Electronic Supplementary Information

Development of colorimetric PEG-based hydrogel sensors for urea detection

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1 Experimental

1.1 Materials

Poly(ethylene glycol) diacrylate (PEG700-DA, M_n = 700 g mol^{-1}), 2-hydroxy-2-methylpropiophenone (Irgacure®1173, 97 %), urease from *Canavalia ensiformis* (Jack bean) 50,000-100,000 units g^{-1} solid, urea (ACS reagent, 99.0-100.5 %) and bovine serum albumin (BSA, heat shot fraction, pH 7, ≥ 98 %) were purchased from Sigma Aldrich. L(+) - Lactic acid (anhydrous, 98 %) was purchased from Alfa Aesar. Phenol red sodium salt, poly(ethylene glycol) (PEGx, where x = M_n = 600 or 1000 or 3000 g mol^{-1}), potassium chloride (KCl), sodium chloride (NaCl), ammonium chloride (NH_4Cl), calcium chloride ($CaCl_2$) and sodium bicarbonate ($NaHCO_3$) were purchased from Fisher Scientific. Phosphate buffer tablets pH = 7 were also purchased by Fisher Scientific. Bioskin® artificial skin. All materials were used as received unless otherwise stated.

1.2 Working solutions

***Preparation of Artificial Sweat*:** The chemical compounds listed in **Table S1**, were mixed in 950 mL of DI H_2O. The pH of the solution was adjusted to pH = 7 using a solution of 0.1 M NaOH. The mixture was transferred to a volumetric flask and made up to a volume of 1 L with DI H_2O. The final concentration of urea was 0.3 mM. A second artificial sweat mixture was also formulated containing 20 mM of urea. Mixtures were kept at ambient temperature for no more than a week.

Table S1. Artificial sweat components

Chemical components	Mass conc. g/L
Potassium Chloride (KCl)	0.37
Sodium Chloride (NaCl)	5.08
Ammonium Chloride (NH_4Cl)	0.11
Calcium Chloride ($CaCl_2$)	0.22
L(+) Lactic Acid	0.90
Urea	0.02
Bovine Serum Albumin or BSA	0.80
Sodium Bicarbonate ($NaHCO_3$)	0.84

1.3 Instrumentation and Methodology

***Nuclear Magnetic Resonance (^{1}H-NMR)*:** NMR spectra were recorded on a Bruker DPX-400 MHz instrument using deuterium oxide (D_2O) or DMSO-d_6 as solvents. Chemical shifts were given as δ in parts per million (ppm) while the analysis was carried out using the ACD/NMR Processor software. Hydrogel kinetic experiments were conducted by transferring pre-cured hydrogel mixtures in NMR tubes initially wrapped in aluminium foil. UV curing followed using a conveyor belt. The LOD for the PEG700-DA crosslinker was determined by analysing various concentrations of the polymer in DMSO-d_6 until the complete disappearance of monomer peaks.

***UV-Vis spectrophotometry*:** The UV absorbance of all studied compounds was recorded on an Agilent Technologies Cary 60 UV-Vis spectrometer using a glass cuvette (from HELLMA Analytics) with a 10 mm optical length. A range of 200-700 nm was selected using distilled water (DI water) or phosphate buffer pH = 7 as solvent.

***UV light source*:** For all curing reactions, a VCP-15-LAB benchtop UV curing conveyor belt system was manufactured by GEW(EC) Ltd (**Figure S1**). The unit was equipped with a 150 mm wide stainless steel conveyor belt (5-70 m min^{-1}) and a E2C®UV ozone-free mercury lamp (UVC region 200-280 nm with a distinct emission at 254 nm, 125 mJ (cm^2)$^{-1}$) integrated with an air exhaust fan. In all curing experiments, a speed of 5 m min^{-1} was used corresponding to ~ 10 sec of irradiation per sample. The standard maximum operating temperature was 40 °C.

Figure S1. The VCP-15-LAB benchtop UV curing conveyor belt used for the hydrogel synthesis.

***Mechanical analysis*:** Mechanical testing was performed on a Shimadzu EZ-LX Universal Testing Instrument equipped with a 50 N load cell. For the compression tests, hydrogels were cured in cylindrical buttons (Diameter: 2.5 cm, Height: 0.7 cm) using plastic transparent moulds, **Figure S2**. A preload force of 0.01N was set with a compression velocity of 5 mm min^{-1}. Experiments were repeated at least 8 times and the mean average values were calculated to determine the ultimate stress (kPa), strain at break (%) and compression modulus (kPa). The compression modulus was calculated at 10% of the stress/strain curve using **Eq.(S1)** :

$$\text{Compression Modulus} = \frac{\text{Stress}}{\text{Strain}}_{(\text{kPa})} \qquad \textbf{Eq. (S1)}$$

***Rheology/Tack testing*:** Rheology was performed on an Anton Parr MCR 302 rheometer equipped with a parallel plate (25 mm diameter) at 25 ℃ or 37 ℃. In all oscillatory sweep experiments, cured hydrogel discs were used (diameter: 25 mm and height: 1.4 mm) (**Figure S3**). Amplitude sweep experiments were conducted at a strain range of 0.01 to 400 % with a constant angular frequency of ω = 10 rad s^{-1}. Frequency sweep measurements were performed at an angular frequency ω = 0.1 to 100 rad s^{-1} using a constant strain of 0.1 % previously determined by the linear viscoelastic region (LVER) of the studied materials. Tack tests were performed at 25 ℃. Hydrogel discs were placed at the bottom plate of the rheometer and the probe was set 0.05 mm above the sample programmed to lower down at a velocity of 0.5 mm s^{-1} for 1 sec and then reverse back at the same rate. The normalised force (F_N) was measured as a function of time. All rheological measurements were performed at least three times using statistical analysis to calculate an average of the total runs.

***Thermogravimetric Analysis (TGA)*:** Experiments were carried out on a Mettler Toledo TGA1/DSC1-STAR^e instrument operated under nitrogen flow (50 mL min^{-1}) at a heating rate of 10 ℃ min^{-1}. Hydrogel samples were heated from 25 ℃ to 600 ℃ in 40 µL aluminium pans containing ~5 mg of sample. The decomposition temperature (T_{deg}) was defined from the inflection points.

***Differential scanning calorimetry (DSC)*:** Thermal analysis was carried out on a Mettler Toledo DSC1-STAR^e differential calorimeter operated under nitrogen flow (50 mL min^{-1}) at a heating rate of 10 ℃ min^{-1} for two heating and cooling cycles. Hydrogel samples were loaded into 40 µL aluminium pans heated from -100 ℃ up to 100 ℃. In every measurement, the glass transition temperature (T_g) values were determined from the midpoint of the step change in the

thermograms of every second cycle to erase thermal history. The melting temperatures (T_m) were determined as the melting maxima while the enthalpies of melting (ΔH_m) were equal to the area of the melting peak.

***Fourier Transform Infrared Spectroscopy (FTIR)*:** Infrared spectroscopy was conducted on a Bruker ALPHA II Fourier transform infrared spectrometer fitted with a crystal plate and a pressure tower running with a speed of 0.5 cm s^{-1} at 65 scans per sample.

***Scanning Electron Microscopy (SEM)*:** Microscopy analysis was conducted on a Zeiss Gemini500 scanning electron microscope equipped with a field emission electron gun. The accelerating voltage was 4 kV while an InLens detector was used at a working distance of ~2 mm. In a typical sample preparation, a hydrogel specimen was initially frozen with liquid nitrogen and further freeze dried to remove water. Freeze dried gels were stuck onto carbon tabs (9 mm) attached to aluminium specimen stubs and their surface was carefully scratched with a scalpel to reveal their interior. Finally, to improve sample imaging, samples were sputter coated with gold (Au) for 15 seconds using a plasma coater.

***Circular Dichroism (CD)*:** Experiments were performed on a Jasco J-1500 circular dichroism spectrophotometer at a sample concentration of 8 mg mL^{-1} (optimum concentration found for this analysis) in phosphate buffer pH = 7. Instrument parameters: wavelength range λ = 300 - 180 nm, D.I.T. = 1 s, scan rate = 100 nm min^{-1} and bandwidth = 2 nm.

***Size Exclusion Chromatography (SEC)*:** SEC analysis was performed on an Agilent Infinity II MDS instrument equipped with differential refractive index (DRI), viscometry (VS), dual angle light scattering (LS) and dual ultraviolet (UV) detectors. The system contained 2 × PLgel Mixed C columns (300 × 7.5 mm) and a PLgel 5 μm guard column while the mobile phase consisted of THF with 0.1 % butylated hydroxytoluene (BHT) at 30 °C with a flow rate of 1 mL min^{-1} and an injection volume of 100 μL. Poly(methyl methacrylate) (PMMA) standards (purchased from Agilent EasyVials) were used as calibrants. Polymers were dissolved in THF and filtered through a GVHP nylon membrane (0.22 μm pore size) before analysis. Dispersity ($Đ$) and experimental molar mass ($M_{n,SEC}$) were calculated using Agilent GPC/SEC software.

***Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-ToF- MS)*:** MALDI-ToF-MS analysis was performed on a Bruker Autoflex Speed MALDI-ToF mass spectrometer, containing a nitrogen laser delivering 2 ns pulses at 337 nm with positive ion ToF detection at an accelerating voltage of 25 kV. Samples were prepared by initially mixing trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene] malononitrile

(DCTB) as a matrix (20 μL) and analyte (10 mg mL^{-1}). Finally, 0.5 μL from this mixture was applied to the target place. Spectra analysis was conducted in linear mode after calibrating with PEG 2,000 Da.

***Smartphone used for RGB quantification and urea detection*:** For the RGB quantification of urea from the surface of hydrogel sensors, an Apple iPhone 8 Plus was equipped with a 12 MP main camera. The camera contained a CMOS image sensor with an aperture of F1.8, a focal length of 28 mm, a sensor size of 1/3'', a pixel size of 1.22 μm and a dual LED flashlight.

1.4 Experimental Procedures

Typical protocol for the preparation of pristine PEG700-DA/PEGx hydrogels

In a typical pristine hydrogel synthesis, DI H_2O (40 mL), PEG700-DA crosslinker (50 mL, 0.08 mol, 1 eq.) and PEGx (where x : 600, 1000, 3000 g mol^{-1}) (30 mL for 24.6 vol%, 60 mL for 39.5 vol% and 100 mL for 52.1 vol%) were mixed in an aluminium foiled jar. Irgacure® 1173 photoinitiator (2 mL, 0.01 mol, 0.125 eq. with respect to the moles of crosslinker) was then added following stirring for ~10 min. Finally, 1 mL of hydrogel pre-cured solution was transferred to in-house PTFE moulds and exposed to UV light (200 - 280 nm) for 10 sec using a conveyor belt.

Typical protocol for the preparation of PEG700-DA/PEGx hydrogel sensors containing 0.73 mg mL^{-1} urease and 0.14 mg mL^{-1} phenol red sodium salt

For the standard preparation of a urease hydrogel sensor, a stock solution, ''Sensor matrix'', containing urease (40 mg, 8 mg mL^{-1}) and phenol red sodium salt (7.5 mg, 1.5 mg mL^{-1}) was initially prepared in 5 mL of phosphate buffer pH = 7. Then, 10 mL of hydrogel premixture solution was mixed with 1 mL of the prepared ''*Sensor matrix*'' leading to a final hydrogel sensor premixture containing 0.73 mg mL^{-1} of urease and 0.14 mg mL^{-1} of phenol red sodium salt. Finally, 1 mL of hydrogel sensor pre-cured solution was transferred to in-house PTFE moulds and exposed to UV light (200 - 280 nm) for 10 sec using a conveyor belt yielding orange-like urea sensor gels.

Swelling kinetic studies

For all swelling experiments, three measurements were performed simultaneously following crosslinking. Hydrogel disc samples were measured immediately after fabrication by placing them in petri dishes filled with DI H_2O. The DI H_2O was replaced regularly to remove any

possible unreacted compounds and avoid the build-up of solute concentration. At predetermined time intervals, samples were removed and gently blotted dry prior recording their weight. The swelling ratio % was determined based on **Eq. (S2)**:

$$\text{Swelling ratio (\%)} = \frac{W_s - W_w}{W_w} \times 100 \qquad \textbf{Eq. (S2)}$$

where W_s is the weight of the swollen hydrogel at a specific time interval and W_w the weight of the initial wet weight before immersing.

Urease kinetic investigations by UV-Vis spectroscopy

For the enzyme kinetic experiments, a stock solution of 0.68 mg mL^{-1} urease (34.2 mg) and 0.03 mg mL^{-1} of phenol red sodium salt (1.5 mg) was prepared in 50 mL of DI H_2O annotated as ‘‘Solution A’’. Stock solutions of urea in DI H_2O were prepared by diluting a 0.8 M solution of urea as shown in **Table S2**. Kinetics were followed by UV-Vis spectroscopy by monitoring the absorbance change of phenol red by time at λ = 559 nm for a total of 8 min. For each measurement, 2 mL of Solution A was added in a glass cuvette following the addition of 100 µL of a selected urea stock solution leading to a final concentration of $[Urea]_{final}$ (substrate) in the cuvette as also indicated in **Table S2**. Experiments were conducted in duplicates.

Table S2. Urea stock solutions and the final concentration of urea when performing urease kinetics.

Preparation of stock solutions from a 0.8 M urea solution	**Stocks solutions of Urea (M)**	**$[Urea]_{final}$ (M)**
25 µL 0.8 M urea + 1975 µL H_2O	0.01	0.0005
50 µL 0.8 M urea + 1975 µL H_2O	0.02	0.0008
105 µL 0.8 M urea + 1975 µL H_2O	0.042	0.002
325 µL 0.8 M urea + 1975 µL H_2O	0.13	0.006
525 µL 0.8 M urea + 1975 µL H_2O	0.21	0.01
1050 µL 0.8 M urea + 1975 µL H_2O	0.42	0.02
-	0.8	0.038

From enzyme kinetic principles,[66] an increase in the concentration of urea (substrate) was expected to increase the velocity of the enzymatic reaction since more molecules would be converted into products as described by the Michaelis-Menten equation, **Eq. (S3)**:

$$V_0 = \frac{V_{max} \times [S]}{K_M + [S]} \qquad \textbf{Eq. (S3)}$$

where V_0 is the initial rate of the enzymatic reaction at the steady state, [S] the substrate concentration at a given time, V_{max} is the maximum rate of reaction when all the active sites of the enzyme are saturated with a substrate and K_M is the Michaelis constant indicative of the enzyme's affinity to the substrate.

Quantification of urea from the surface of hydrogel sensors by the RGB methodology

In a typical RGB quantification experiment, hydrogel sensor disc specimens were fabricated and reacted with 20 μL of different urea concentrations. To ensure the complete hydrolysis of urea on the surface of the gels, sensors were left to develop for at least 10 min. Then, the specimens were transferred in a dark room and images were taken using the LED flashlight of a smartphone. Selected photos were uploaded to ImageJ software to determine the RGB values of the fuchsia-coloured spots and attain a relation between the RGB levels and the concentration of urea. The Blank intensity values were attained from the RGB readings of unreacted spots on the surface of the sensors from the same image to keep ambient light interference constant. The RGB levels were then transformed into absorbance RGB intensities and graphed against several concentrations of urea to attain a calibration curve for every different colour channel. For comparison, principal component analysis (PCA) using the average RGB levels of sensor standards was performed using OriginLab software. The PC1 data were then plotted against the logarithm of the concentration of urea to attain a calibration curve. The determination of the LOD and LOQ values was achieved using the method based on the SD of the 'Y' intercept after analysing 8 replicates for every different urea concentration.

Steps for RGB determination in ImageJ software

1. Open the desired image with the sensors. Select the desired fuchsia spot using the cursor.
2. Select the desired fuchsia spot by drawing a circle using the "circle tool". To use the same Pixel Area for every fuchsia spot, set *via* the ROI Manager: Analyse → Tools → ROI Manager. Measure the Pixel Area with Analyse → Measure. For each analysis the used Pixel Area was 9,946 pixels^2.
3. Plugins → Analyse → Measure RGB

Leachables study

Leachable experiments were performed on artificial skin (Bioskin®) that mimicked the human's skin texture. Hydrogels (×3 replicates) were cast in squares and applied on the top of the fake skin prior wetting its surface with 30 μL of DI H_2O to mimic real skin's conditions. Hydrogels were sealed in a vial and incubated for 1 hr at 37 ℃. After 1 hr, the gels were removed, and the fake skin was extracted with 5 mL of DI H_2O for 24 hr to assure complete extraction of components. After removal of DI H_2O *in vacuo*, the crude solids were analysed by NMR and GC-FID.

Hydrogels extraction process

Standard pristine PEG700-DA/PEGx hydrogel formulations and their sensor analogues were extracted to analyse their extractables. Soxhlet extractions were performed using MeOH as the solvent (150 mL). A standard Soxhlet set-up was used placing pre-weighted gel samples in separate glass-wool Soxhlet thimbles and refluxed at 100 ℃ for 2 hr. The solvent was then removed *in vacuo* to analyse the amount of extracted PEG free chains using SEC using the below equation, **Eq.(S1)**:

$$RI\ (mV) = K_{RI} \times dn/dc \times C_{pol.} \times V_{inj.} \quad \textbf{Eq. (S1)}$$

2 Supporting Information

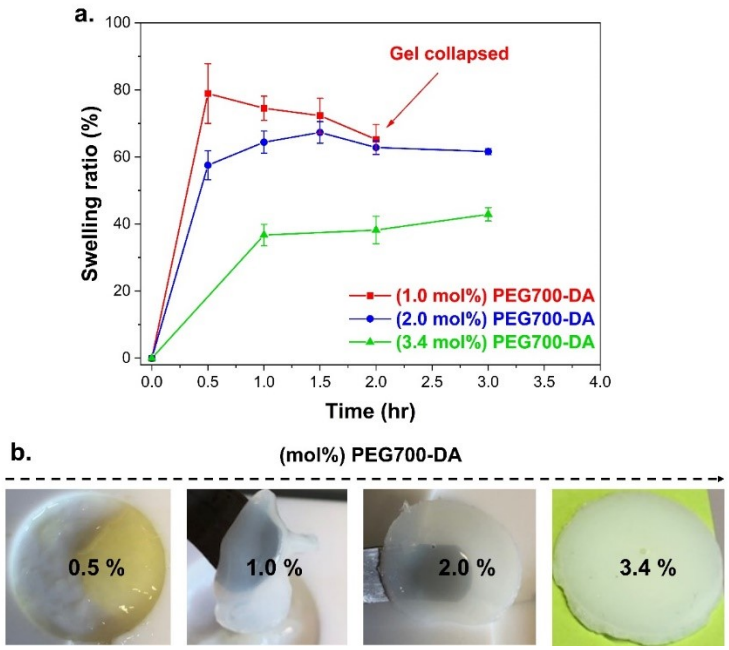

Figure S2. (a) Comparison of the swelling kinetics of PEG700-DA/PEG1000 (39.5 vol%) hydrogels containing different mol% of PEG700-DA crosslinker (×4 replicates) in DI water at ambient temperature along with (b) representative images of the examined hydrogels synthesized with 0.5, 1.0, 2.0 and 3.4 mol% of crosslinker. The highest the mol% the stronger the hydrogel.

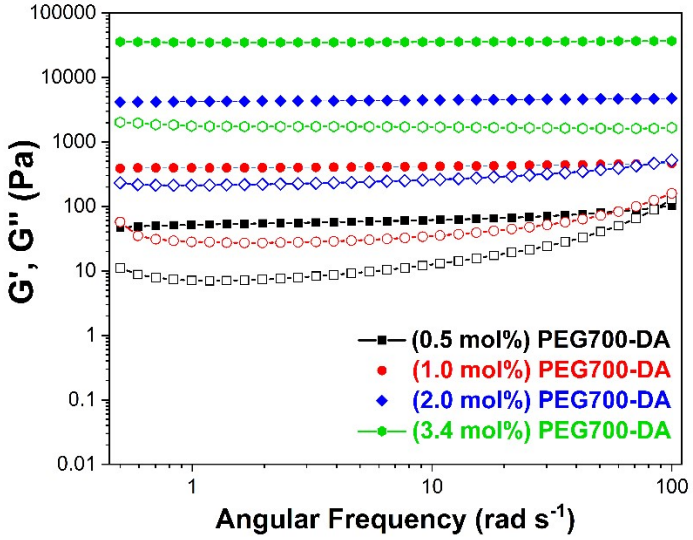

Figure S3. Frequency sweeps of PEG700-DA/PEG1000 (39.5 vol%) formulations synthesised by 0.5, 1.0, 2.0 and 3.4 mol% of PEG700-DA crosslinker at a constant strain of $\gamma = 0.1\%$.

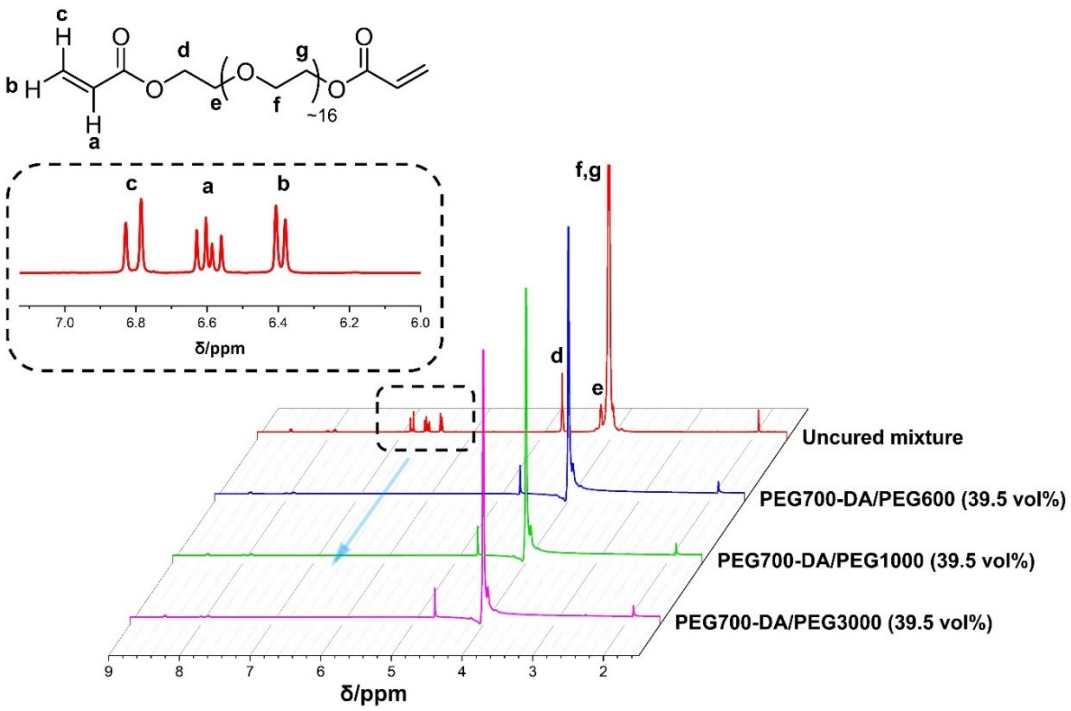

Figure S4. ^{1}H-NMR (400 MHz, D_2O) spectra of an uncured mixture and three fully cured hydrogels: PEG700-DA/PEG600 (39.5 vol%), PEG700-DA/PEG1000 (39.5 vol%) and PEG700-DA/PEG3000 (39.5 vol%) after 10 sec exposure in high intensity UV light (×1 pass in the conveyor belt) demonstrating >99% monomer conversion.

Figure S5. Cylindrical hydrogel samples used for compression testing

Table S3 Tabulated rheological results of PEG700-DA/PEGx hydrogels at 37 ℃ using oscillation mode, examining the effect of mol% of crosslinker and different vol% and M_n of PEGx. Data

Entry	PEG700-DA (mol%)	Storage Modulus (G') (Pa)	Loss Modulus (G'') (Pa)	Crossover point (%)
PEG700-DA	3.5	104,615.0 ± 2,000.0	2,870.8 ± 435.5	9.3 ± 0.2
PEG700-DA/PEG1000 **(24.6 vol%)**	3.4	53,320.0 ± 2,900.0	903.2 ± 88.0	9.4 ± 0.0
PEG700-DA/PEG1000 **(39.5 vol%)**	0.5	53.7 ± 9.2	10.1 ± 2.2	N/A
PEG700-DA/PEG1000 **(39.5 vol%)**	1.0	401.5 ± 74.2	25.4 ± 5.1	N/A
PEG700-DA/PEG1000 **(39.5 vol%)**	2.0	4,314.1 ± 853.4	220.8 ± 48.3	N/A
PEG700-DA/PEG1000 **(39.5 vol%)**	3.4	35,173.5 ± 550.0	17,560.0 ± 47.0	55.3 ± 5.2
PEG700-DA/PEG1000 **(52.1 vol%)**	3.3	28,667.0 ± 2,000.0	1,069.5 ± 176.2	141.7 ± 0.7
PEG700-DA/PEG600 **(39.5 vol%)**	3.3	42,050.0 ± 3,000.0	30,817.0 ± 13868.0	41.7 ± 2.5
PEG700-DA/PEG3000 **(39.5 vol%)**	3.5	106,608.0 ± 51,100.0	1,760 ± 47.0	15.8 ± 3.4

represented as a Mean ± SD value from at least ×3 replicates.

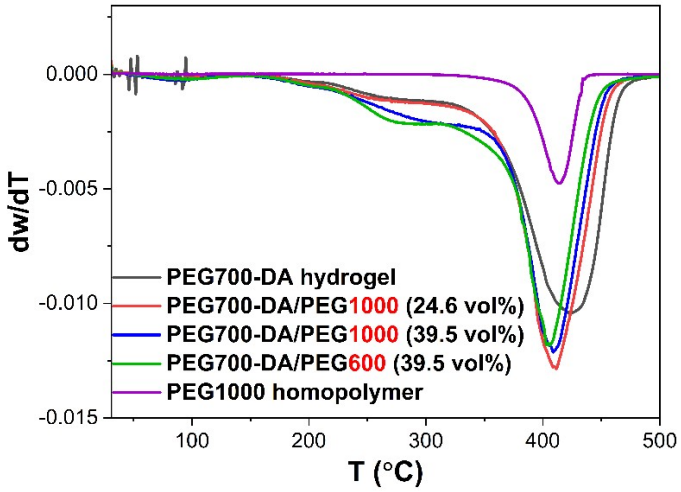

Figure S6. TGA first derivative curves

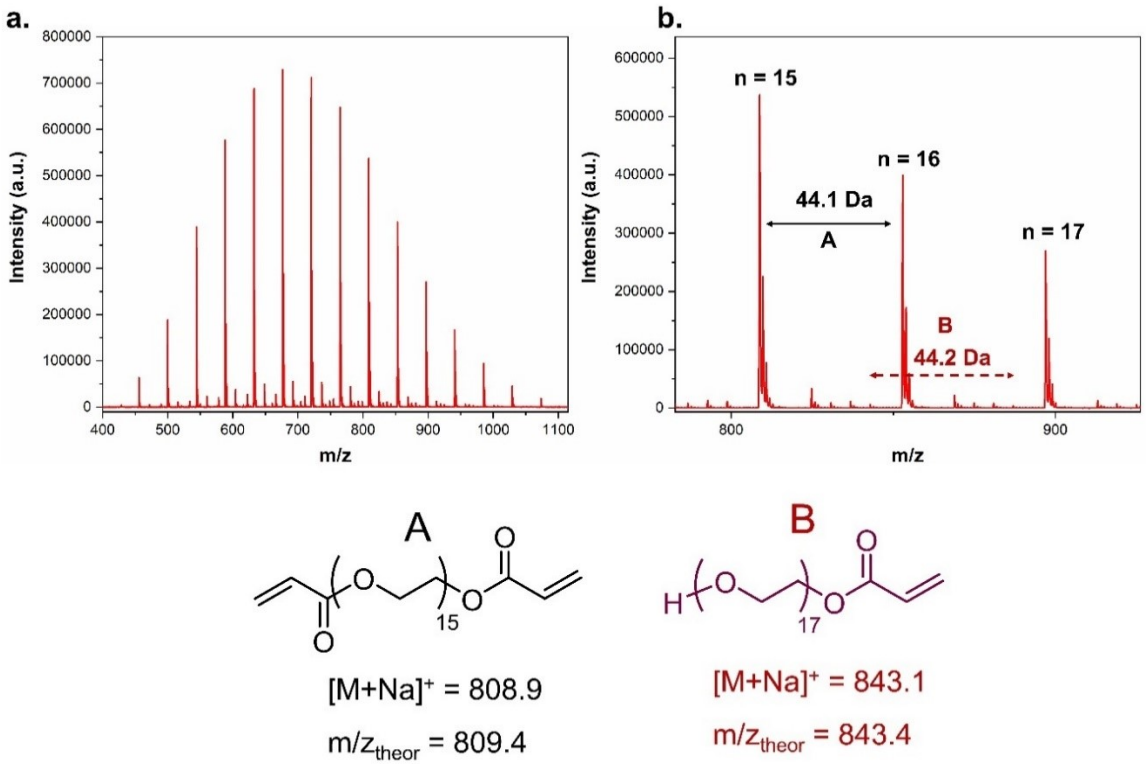

Figure S7. MALDI-ToF analysis of commercial PEG700-DA crosslinker (a) MALDI spectra along with (b) zoomed region where two species are detected A and B.

Table S4. DSC analysis results for PEG700-DA/PEGx hydrogels and their components as found from the 2nd thermal cycle.

Entry	$T_{g,\ midpoint}$ (℃)	$T_{g,\ onset}$ (℃)	T_m (℃)
PEG700-DA	-	-	16.0
PEG700-DA gel	-39.2	-43.5	-
PEG1000	-	-	40.0
PEG700-DA/PEG600 (24.6 vol%)	-43.4	-48.5	20.2
PEG700-DA/PEG1000 (24.6 vol%)	-41.0	-46.0	38.7
PEG700-DA/PEG3000 (24.6 vol%)	-39.3	-43.4	62.1

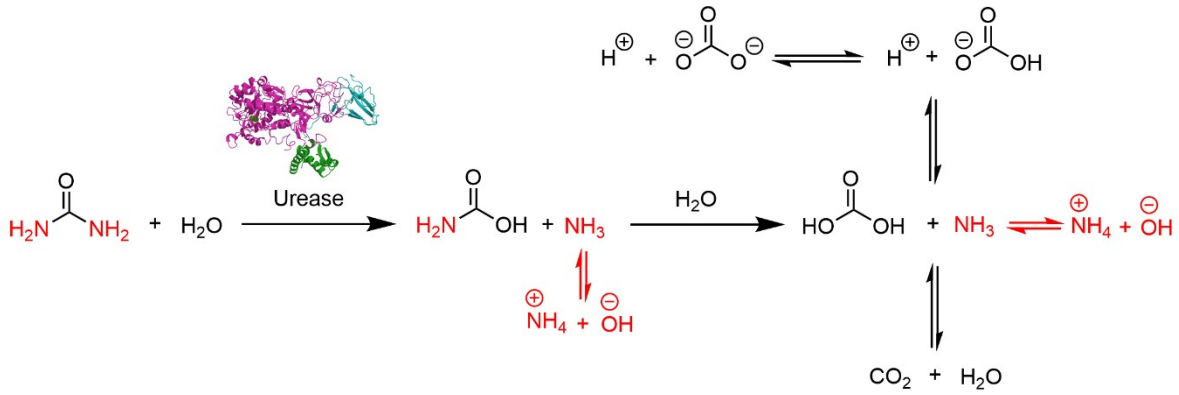

Figure S8. The catalytic hydrolysis of urea from urease enzyme in water.

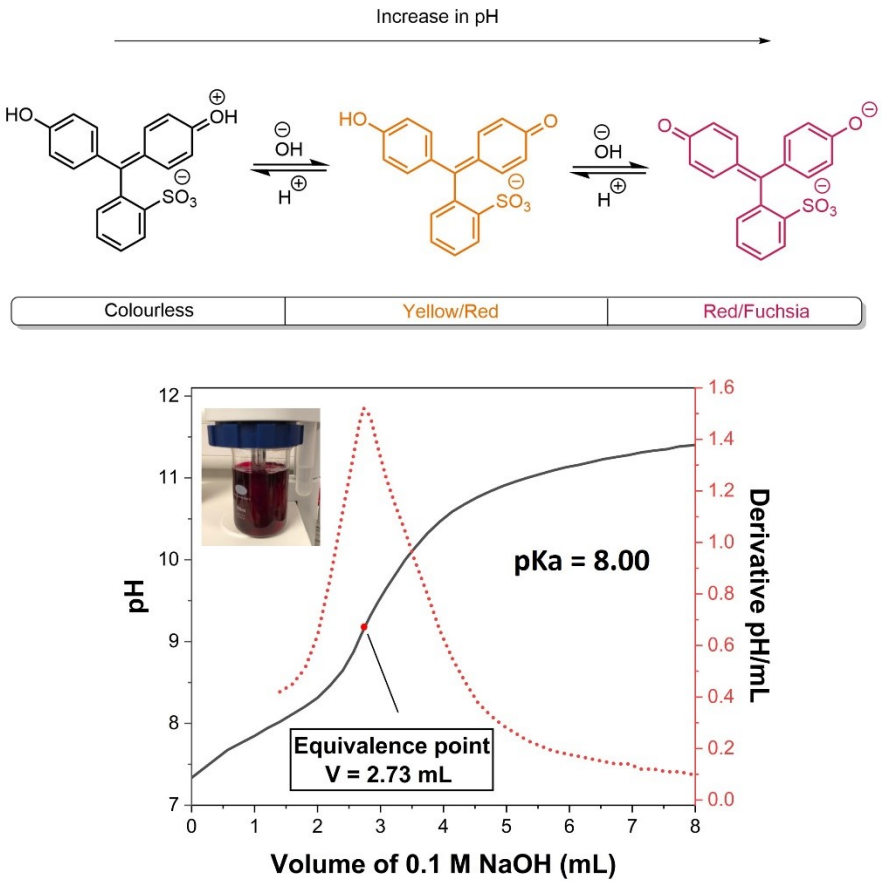

Figure S9. The three forms of phenol red indicator at different pH environments along with their distinct colours and titration results of a 0.01 M solution of phenol red titrated with 0.1 M NaOH.

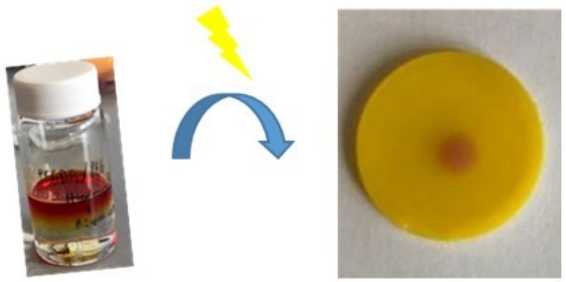

Figure S10. Preliminary sensor test after addition of 20 µL of 0.015 M urea solution on the surface of a sensor gel.

Table S5. Tabulated response times after addition of 20 µL of urea solutions in duplicates. The response times correspond to the first visualization of fuchsia colour on the surface of PEG700-DA/PEG1000 hydrogels.

Entry	Urea (M)	Response 1 (min)	Response 2 (min)
PEG700-DA/PEG1000 (24.6 vol%)	0.02	8	8
	0.015	6	6
	0.002	8	7
PEG700-DA/PEG1000 (39.5 vol%)	0.02	4	4
	0.015	4	4
	0.002	4	4
PEG700-DA/PEG1000 (52.1 vol%)	0.02	>15	>15
	0.015	-	-
	0.002	-	-

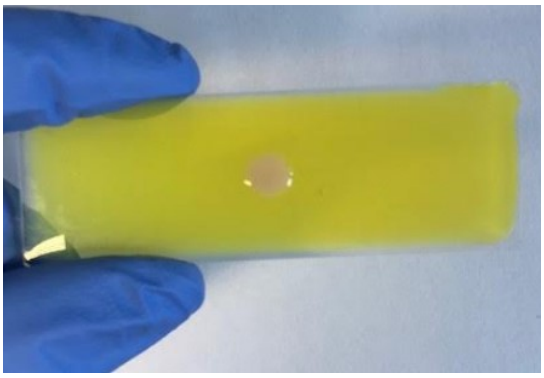

Figure S11. PEG700-DA/PEG1000 (39.5 vol%) sensor gels of 0.65 mm thickness containing 1.46 mg mL^{-1} of urease and 0.28 mg mL^{-1} of phenol red.

Table S6. Tabulated rheological results of PEG700-DA/PEG1000(39.5 vol%) hydrogels containing different amounts of phenol red (0.05, 0.1, 0.2, 0.4 mg mL^{-1}) at 37 °C using oscillation mode. Data represented as a Mean ± SD value from at least (3 replicates).

[Ph. Red] (mg mL^{-1})	PEG700-DA (mol%)	Irgacure® 1173 (mol%)	Solid content (wt%)	G' (Pa)	G'' (Pa)	Crossover point (%)
Blank	2.5	0.4	69.5	17,430 ± 890	795 ± 80	62.0
0.05	2.5	0.4	69.5	18,332 ± 256	592 ± 89	62.0
0.1	2.5	0.4	69.5	15,584 ± 245	805 ± 83	62.0
0.2	2.5	0.4	69.5	16,319 ± 320	827 ± 80	62.0
0.4	2.5	0.4	69.5	14,500 ± 170	675 ± 97	62.0

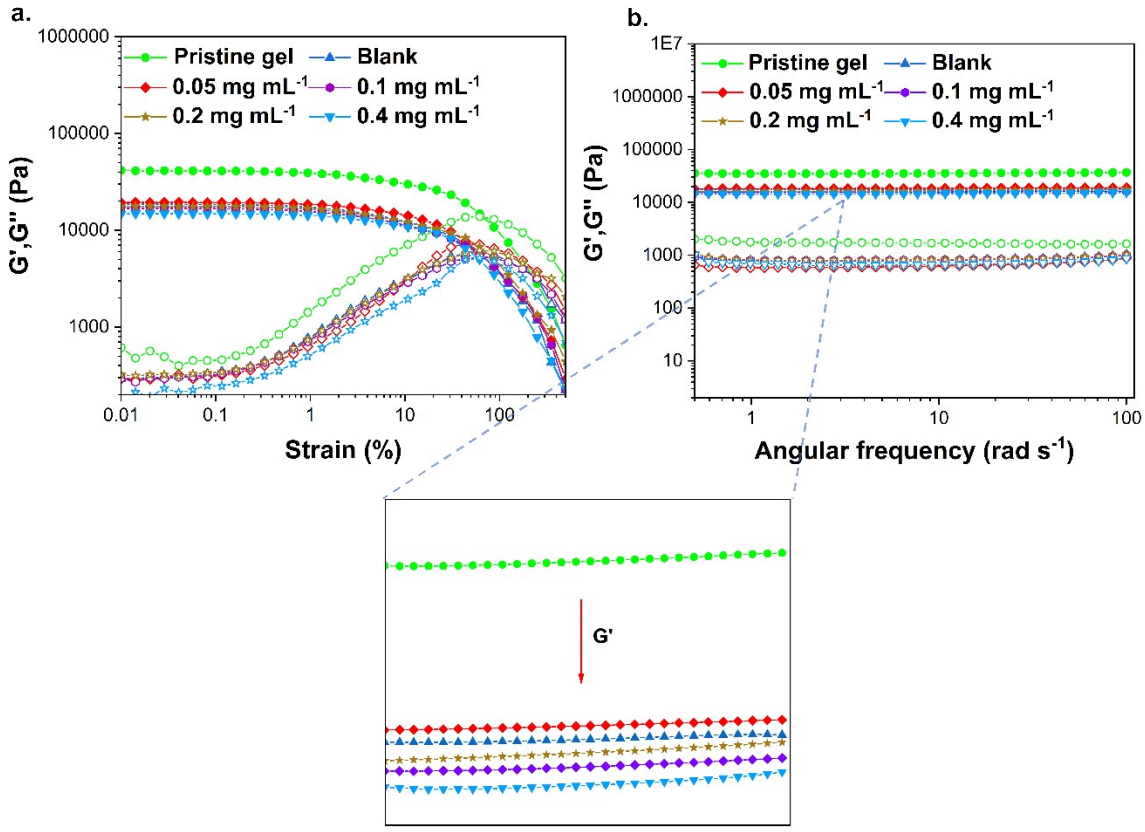

Figure S12. (a) Amplitude (γ = 0.01-600%, $\omega_{constant}$ = 10 rad s^{-1}) and (b) frequency sweep experiments ($\gamma_{constant}$ = 0.1%, ω = 0.5-100 rad s^{-1}) of PEG700-DA/PEG1000(39.5 vol%) sensor gels containing various amounts of phenol red indicator. Experiments were conducted in triplicates at 37 °C using an oscillatory mode where storage modulus (G', filled symbols) and loss modulus (G'', empty symbols).

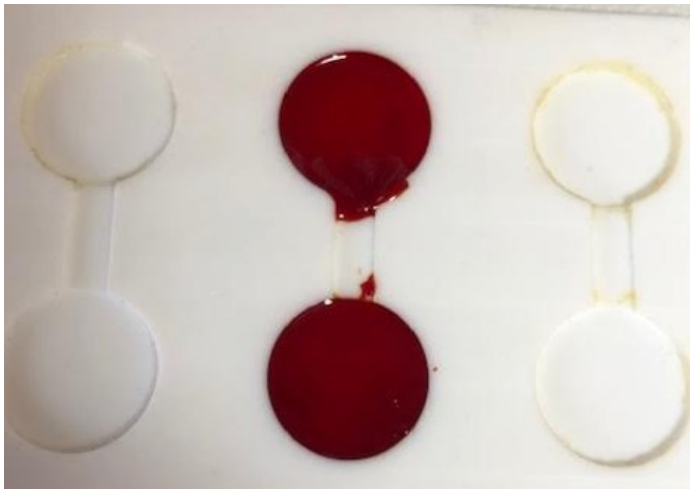

Figure S13. Sensors containing a high concentration of phenol red (10 mg mL^{-1}) failing to cure.

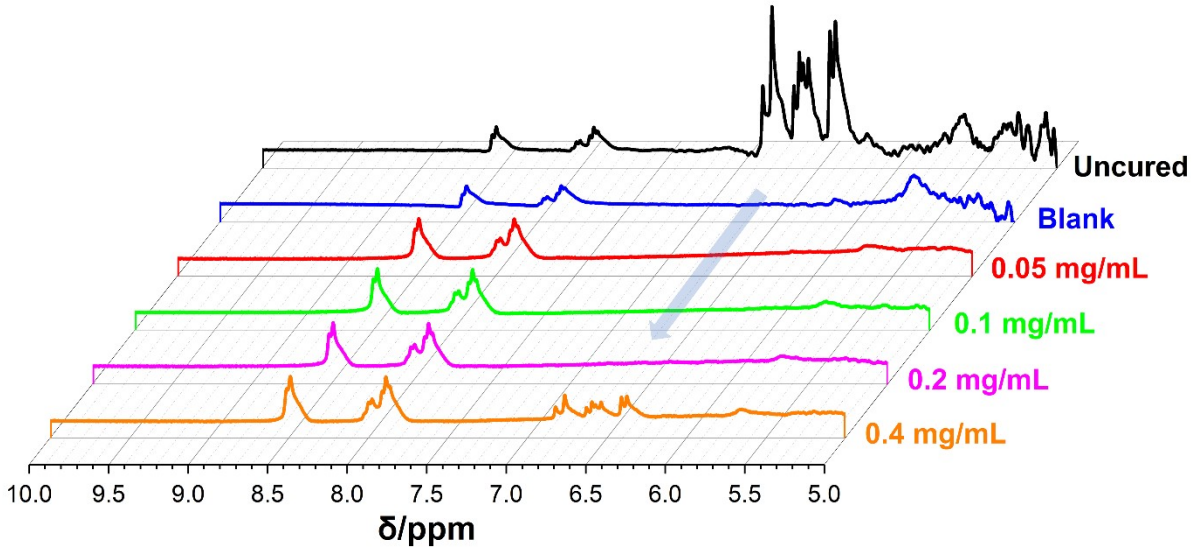

Figure S14. ^{1}H-NMR (400 MHz, D_2O) spectra comparison of an uncured PEG700-DA/PEG1000 (39.5 vol%) mixture, a cured Blank and four cured ones containing different concentrations of phenol red (0.05, 0.1, 0.2 and 0.4 mg mL^{-1}) after 10 sec exposure under high intensity UV light (×1 pass in the conveyor belt inside NMR tubes).

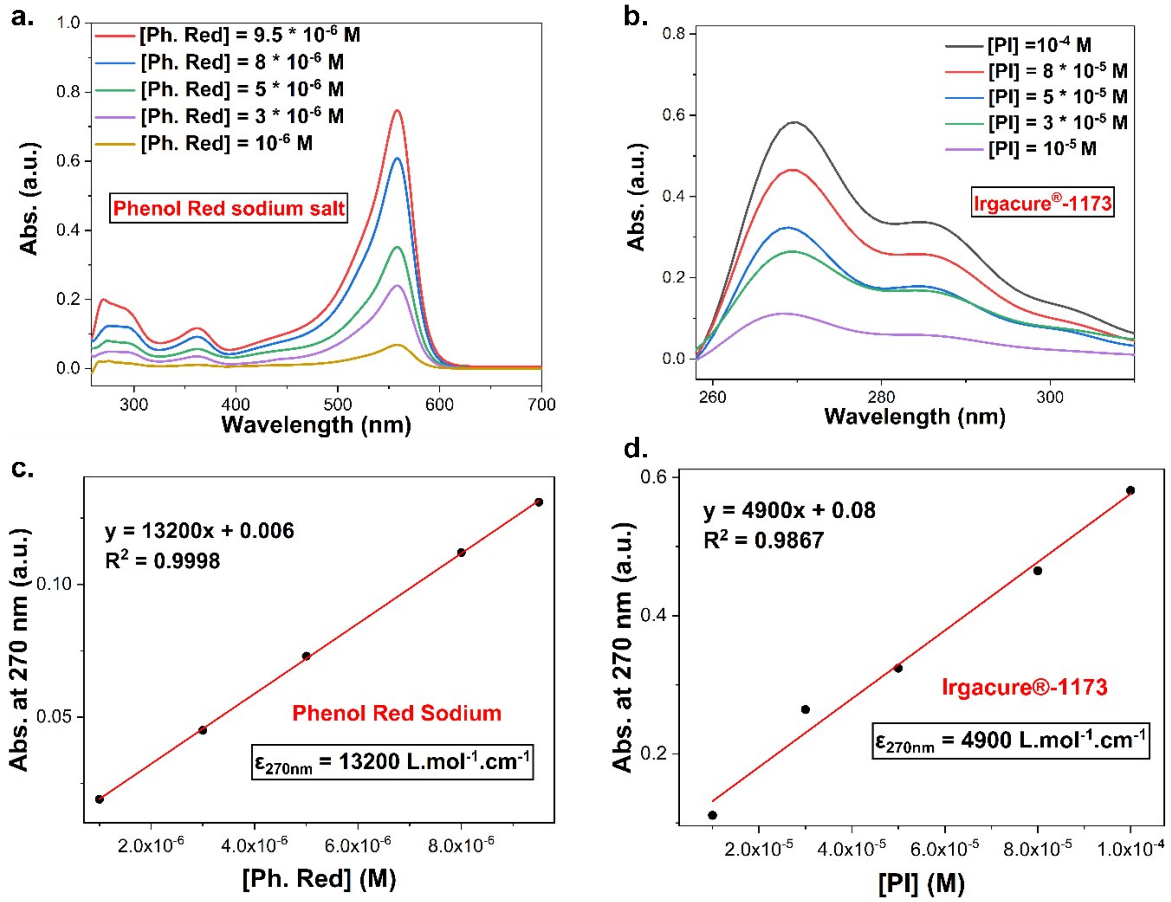

Figure S15. UV-Vis absorption spectra of (a) phenol red (b) Irgacure®1173 photoinitiator at various concentrations along with their calibration curves (c), (d) at λ = 270 nm for the determination of the molar extinction coefficient (ε) values.

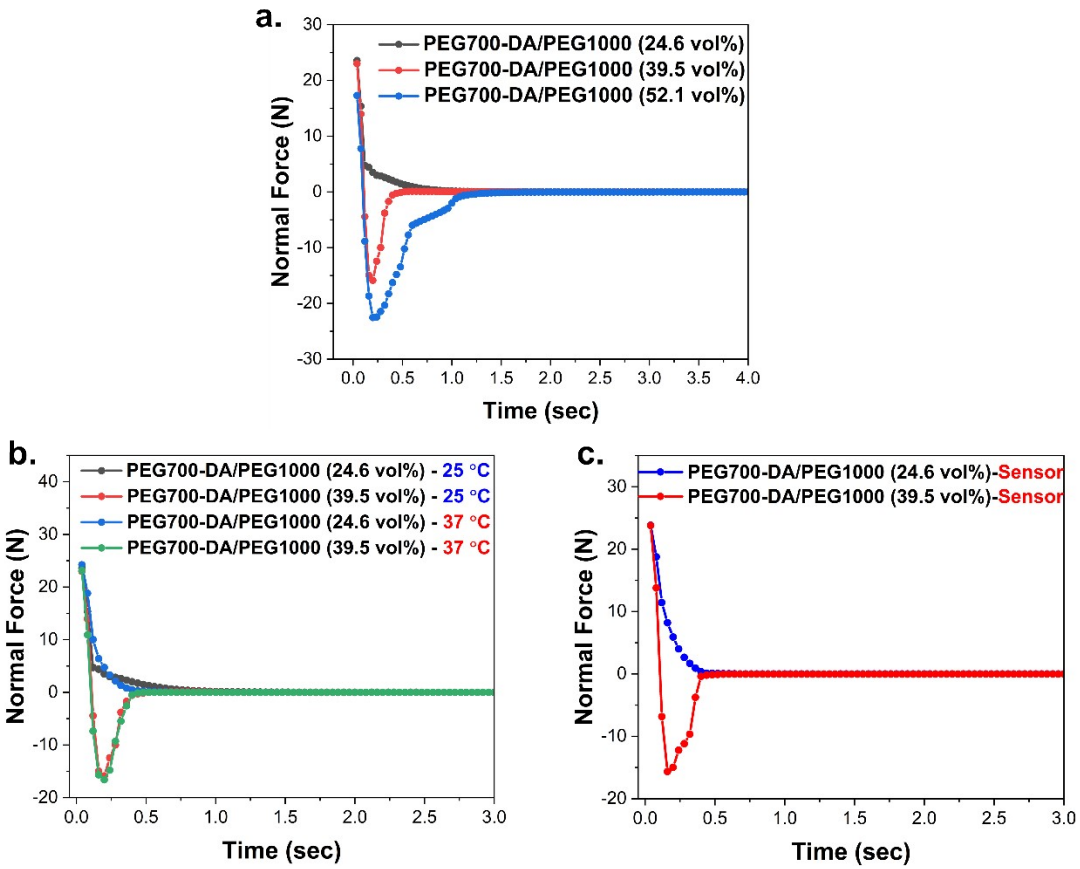

Figure S16. Normal force-time profiles for (a) pristine PEG700-DA/PEG1000 formulations containing different vol% of PEG1000, (b) pristine PEG700-DA/PEG1000 formulations at different temperatures and (c) PEG700-DA/PEG1000 sensor gels containing 0.73 mg mL^{-1} of urease and 0.14 mg $mL^{-1}$of phenol red at 37 °C. Tests were performed in duplicate.

Table S7. Tabulated tack tests results using the inverted probe technique.

PEG1000 (vol%)	PEG700-DA (mol%)	T (°C)	Peak Force (N)	Area
24.6	3.4	25	0	0
24.6	3.4	37	0	0
24.6	2.5	37	0	0
39.5	3.4	25	-16.95 ± 0.78	2.51 ± 0.62
39.5	3.4	37	-17.10 ± 0.85	2.76 ± 0.66
39.5	2.5	37	-17.10 ± 0.89	2.86 ± 1.14
52.1	3.3	25	-23.02 ± 2.16	9.63 ± 3.53

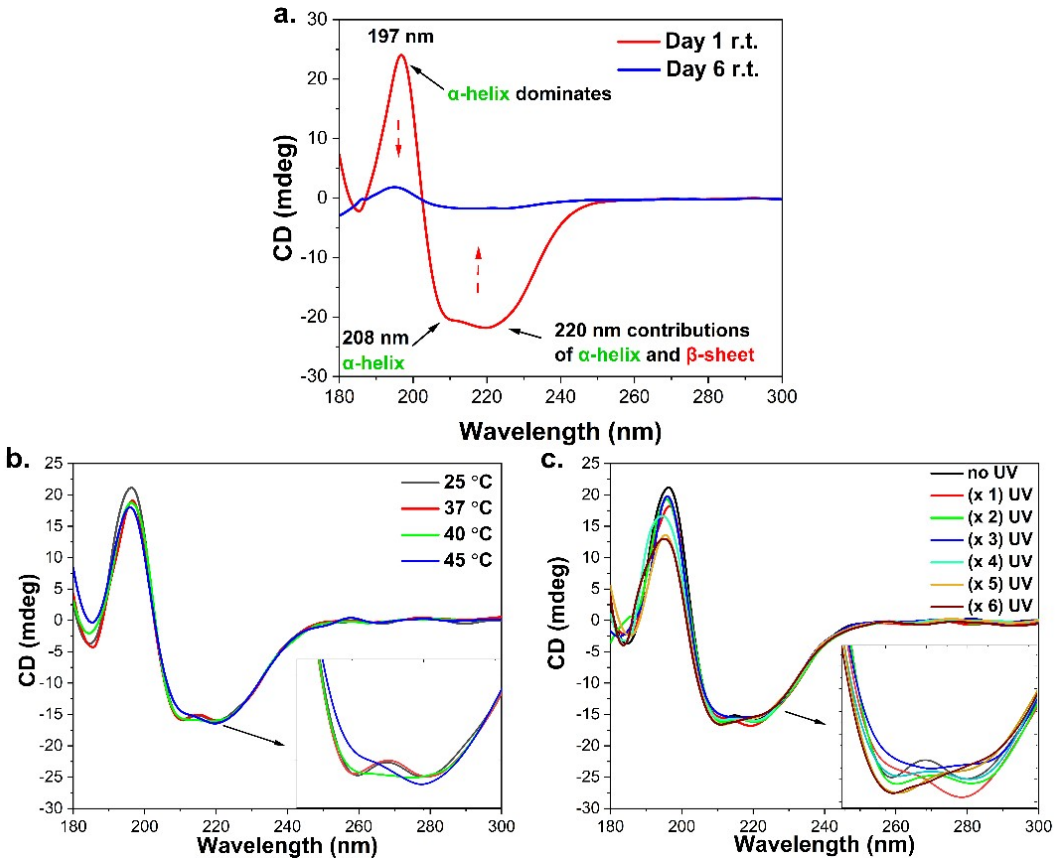

Figure S17. Circular dichroism (CD) spectra of free urease (a) after 6 days at ambient temperature, (b) at various increasing temperatures and (c) at different UV (λ = 200-280 nm) exposure times.

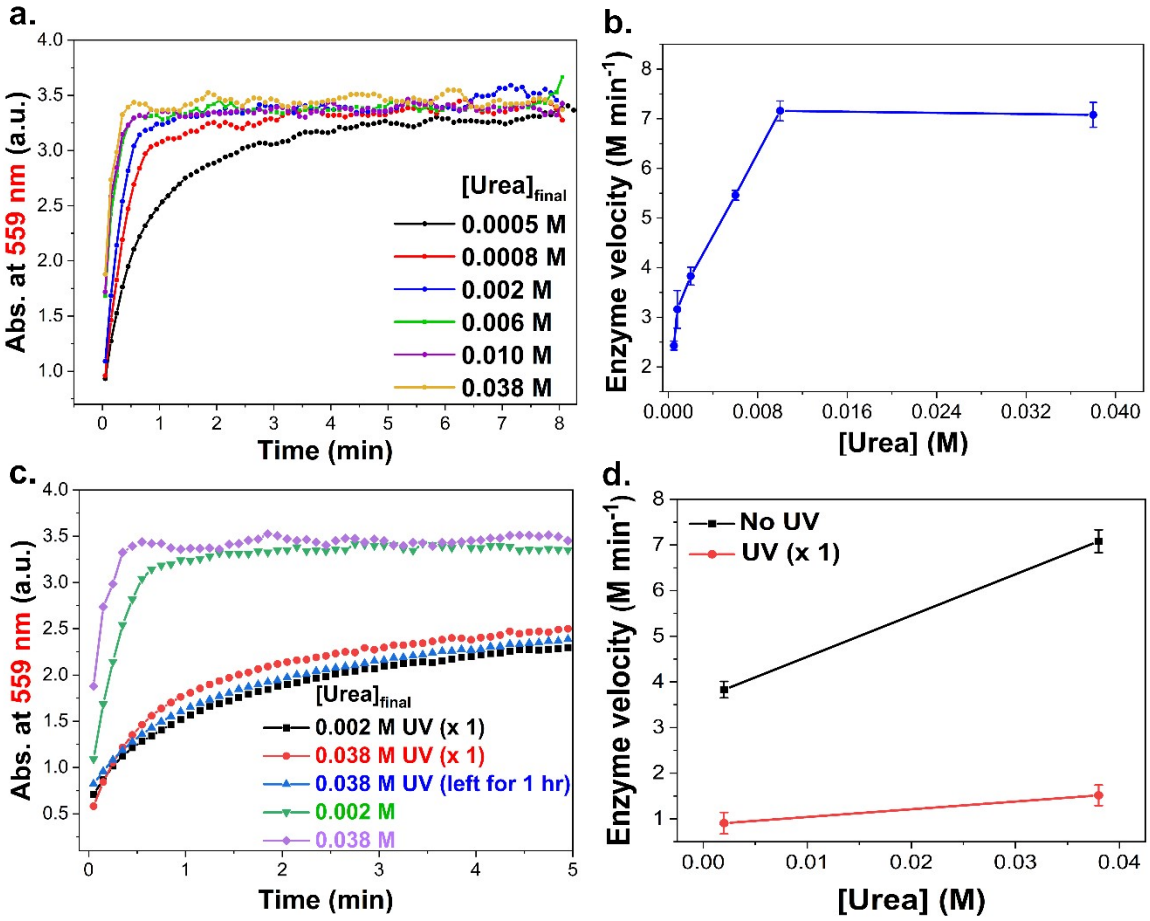

Figure S18. (a) Absorption at 559 nm against time for different concentrations of urea (b) dependence of enzyme's velocity on the concentration of urea (c) absorption at 559 nm against time for 0.002 and 0.038 M of urea after (×1) pass under UV light and after letting the solution settle for 1 hr at ambient temperature (d) comparison of enzyme's before and after exposure to UV light at 0.002 and 0.038 M of urea. All kinetic experiments were conducted at ambient temperature in duplicates using a UV light of 200-280 nm.

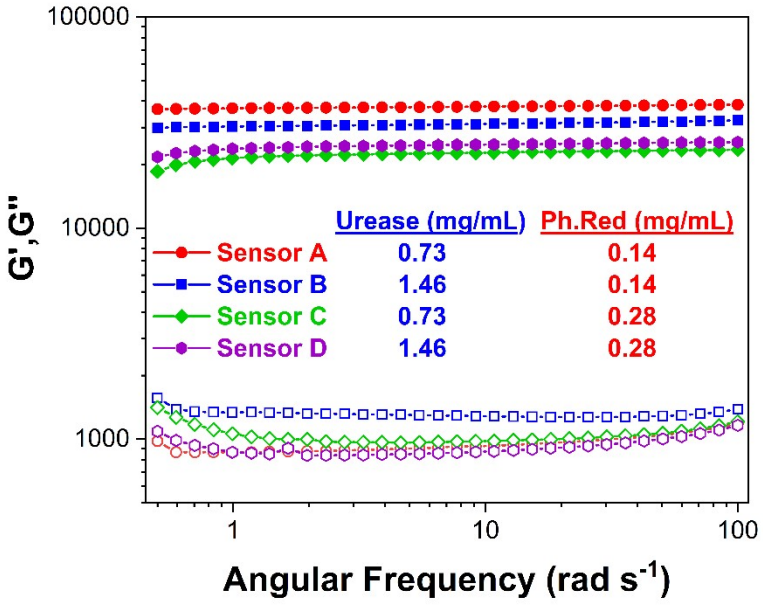

Figure S19. Frequency sweeps of PEG700-DA/PEG1000(39.5 vol%) sensor formulations with various amounts of urease and phenol red using a constant strain of $\gamma = 0.1\%$. Experiments were conducted at 37 °C using oscillatory mode where storage modulus (G', filled symbols) and loss modulus (G'', empty symbols).

Figure S20. Hydrogel sensor against a control model sweat solution without urea showing no colorimetric response.

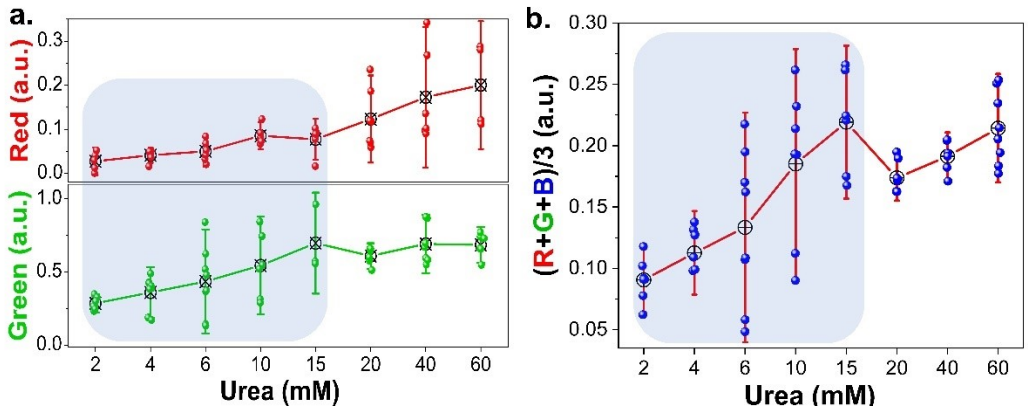

Figure S21. (a) Calibration curves of Red, Green, Blue and (b) average (R+G+B)/3 absorption intensities with concentrations of 2-60 mM shown as an average of 8 replicates. Linear regions are highlighted with blue.

Table S8. Principal component analysis (PCA) of the average RGB data from the pictures of the sensor standards. The PC1 values were selected.

[Urea], mM	Log(urea), mM	Red	Green	Blue	PC
2	0.30	0.028	0.29	-0.02	-1.32
4	0.60	0.040	0.36	-1.4	-0.54
6	0.78	0.050	0.43	-0.7	-0.08
10	1.00	0.070	0.54	-1.0	0.64
15	1.18	0.080	0.68	-0.9	1.30

Standardised; eigenvalue = 0.997; % variance = 99.7%

Figure S22. PEG700-DA/PEG1000 (39.5 vol%) sensor gels (×3 replicates) on the top of Bioskin® specimens for leachable studies.

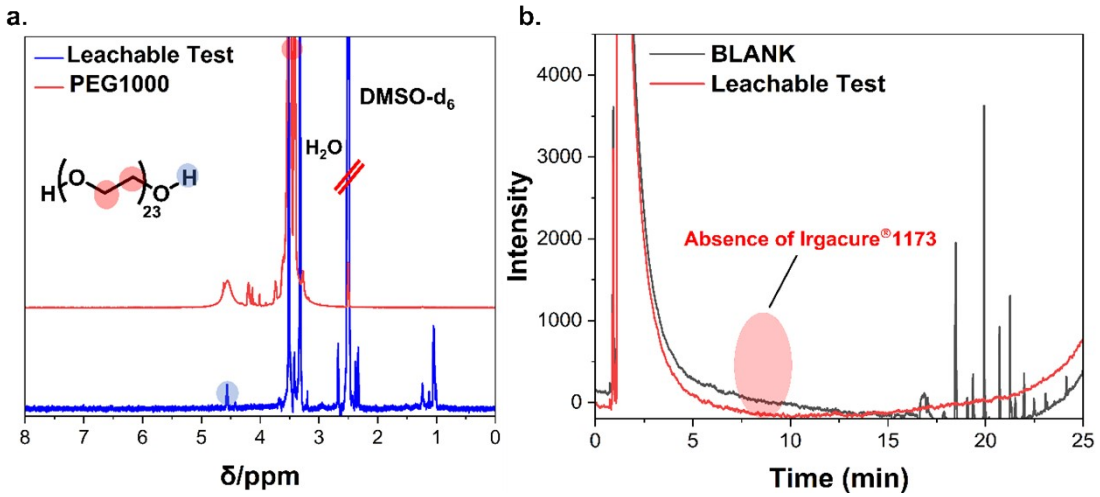

Figure S23. (a) Overlayed ^{1}H-NMR (400 MHz, DMSO-d_6) spectra of crude leachable product from a PEG700-DA/PEG1000 (39.5 vol%) pristine gel compared to a PEG1000 spectrum along with the (b) GC-FID chromatogram in $CHCl_3$ showing the absence of Irgacure®1173.

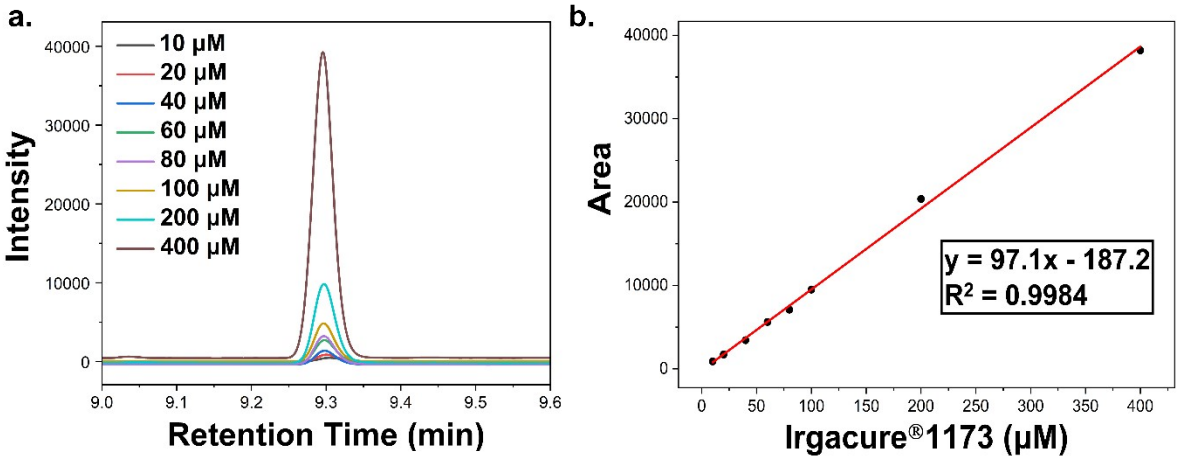

Figure S24. (a) GC-FID chromatograms of Irgacure®1173 in the range of 10-400 µM (b) calibration curve of Irgacure®1173 in $CHCl_3$.

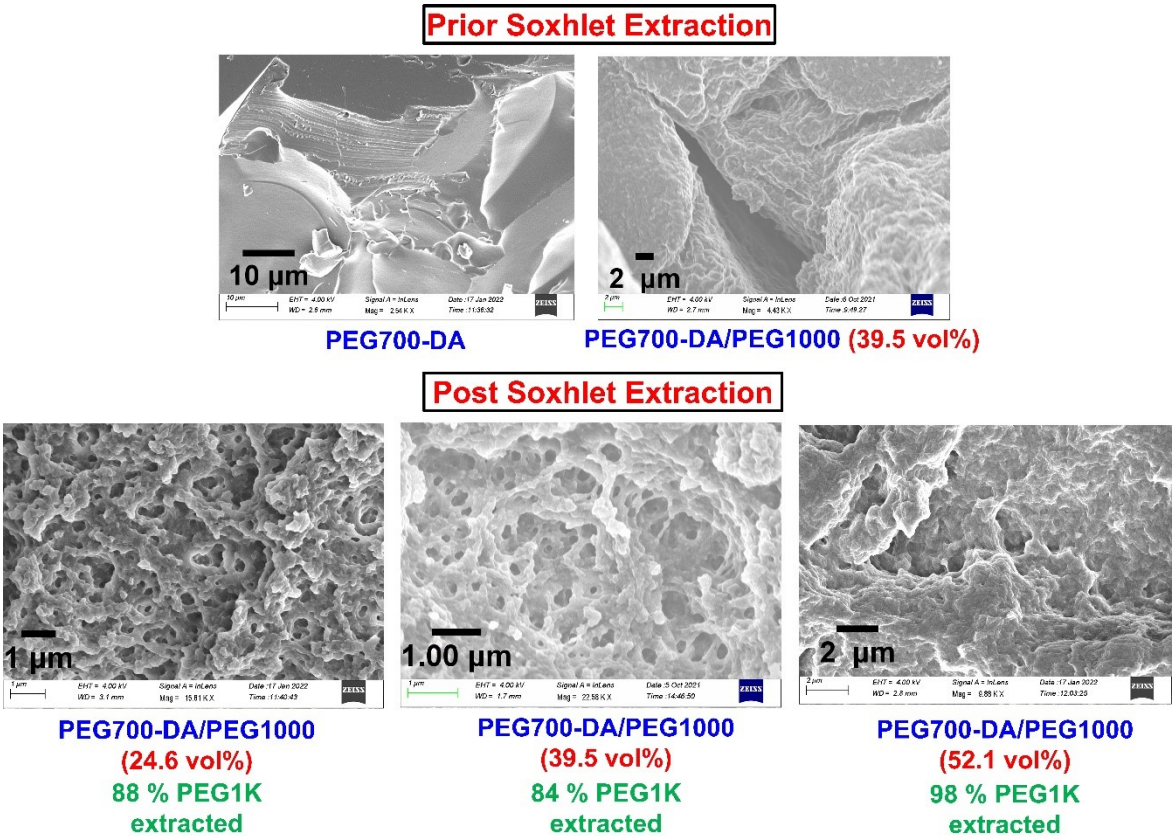

Figure S25. SEM images of PEG700-DA and PEG700-DA/PEG1000 pristine gels containing different vol% of PEG1000 prior and post Soxhlet extraction for 2 hr in MeOH to remove the unbound PEG homopolymers.

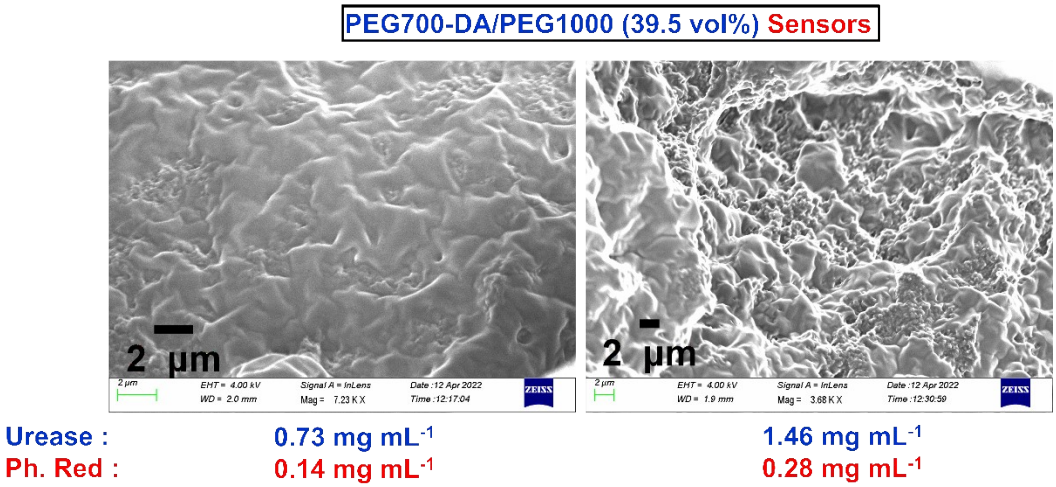

Figure S26. SEM images PEG700-DA/PEG1000 (39.5 vol%) sensor gels containing different amounts of urease and phenol red.