

Supplementary Information

Development of Enzymatically Crosslinked Natural Deep Eutectogels: Versatile Gels for Enhanced Drug Delivery

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Supplementary Figures

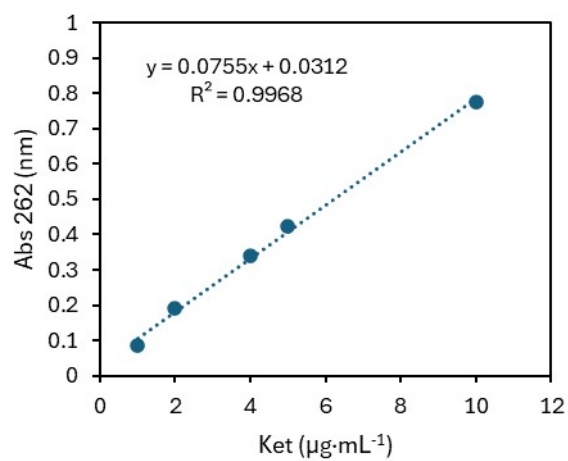
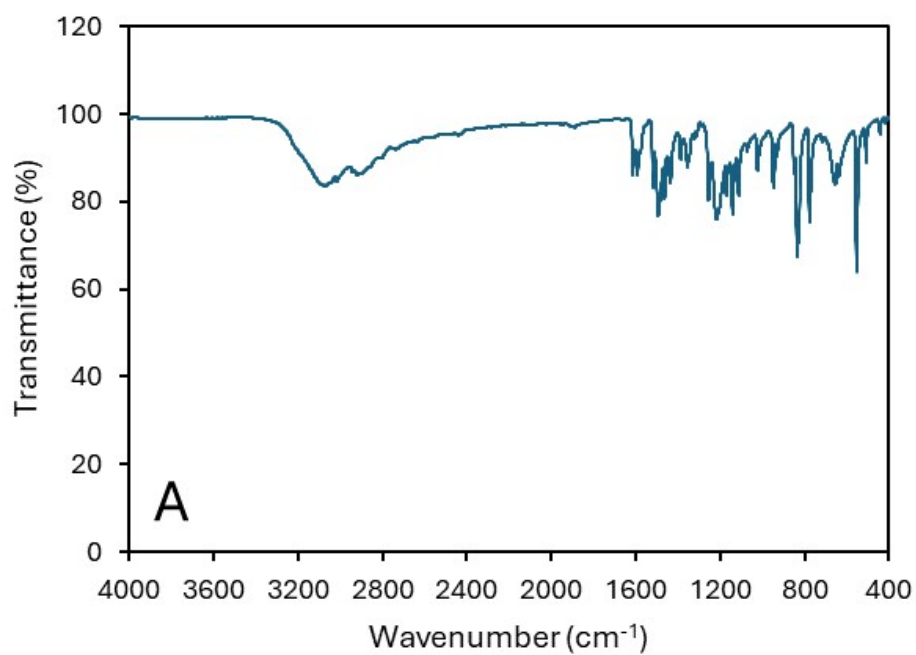


Figure S1 - Calibration curve of ketoprofen in PBS in concentration ranging from 10 to 1 $\mu\text{g}\cdot\text{mL}^{-1}$ and linear regression used for the quantification of ketoprofen on the release studies.



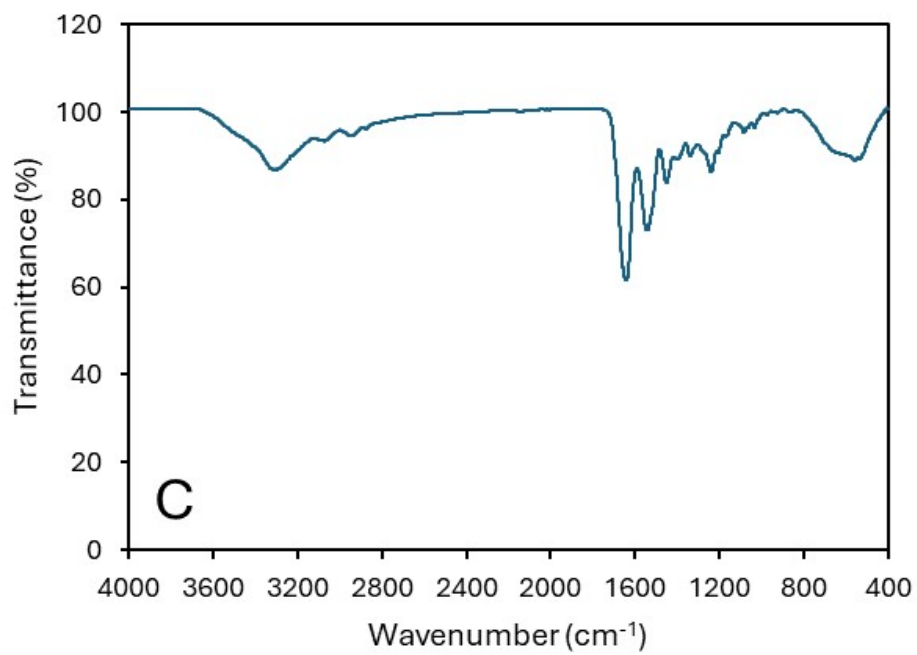
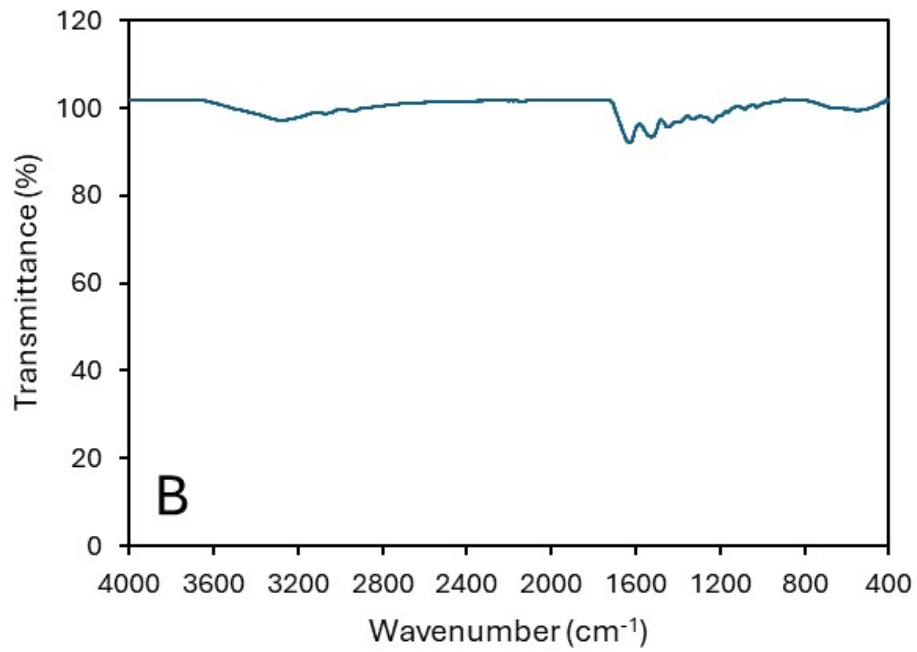


Figure S2 – ATR-FTIR spectra obtained for A) tyramine hydrochloride, B) gelatine, C) Glt-Ph conjugate. The spectra were obtained between 4000 and 400 cm⁻¹, in transmittance mode.

Supplementary Tables

Table S1 – Gelation time obtained for each individual replica of gels prepared with different concentrations of HRP.

HRP (U·mL ⁻¹)	Gelation time (secs)		
	R1	R2	R3
5	42	57	57
10	24	30	36

Table S2 – Data obtained for the measurements of viscosity (mPa·s) as function of shear rate (1/s) for three individual replicas of the mixture 5 %w/v Glt-Ph in 90BGly.

Shear Rate 1/s	R1	R2	R3
	Viscosity mPa·s	Viscosity mPa·s	Viscosity mPa·s
0.00998	6.76E+06	2.11E+06	2.11E+06
0.0149	5.72E+06	1.37E+06	1.37E+06
0.0221	4.43E+06	9.78E+05	9.78E+05
0.0329	3.98E+06	6.68E+05	6.68E+05
0.0489	2.78E+06	3.49E+05	3.49E+05
0.0728	2.53E+06	3.72E+05	3.72E+05
0.108	1.71E+06	2.41E+05	2.41E+05
0.161	9.60E+05	3.36E+05	3.36E+05
0.24	4.23E+05	2.43E+05	2.43E+05
0.356	2.54E+05	1.43E+05	1.43E+05
0.53	95804	1.16E+05	1.16E+05
0.788	1.04E+05	1.08E+05	1.08E+05
1.17	45888	57810	57810
1.74	37231	54453	54453
2.59	28685	29744	29744
3.86	21800	23969	23969
5.74	16897	17113	17113
8.53	12588	10556	10556
12.7	9908.2	8647	8647

Table S3 - Storage (G') and loss (G'') moduli as a function of frequency ($\text{rad}\cdot\text{s}^{-1}$) obtained for three individual replicas of hydrogel prepared with HRP concentration of $10 \text{ U}\cdot\text{mL}^{-1}$.

Frequency $\text{rad}\cdot\text{s}^{-1}$	R1		R2		R3	
	G'' Pa	G' Pa	G'' Pa	G' Pa	G'' Pa	G' Pa
11	94.917	752.11	37.663	597.89	41.087	673.2
8.63	92.635	828.96	36.397	668.65	40.553	748.31
6.8	89.966	875.47	34.897	711.44	39.6	792.47
5.36	86.995	904.56	33.531	737.79	38.695	818.24
4.22	84.107	922.56	32.168	753.37	37.914	832.48
3.33	81.535	935.32	30.869	762.4	36.527	839.72
2.62	79.131	944.93	30.087	767.57	35.645	842.76
2.07	76.73	951.33	29.109	769.32	34.607	841.65
1.63	74.463	955.25	28.204	768.86	33.562	838.08
1.28	73.171	956.92	27.359	766	32.371	832.76
1.01	71.766	958.67	27.29	762.55	31.591	825.12
0.797	69.893	960.92	26.016	759.83	30.9	818.45
0.628	69.621	961.99	25.629	755.99	30.291	812.06

Table S4 - Storage (G') and loss (G'') moduli as a function of frequency ($\text{rad}\cdot\text{s}^{-1}$) obtained for two individual replicas of hydrogel prepared with HRP concentration of $5 \text{ U}\cdot\text{mL}^{-1}$.

Frequency $\text{rad}\cdot\text{s}^{-1}$	R1		R2	
	G'' Pa	G' Pa	G'' Pa	G' Pa
11	74.75	597.64	32.285	775.17
8.63	73.66	670.49	32.121	855.96
6.8	74.169	713.25	32.055	903.67
5.36	74.737	737.22	31.882	932.1
4.22	76.168	748.27	31.949	947.85
3.33	77.316	752.26	32.042	956.03
2.62	78.437	752.07	32.141	959.71
2.07	79.162	747.48	32.108	959.04
1.63	79.634	740.33	32.201	956.37
1.28	79.495	731.91	31.876	951.82
1.01	79.897	721.15	33.626	945.06
0.797	79.935	710.29	32.639	939.78
0.628	81.001	699.55	32.637	934.52

Table S5 - Storage (G') and loss (G'') moduli as a function of frequency ($\text{rad}\cdot\text{s}^{-1}$) obtained for three individual replicas of eutectogel prepared with HRP concentration of $10 \text{ U}\cdot\text{mL}^{-1}$, at different time points (0, 1 month, 3 months and 1 year).

Time 0						
	R1		R2		R3	
Frequency $\text{rad}\cdot\text{s}^{-1}$	G'' Pa	G' Pa	G'' Pa	G' Pa	G'' Pa	G' Pa
11	36.492	50.831	32.688	254.03	30.332	400.26
8.63	32.835	123.76	28.486	328.41	26.882	475.83
6.8	29.966	168.48	25.096	373.99	23.964	521.79
5.36	27.43	195.06	22.245	401.17	21.707	548.95
4.22	25.415	211.46	19.987	417.79	20.115	566.12
3.33	23.907	221.4	18.24	427.56	18.492	576.66
2.62	22.818	229.51	17.04	435.04	17.54	582.46
2.07	21.915	234.44	15.998	438.56	16.692	587.27
1.63	21.265	239.13	15.206	441.3	16.062	591.49
1.28	20.884	244.81	14.708	444.22	15.692	596.84
1.01	20.872	251.89	14.845	448.42	15.968	602.64
0.797	21.534	259.79	15.398	451.62	17.25	608.36
0.628	22.15	268.89	15.816	455.95	18.017	615.69

1 month						
	R1		R2		R3	
Frequency $\text{rad}\cdot\text{s}^{-1}$	G'' Pa	G' Pa	G'' Pa	G' Pa	G'' Pa	G' Pa
11	84.737	170.08	69.36	122.58	62.218	78.616
8.63	74.397	253.37	94.285	153.23	66.532	126.82
6.8	65.594	301.69	85.35	200.8	59.565	179.45
5.36	57.793	329.34	77.784	227.81	53.546	210.27
4.22	51.396	345.77	71.41	242.73	48.515	228.55
3.33	45.656	355.49	65.744	251.39	44.222	239.18
2.62	40.87	362.21	60.874	256.96	40.65	246.19
2.07	36.498	365.53	56.314	258.95	37.443	249.14
1.63	32.692	367.95	52.412	260.08	34.809	250.81
1.28	29.582	369.8	48.846	261.13	32.477	251.72
1.01	26.862	371.93	45.933	262.5	30.738	252.25
0.797	24.448	373.38	43.014	263.42	29.188	252.2
0.628	22.69	374.78	40.758	264.47	27.808	251.6

3 months						
	R1		R2		R3	
Frequency rad·s ⁻¹	G'' Pa	G' Pa	G'' Pa	G' Pa	G'' Pa	G' Pa
11	73.266	110.76	77.168	382.17	63.2	92.549
8.63	75.478	127.33	67.822	452.52	69.584	119.14
6.8	66.332	167.89	59.602	493.79	61.961	160.35
5.36	58.707	190.23	52.466	517.14	55.594	184.26
4.22	52.078	202.66	46.453	529.99	50.058	197.44
3.33	46.341	209.23	41.406	536.53	45.331	204.4
2.62	41.373	213.86	37.185	539.4	41.265	208.91
2.07	36.841	214.88	33.527	539.21	37.561	209.74
1.63	32.923	215	30.382	537.82	34.254	209.68
1.28	29.391	214.71	27.799	535.69	31.293	209.1
1.01	26.335	214.37	25.672	533.16	28.591	208.61
0.797	23.634	213.96	24	530.44	26.144	207.95
0.628	21.282	213.53	22.538	527.81	24.12	207.24

1 year						
	R1		R2		R3	
Frequency rad·s ⁻¹	G'' Pa	G' Pa	G'' Pa	G' Pa	G'' Pa	G' Pa
11	71.751	374.24	87.079	766.83	71.515	372.69
8.63	62.68	451.44	77.644	843.02	64.865	450.58
6.8	54.652	497.51	69.301	888.2	59.413	497.84
5.36	48.082	524.63	62.223	914.19	56.381	523.79
4.22	42.403	540.11	56.28	928.58	53.088	538.11
3.33	37.693	548.37	51.438	935.63	50.34	544.59
2.62	33.938	552.78	47.526	938.75	49.962	549.76
2.07	30.628	554.14	44.169	939.01	48.662	552.48
1.63	27.914	554.01	41.447	937.95	47.012	552.35
1.28	25.69	553.1	39.376	935.69	46.275	545.74
1.01	23.908	551.69	38.044	935.01	44.458	541.48
0.797	22.399	549.97	36.46	935.17	43.96	536.99
0.628	21.195	548.17	35.569	931.79	43.684	531.51

Table S6 - Storage (G') and loss (G'') moduli as a function of frequency ($\text{rad}\cdot\text{s}^{-1}$) obtained for individual replicas of eutectogel prepared with HRP concentration of $5 \text{ U}\cdot\text{mL}^{-1}$, at different time points (0, 1 month, 3 months and 1 year).

Time 0				
Frequency $\text{rad}\cdot\text{s}^{-1}$	R1		R2	
	G'' Pa	G' Pa	G'' Pa	G' Pa
11	30.574	60.613	32.597	150.06
8.63	27.114	143.19	29.102	231.38
6.8	23.759	192.98	26.17	279.46
5.36	21.261	222.88	23.548	308.12
4.22	19.041	240.4	21.415	323.86
3.33	17.153	250.65	19.707	331.79
2.62	15.81	256.61	18.57	335.18
2.07	14.421	258.86	17.423	334.38
1.63	13.377	259.37	16.595	331.57
1.28	12.515	258.75	16.011	326.73
1.01	11.825	257.18	15.322	321.04
0.797	11.416	255.01	14.276	315.4
0.628	11.048	252.86	13.772	309.18

1 month						
Frequency $\text{rad}\cdot\text{s}^{-1}$	R1		R2		R3	
	G'' Pa	G' Pa	G'' Pa	G' Pa	G'' Pa	G' Pa
11	49.33	73.878	49.155	46.938	65.098	133.95
8.63	45.832	101.43	43.163	140.39	66.317	198.1
6.8	40.249	156.27	37.925	196.38	57.505	249.17
5.36	35.492	188.96	33.18	229.94	51.71	279.49
4.22	31.221	208.33	29.195	249.85	45.814	297.5
3.33	27.681	219.6	25.89	261.3	40.762	307.41
2.62	24.807	226.73	23.097	267.86	36.766	312.9
2.07	22.211	229.66	20.594	270.79	33.103	316.21
1.63	20.038	230.85	18.58	271.9	29.945	317.36
1.28	18.129	231.01	16.914	271.97	27.081	318.23
1.01	16.536	230.61	15.561	271.41	24.805	319.07
0.797	15.237	229.89	14.431	270.45	22.876	319.19
0.628	14.116	228.88	13.492	269.43	21.054	318.47

3 months						
	R1		R2		R3	
Frequency rad·s ⁻¹	G'' Pa	G' Pa	G'' Pa	G' Pa	G'' Pa	G' Pa
11	66.266	303.44	71.005	177.51	60.072	211.8
8.63	57.041	375.22	61.221	255.09	51.781	289.78
6.8	49.135	418	52.874	301.47	44.57	336.44
5.36	42.486	443.48	45.63	328.62	38.446	363.88
4.22	36.848	457.93	39.532	344.37	33.295	379.94
3.33	32.083	465.33	34.254	353	28.903	389
2.62	28.146	468.78	29.897	358.23	25.365	394
2.07	24.791	469.53	26.067	359.87	22.22	395.82
1.63	22.02	468.61	22.928	360.02	19.597	396.05
1.28	19.551	466.4	20.307	359.28	17.451	395.35
1.01	17.566	463.55	18.119	358	15.681	394.05
0.797	15.801	460.64	16.357	356.37	14.228	392.42
0.628	14.255	457.2	14.908	354.66	12.994	390.77

1 year						
	R1		R2		R3	
Frequency rad·s ⁻¹	G'' Pa	G' Pa	G'' Pa	G' Pa	G'' Pa	G' Pa
11	78.63	427.99	74.739	444.66	76.141	201.03
8.63	68.259	509.8	65.525	528.85	68.189	283.69
6.8	59.405	558.73	57.909	578.12	60.823	332.56
5.36	52.169	587.3	51.355	606.96	54.704	360.27
4.22	46.062	603.46	46.061	623.17	49.748	375.34
3.33	40.933	612.17	42.122	635.04	45.564	383
2.62	36.729	616.13	38.258	643.73	42.175	385.42
2.07	33.166	617.27	34.939	644.93	38.945	385.13
1.63	30.285	616.8	32.431	643.29	36.488	383.64
1.28	27.937	615.33	30.551	640.13	34.435	380.95
1.01	25.984	613.26	29.113	636.1	32.557	379.55
0.797	24.317	611.08	27.845	631.53	31.185	378.09
0.628	23.028	608.67	28.558	623.66	30.268	376.13

Table S7 – Absorbance (Abs) obtained at 262 nm, and dilution factor (df), of the samples of the drug release from BGly.

Time days	BGly1		BGly2		BGly3	
	Abs	df	Abs	df	Abs	df
0.00	0.075	-	0.049	-	0.06	-
0.02	0.112	-	0.059	-	0.467	-
0.04	0.13	-	0.073	-	0.634	8
0.08	0.156	-	0.437	-	0.562	8
0.17	0.532	4	0.934	16	0.562	8
0.25	0.652	16	0.582	32	0.687	32
0.33	0.588	32	0.785	32	0.468	32
1.00	0.560	32	0.725	64	0.743	32
2.00	0.498	50	0.594	50	0.553	50
3.00	0.639	50	0.586	50	0.509	50
6.00	0.721	50	0.553	50	0.577	50
8.00	0.675	50	0.577	50	0.669	50
10.00	0.72	50	0.651	50	0.659	50

Table S8 – Absorbance (Abs) obtained at 262 nm, and dilution factor (df), of the samples of the drug release from eutectogel.

Time days	Eutectogel 1		Eutectogel 2		Eutectogel 3	
	Abs	df	Abs	df	Abs	df
0.00	0.257	-	0.098	-	0.097	-
0.02	0.421	-	0.174	-	0.112	-
0.04	0.424	-	0.45	-	0.152	-
0.08	0.709	-	0.447	-	0.919	-
0.17	1.095	16	0.51	8	0.603	16
0.25	0.626	32	0.56	8	1.066	16
0.33	0.884	32	0.467	8	1.096	16
1.00	0.481	64	0.911	16	0.611	32
2.00	0.525	50	0.459	50	0.388	50
3.00	0.561	50	0.501	50	0.478	50
6.00	0.642	50	0.581	50	0.51	50
8.00	0.605	50	0.61	50	0.605	50
10.00	0.634	50	0.762	50	0.708	50

Table S9 – Absorbances obtained from the MTS assay that were used to determine the cell viability in the presence of 90BGly 125 mg·mL⁻¹, Glt-Ph 1.04 %w/v, H₂O₂ 0.75 mM, and HRP 2.44 U·mL⁻¹.

Sample	<i>n = 1</i>			<i>n = 2</i>		
	Abs 1	Abs 2	Abs 3	Abs 1	Abs 2	Abs 3
Control cells	0.293	0.291	0.293	0.280	0.326	0.304
90BGly	0.241	0.251	0.215	0.233	0.233	0.229
Glt-Ph	0.208	0.211	0.232	0.319	0.331	0.315
H₂O₂	0.272	0.267	0.295	0.299	0.312	0.302
HRP	0.271	0.284	0.289	0.309	0.314	0.297