503	$2.50 \cdot 10^{-3}$	52.38	9.08
MA 503	$3.63 \cdot 10^{-3}$	30.95	9.22	GA 508	$2.38 \cdot 10^{-3}$	54.76	9.50
MA 508	$3.50 \cdot 10^{-3}$	33.33	9.64	GA 5024	$2.18 \cdot 10^{-3}$	58.57	10.16
MA 5024	$3.38 \cdot 10^{-3}$	35.71	10.31				

S1 Table. Results of determination of number of free amino groups and the cross-linking degree of chitosan and hydrogel samples with conductometric titration. n_A: x mol of free amino group per 1 g chitosan/chitosan hydrogel. CR: cross-linking degree (%). CE: cross-linking efficacy.

3. Determination of flow properties



S3 Figure. Viscosity curves of chitosan hydrogels, cross-linked with malic and glutaric acid at different reaction temperatures. The continuous lines remark the upward curves, and the dotted lines remark the downward curves.

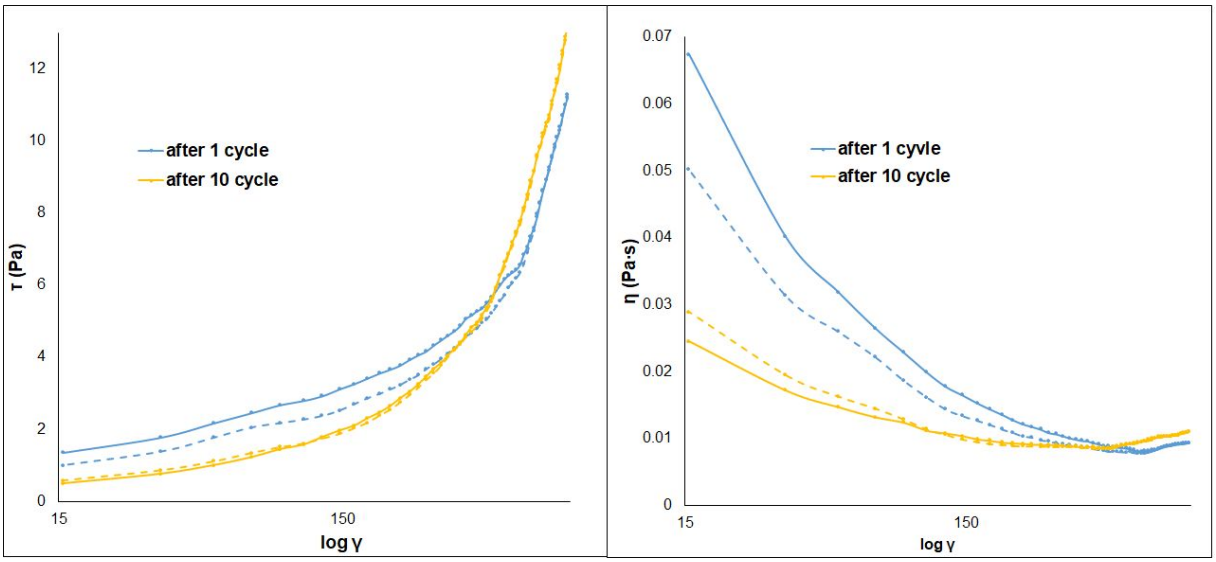
Sample	Bingham plasticity parameters			Hysteresis area (Pa/s·ml)	Apparent viscosity (Pa·s)
MA 298.15	**τ_B (Pa)**	**η_B (Pa·s)**	**R^2**		
MA 250	0.010	$2.09 \cdot 10^{-3}$	0.9900	16.744	$4.44 \cdot 10^{-3}$
MA 251.5	0.018	$2.07 \cdot 10^{-3}$	0.9822	20.301	$4.55 \cdot 10^{-3}$
MA 253	0.077	$2.41 \cdot 10^{-3}$	0.9868	25.606	$4.08 \cdot 10^{-3}$
MA 25.45	0.143	$3.04 \cdot 10^{-3}$	0.9715	26.204	$4.57 \cdot 10^{-3}$
MA 256	0.148	$4.29 \cdot 10^{-3}$	0.9281	28.000	$5.21 \cdot 10^{-3}$
MA 258	0.997	$5.01 \cdot 10^{-3}$	0.9878	34.484	$6.85 \cdot 10^{-3}$
MA 2524	1.462	$5.51 \cdot 10^{-3}$	0.9664	54.874	$7.68 \cdot 10^{-3}$
MA 313.15 K	**τ_B (Pa)**	**η_B (Pa·s)**	**R^2**	**Hysteresis area (Pa/s·ml)**	**Apparent viscosity (Pa·s)**
MA 401.5	0.044	$2.77 \cdot 10^{-3}$	0.9609	23.779	$5.03 \cdot 10^{-3}$
MA 403	0.053	$3.90 \cdot 10^{-3}$	0.9695	25.698	$5.99 \cdot 10^{-3}$
MA 404.5	0.730	$5.43 \cdot 10^{-3}$	0.9916	35.989	$7.98 \cdot 10^{-3}$
MA 406	0.747	$6.01 \cdot 10^{-3}$	0.9325	54.885	$8.54 \cdot 10^{-3}$
MA 408	1.764	$6.11 \cdot 10^{-3}$	0.9619	59.790	$9.14 \cdot 10^{-3}$
MA 4024	2.189	$6.43 \cdot 10^{-3}$	0.9422	70.833	$9.88 \cdot 10^{-3}$
MA 323.15 K	**τ_B (Pa)**	**η_B (Pa·s)**	**R^2**	**Hysteresis area (Pa/s·ml)**	**Apparent viscosity (Pa·s)**
MA 50.15	0.491	$3.80 \cdot 10^{-3}$	0.9798	22.456	$6.00 \cdot 10^{-3}$
MA 503	0.617	$4.06 \cdot 10^{-3}$	0.9297	34.632	$6.22 \cdot 10^{-3}$
MA 504.5	1.174	$4.25 \cdot 10^{-3}$	0.9516	45.619	$6.78 \cdot 10^{-3}$
MA 506	1.175	$4.92 \cdot 10^{-3}$	0.9384	73.468	$8.27 \cdot 10^{-3}$
MA 508	1.480	$5.39 \cdot 10^{-3}$	0.9876	85.343	$8.72 \cdot 10^{-3}$
MA 5024	2.338	$3.43 \cdot 10^{-2}$	0.9468	113.316	$8.67 \cdot 10^{-3}$

S2 Table. Rheological parameters of chitosan hydrogels cross-linked with malic acid at different temperatures. Chitosan hydrogels show Bingham plastic behaviour. The τB is the yield stress needed to flow (Pa), ηB is Bingham viscosity (Pa·s). R2 provide information about

the fit of Bingham plasticity mathematical model. Hysteresis area values were calculated from the area of hysteresis loop.

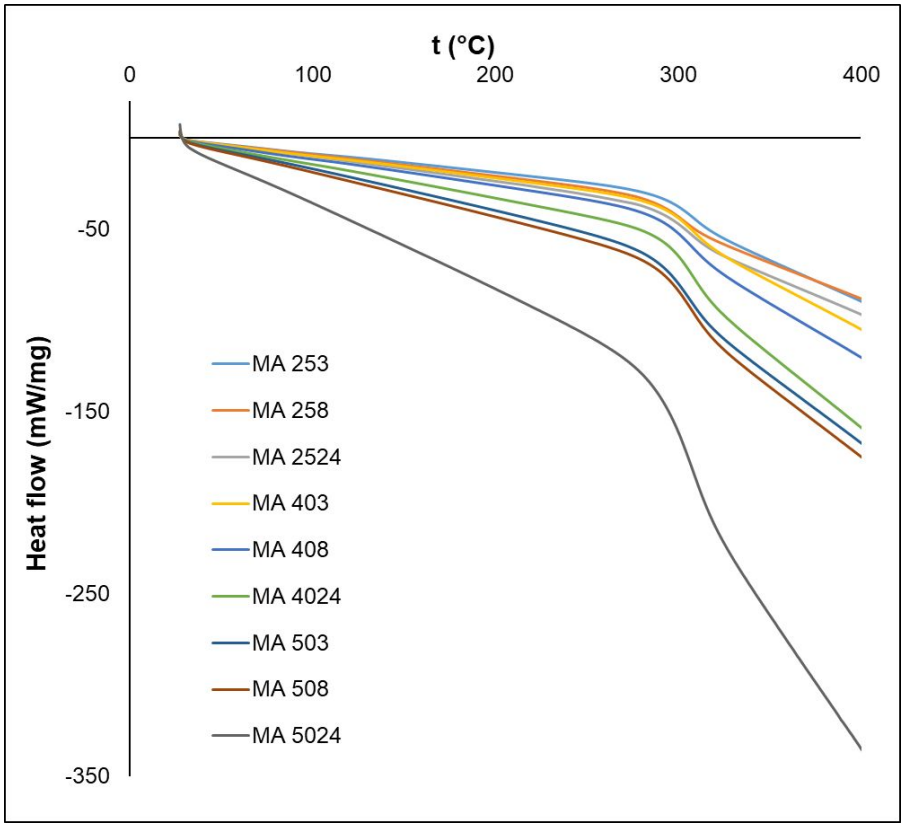
Sample	Bingham plasticity parameters			Hysteresis area (Pa/s·ml)	Apparent viscosity (Pa·s)
GA 298.15 K	**τ_B (Pa)**	**η_B (Pa·s)**	**R^2**		
GA 250	0.095	$6.14 \cdot 10^{-3}$	0.9813	14.813	$5.39 \cdot 10^{-3}$
GA 251.5	0.225	$6.30 \cdot 10^{-3}$	0.9598	19.463	$7.96 \cdot 10^{-3}$
GA 253	0.582	$6.94 \cdot 10^{-3}$	0.9691	26.439	$8.59 \cdot 10^{-3}$
GA 254.5	0.708	$7.11 \cdot 10^{-3}$	0.9519	26.820	$8.65 \cdot 10^{-3}$
GA 256	0.721	$7.13 \cdot 10^{-3}$	0.9570	34.859	$8.95 \cdot 10^{-3}$
GA 258	1.234	$7.74 \cdot 10^{-3}$	0.9788	39.252	$9.34 \cdot 10^{-3}$
GA 2524	1.521	$8.55 \cdot 10^{-3}$	0.9820	60.820	$9.38 \cdot 10^{-3}$
GA 313.15 K	**τ_B (Pa)**	**η_B (Pa·s)**	**R^2**	**Hysteresis area (Pa/s·ml)**	**Apparent viscosity (Pa·s)**
GA 401.5	0.502	$6.11\ 10^{-3}$	0.9511	55.463	$8.32 \cdot 10^{-3}$
GA 403	0.913	$6.11 \cdot 10^{-3}$	0.9240	70.595	$8.92 \cdot 10^{-3}$
GA 404.5	1.194	$6.35 \cdot 10^{-3}$	0.9790	76.055	$8.69 \cdot 10^{-3}$
GA 406	1.503	$6.55 \cdot 10^{-3}$	0.9691	77.651	$9.24 \cdot 10^{-3}$
GA 408	1.628	$6.60 \cdot 10^{-3}$	0.9632	91.418	$9.83 \cdot 10^{-3}$
GA 4024	2.002	$7.58 \cdot 10^{-3}$	0.9693	98.155	$1.03 \cdot 10^{-2}$
GA 323.15 K	**τ_B (Pa)**	**η_B (Pa·s)**	**R^2**	**Hysteresis area (Pa/s·ml)**	**Apparent viscosity (Pa·s)**
GA 501.5	1.156	$6.59 \cdot 10^{-3}$	0.9815	52.319	$8.69 \cdot 10^{-3}$
GA 503	1.406	$7.11 \cdot 10^{-3}$	0.9743	68.531	$9.48 \cdot 10^{-3}$
GA 504.5	1.468	$7.41 \cdot 10^{-3}$	0.9571	73.308	$1.01 \cdot 10^{-2}$
GA 506	1.561	$7.96 \cdot 10^{-3}$	0.9718	84.826	$1.04 \cdot 10^{-2}$
GA 508	1.874	$8.05 \cdot 10^{-3}$	0.9532	89.969	$1.07 \cdot 10^{-2}$
GA 5024	2.390	$9.12 \cdot 10^{-3}$	0.9693	117.505	$1.12 \cdot 10^{-2}$

S3 Table. Rheological parameters of chitosan hydrogels cross-linked with glutaric acid at different temperatures. Chitosan hydrogels show Bingham plastic behaviour. The τ_B is the yield stress needed to flow (Pa), η_B is Bingham viscosity (Pa·s). R^2 provide information about the fit of Bingham plasticity mathematical model. Hysteresis area values were calculated from the area of hysteresis loop.

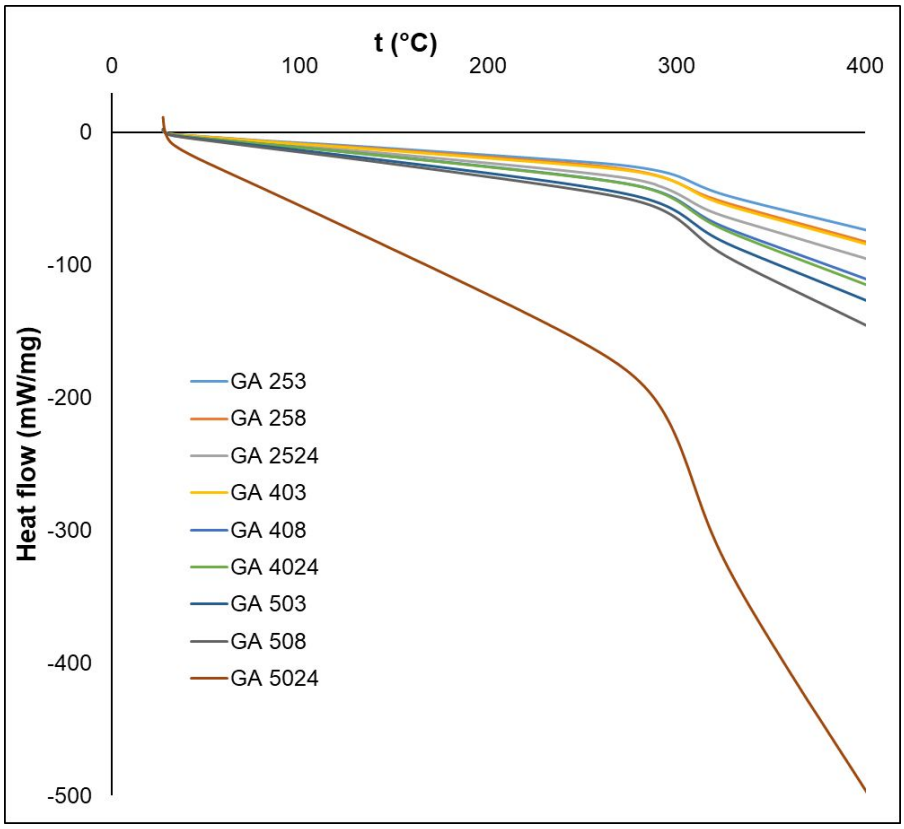


S4 Figure. Flow (A) and viscosity (B) curves of GA 2524 hydrogel sample after 1st and 10th measurement cycle. The continuous lines remark the upward curves, and the dotted lines remark the downward curves.

4. DSC measurements of hydrogels

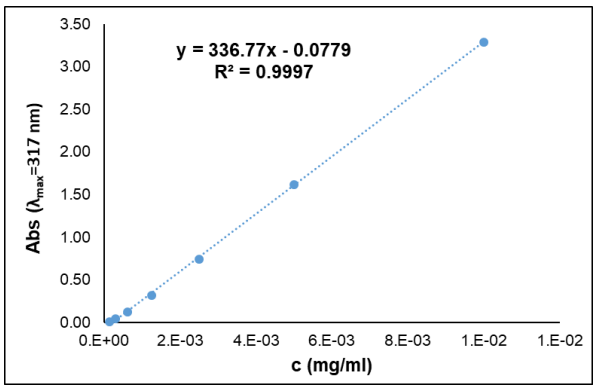


S5. Figure: DSC curve of hydrogels cross-linked with malic acid.

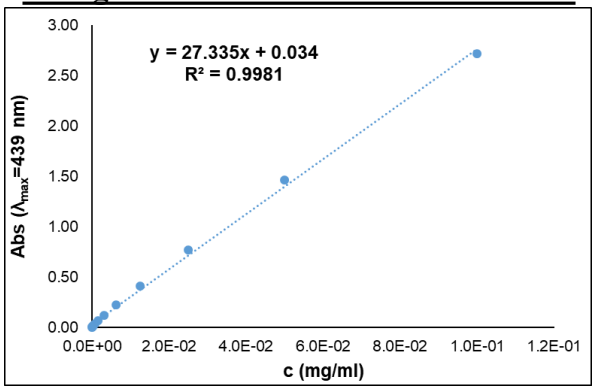


S6. Figure: DSC curve of hydrogels cross-linked with glutaric acid.

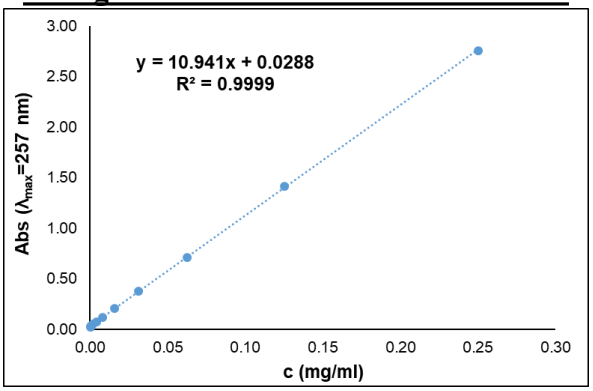
5. Characterization of API adsorption of hydrogels



S7 Figure: Calibration curve of baicalin.



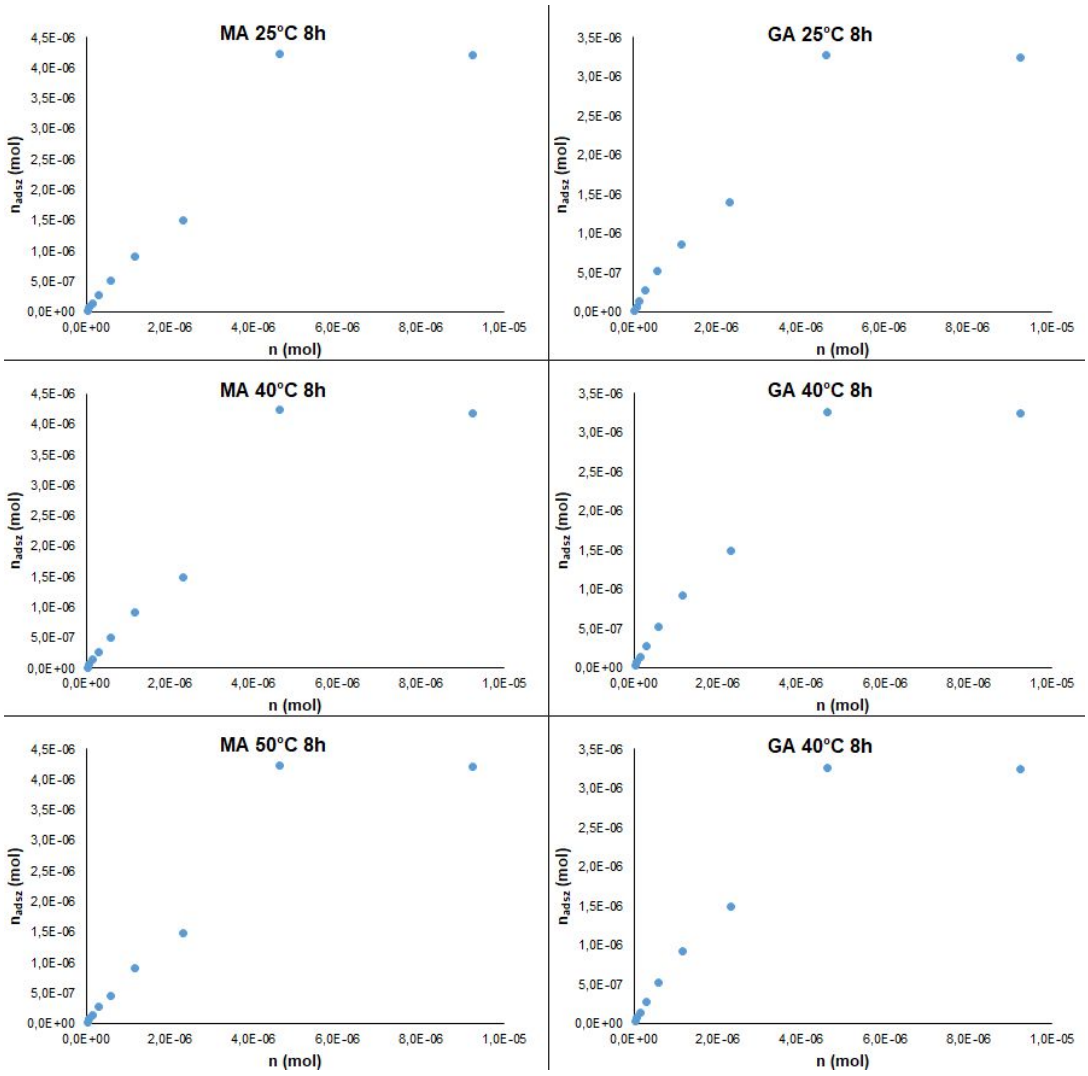
S8 Figure: Calibration curve of emodin.



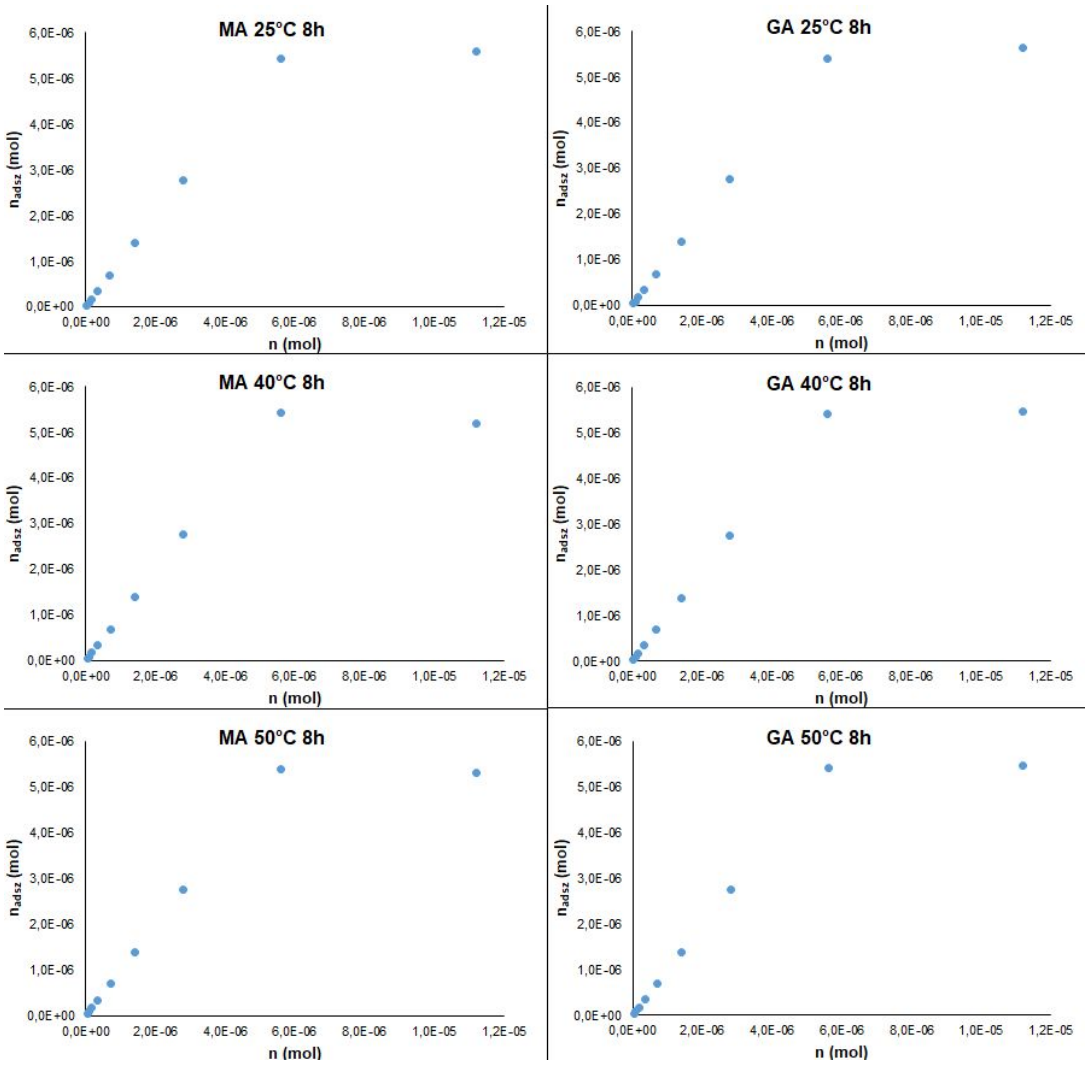
S9 Figure: Calibration curve of glycyrrhizic acid.

API	Linearity	LOD (mg/ml)	LOQ (mg/ml)
baicalin	0.997	0.000106	0.000321
emodin	0.9981	0.00236	0.00715
glycyrrhizic acid	0.9999	0.00126	0.00367

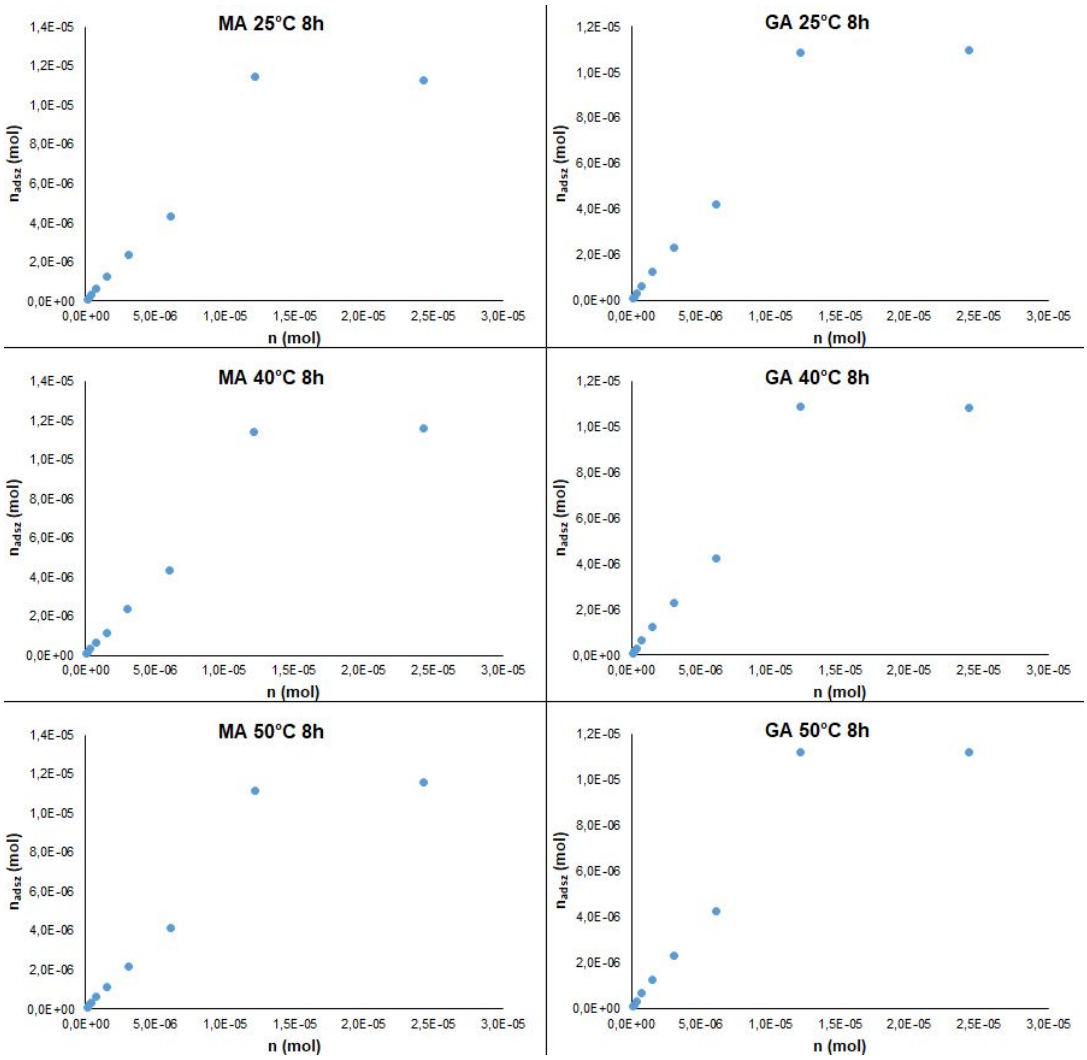
S4 Table: General analytical parameters of APIs.



S10. Figure: Adsorption isotherm of chitosan hydrogels with emodin. MA: hydrogels cross-linked with malic acid. GA: hydrogels cross-linked with glutaric acid.

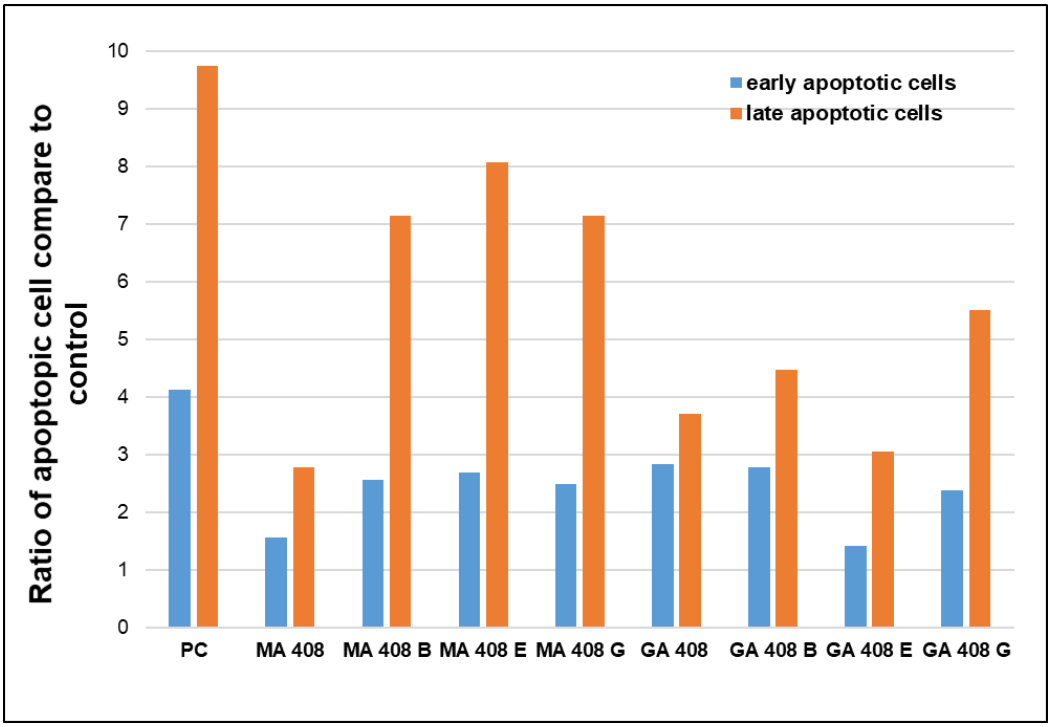


S11. Figure: Adsorption isotherm of chitosan hydrogels with baicalin. MA: hydrogels cross-linked with malic acid. GA: hydrogels cross-linked with glutaric acid.

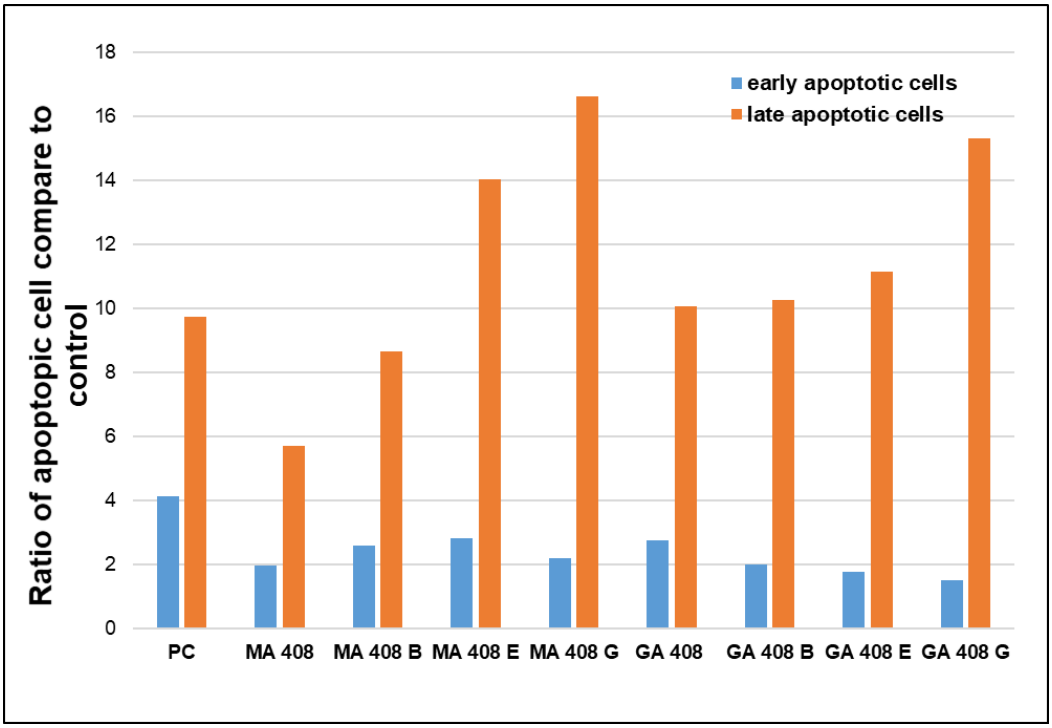


S12. Figure: Adsorption isotherm of chitosan hydrogels with glycyrrhizic acid. MA: hydrogels cross-linked with malic acid. GA: hydrogels cross-linked with glutaric acid.

6. Results of cytotoxicity measurements of hydrogels



S13. Figure: Results of cytotoxicity measurements of HEK 293 cell lines. The ratio of apoptotic cells was calculated to the untreated sample. The concentration of hydrogels were 0.1 mg/ml. PC: positive control (3 V/V% DMOS solution). B: baicalin. E: emodin. G: glycyrrhizic acid.



S14. Figure: Results of cytotoxicity measurements of HEK 293 cell lines. The ratio of apoptotic cells was calculated to the untreated sample. The concentration of hydrogels were 10 mg/ml. PC: positive control (3 V/V% DMOS solution). B: baicalin. E: emodin. G: glycyrrhizic acid.