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Electronic Supplementary Information

Maleimide-bearing nanogels as novel mucoadhesive materials for drug delivery

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General analytical considerations

Dynamic light scattering and ζ-potential measurements

The z-average hydrodynamic diameter and ζ-potential were determined using a Nano Zetasizer (Zetasizer Nano ZS with Laser Doppler Microelectrophoresis) at 25 ºC using 1 mg mL^{-1} samples in DI H_2O with 100-fold dilution.

Morphology of the nanogels

The PVP, protected nanogels and deprotected nanogels were observed using Transmission Electron Microscope (TEM) for particle size and shape assessment. The sample was diluted with deionised water, then placed in sonicator bath for 30 minutes. The sample was then dropped onto a carbon-coated copper grid and allowed to dry before being stained with uranyl acetate (1.5%w/v. solution in deionised H2O). Then, the sample was observed using a 200 kV TEM microscope (Philips CM 20, UK).

Thermogravimetric Analysis (TGA)

The composition of the nanogels was analysed using a TGA Q50 (TA instruments, TA universal analysis software, UK). Samples (5-10 mg) were placed in aluminium sample pans and heated, under a nitrogen atmosphere, at a rate of 10 °C/min to 500 °C. Nitrogen was introduced to the samples at a rate of 25 mL/min to maintain an oxidizing environment around the sample.

Fourier transform infrared spectrophotometry (FT-IR)

The Fourier transform infrared spectroscopy was carried out using a PerkinElmer spectrum 100 infrared spectrophotometer and Spectrum software with a wave number range of 600–4000 cm^{-1}.

Nuclear magnetic resonance spectroscopy (NMR)

^1H NMR spectra were recorded on a Bruker Ultrasheild 400 plus spectrometer at 298 K. All chemical shifts were reported as δ in parts per million (ppm), using the chemical shift of the residual solvent resonances as references (DMSO: δ = 2.50 ppm, $CHCl_3$: δ = 7.26 ppm).

Statistical analysis

All experimental measurements were collected in triplicate and the values are expressed as the mean ± standard deviation (SD). The statistical significance of the differences in each experiment was examined using one-way analysis of variance (ANOVA), followed by a least significant difference (LSD) post hoc test. The differences were significant at $p < 0.05$.

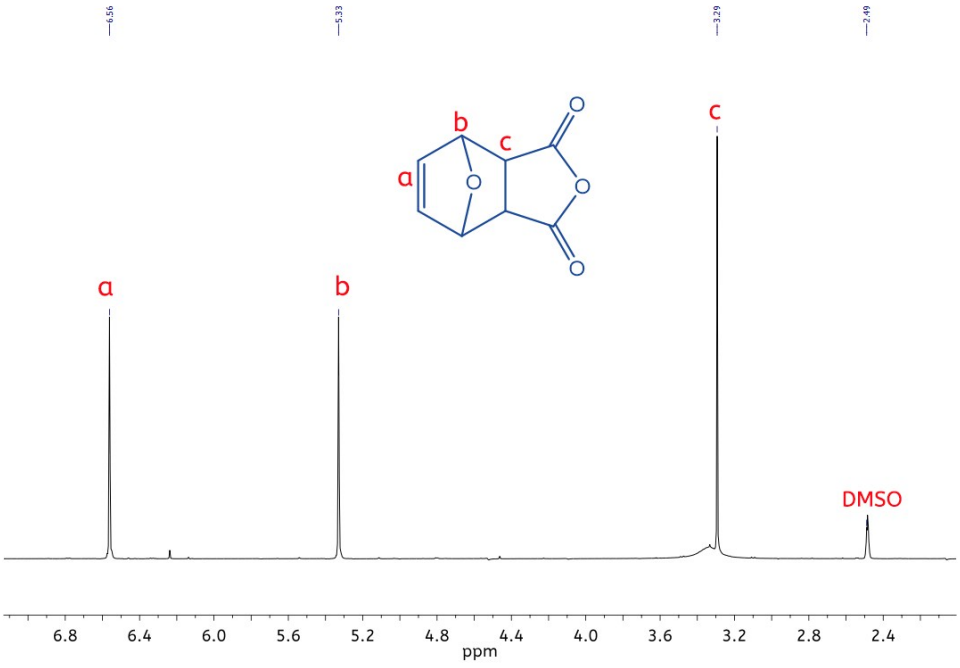

Fig. S1 ^1H NMR spectrum of 2,5-Dimethylfuran-protected anhydride (400 MHz, 25 ˚C, DMSO-d_6).

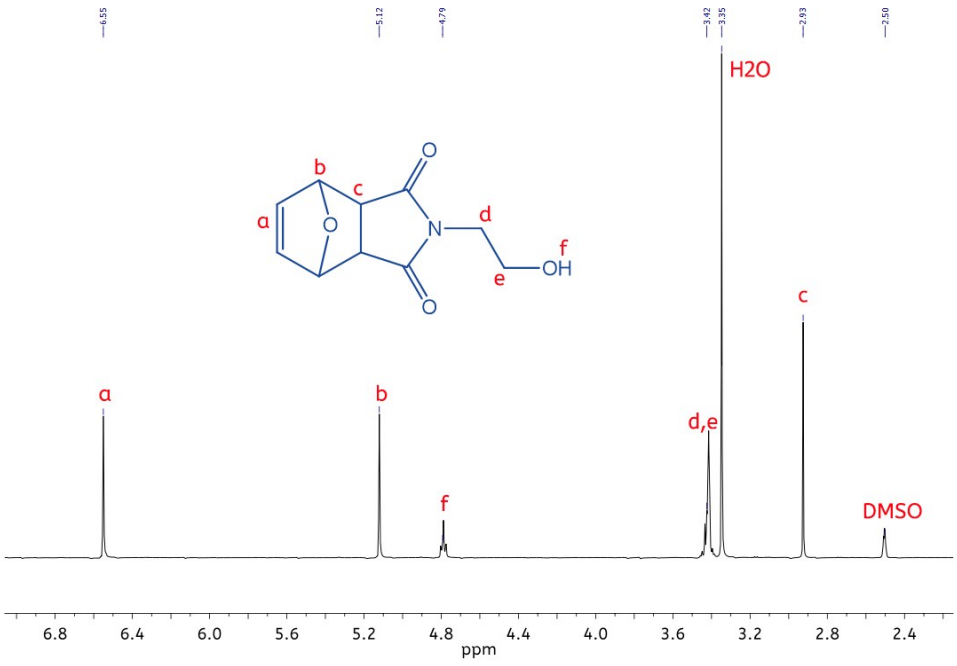

Fig. S2 ^{1}H NMR spectrum of 2,5-Dimethylfuran-protected 3-maleimido ethylalcohol (400 MHz, 25 ˚C, DMSO-d_6).

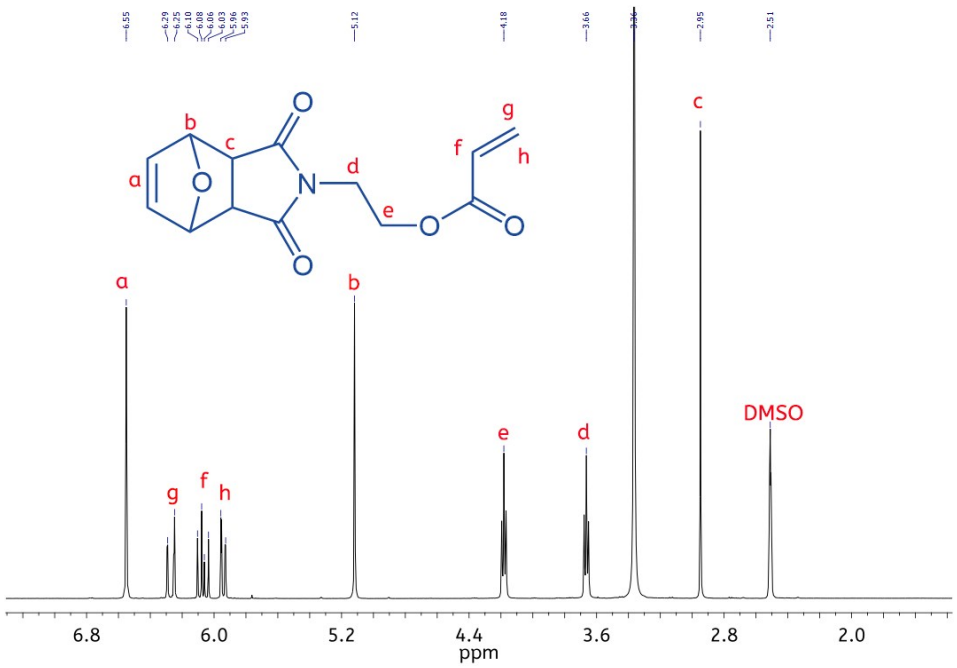

Fig. S3 ^{1}H NMR spectrum of protected maleimide acrylate monomer (400 MHz, 25 ˚C, DMSO-d_6).

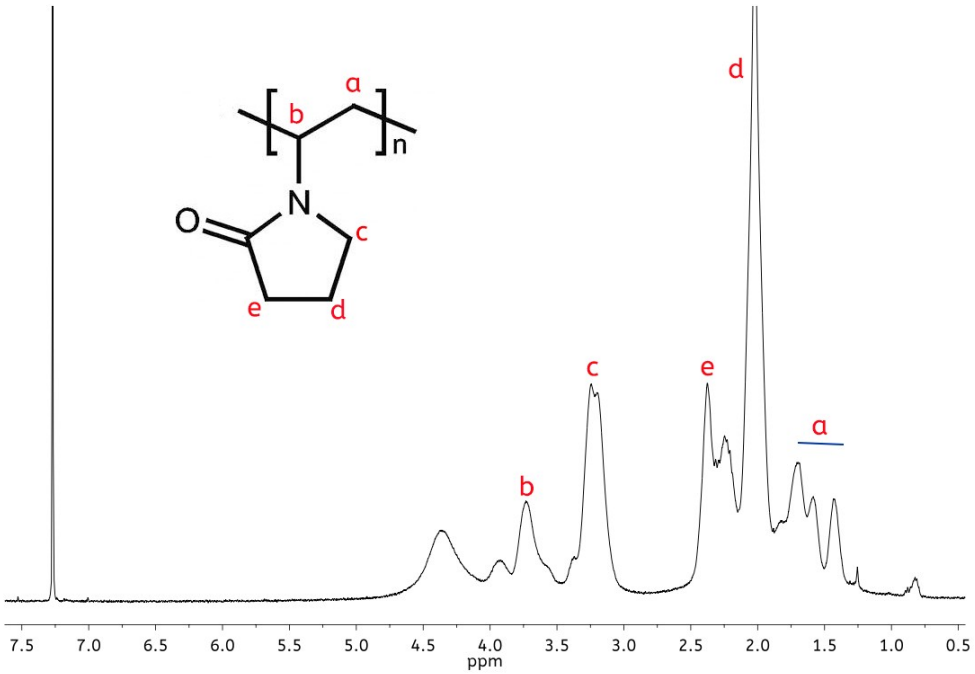

Fig. S4 ^{1}H NMR spectrum of PVP nanogels (400 MHz, 25 ˚C, $CDCl_3$).

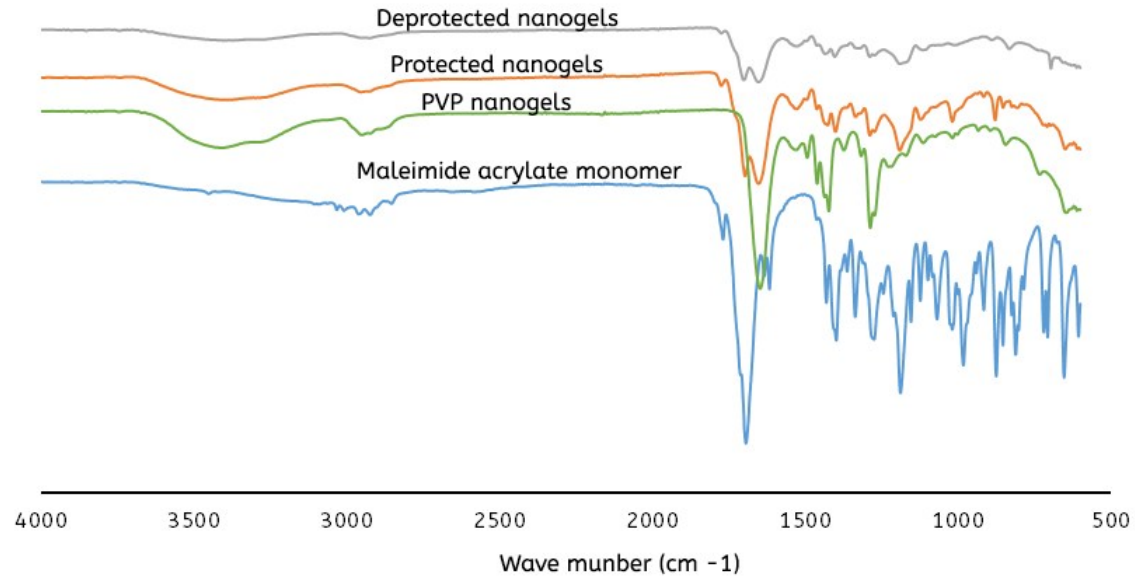

Fig. S5 FT-IR spectra of (i) deprotected nanogels, (ii) protected nanogels, (iii) PVP nanogels, and (iv) protected maleimide acrylate monomer

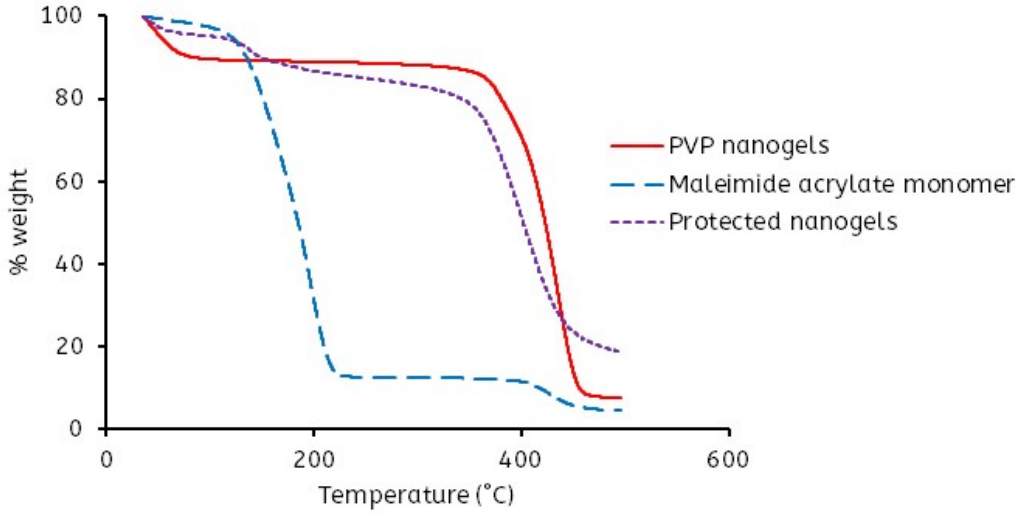

Fig. S6 TGA thermograms of protected maleimide acrylate monomer, PVP nanogels and furan-protected maleimide-PVP nanogels

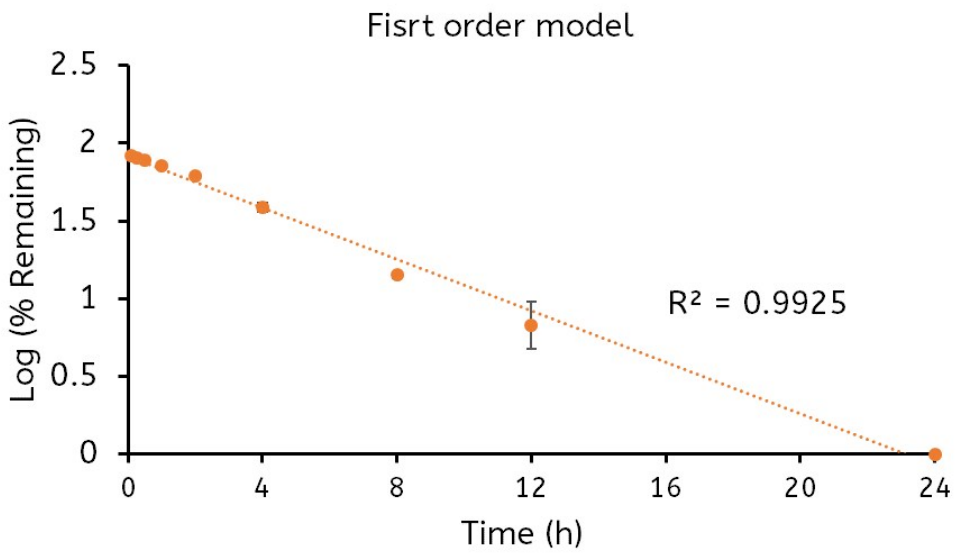

Fig.S7 Fitting of release kinetic of fluorescein sodium from the nanogels with the first order model

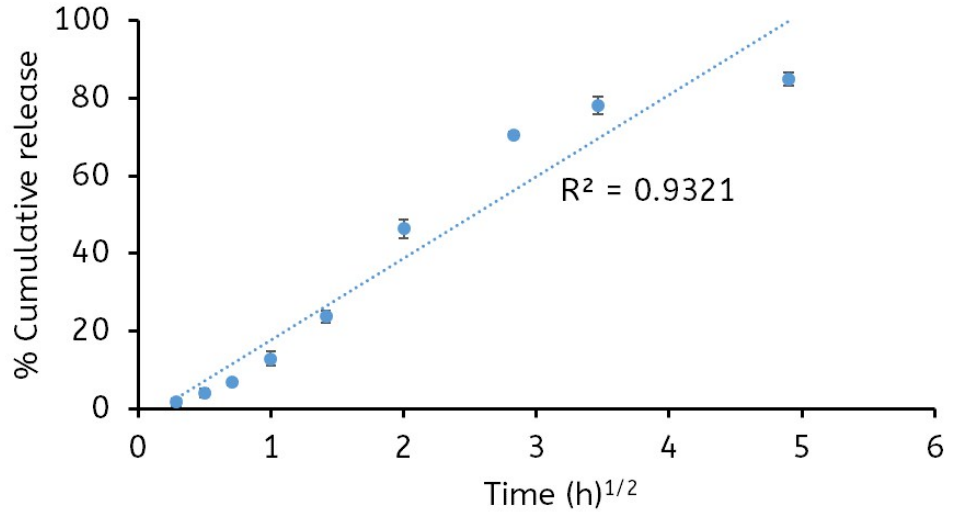

Fig.S8 Fitting of release kinetic of fluorescein sodium from the nanogels with the Higuchi model

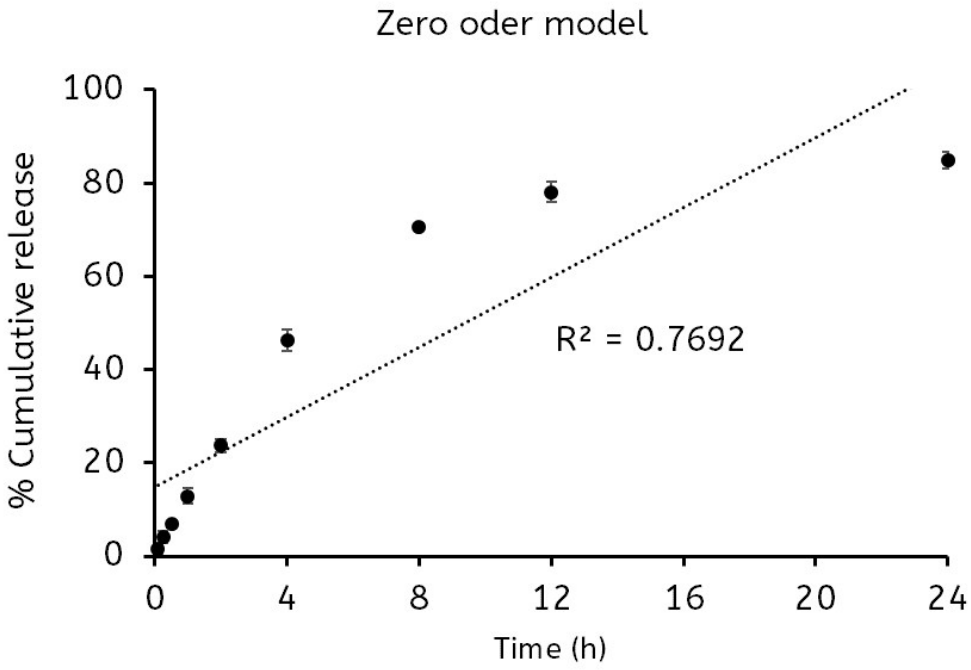

Fig.S9 Fitting of release kinetic of fluorescein sodium from the nanogels with the zero order model

Table S1 The statistical significance of the differences in %retention of the test solution on bovine conjunctival tissue.

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.077	3	.026	19.215	.001
Within Groups	.011	8	.001		
Total	.088	11			

Table S2 A least significant difference (LSD) post hoc test of the differences in %retention of the test solution on bovine conjunctival tissue.

Multiple Comparisons

LSD

(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.04866	.02983	.141	-.1174	.0201
	3	.10716*	.02983	.007	.0384	.1759
	4	.15081*	.02983	.001	.0820	.2196
2	1	.04866	.02983	.141	-.0201	.1174
	3	.15582*	.02983	.001	.0870	.2246
	4	.19947*	.02983	.000	.1307	.2682
3	1	-.10716*	.02983	.007	-.1759	-.0384
	2	-.15582*	.02983	.001	-.2246	-.0870
	4	.04364	.02983	.182	-.0251	.1124
4	1	-.15081*	.02983	.001	-.2196	-.0820
	2	-.19947*	.02983	.000	-.2682	-.1307
	3	-.04364	.02983	.182	-.1124	.0251

*. The mean difference is significant at the 0.05 level. 1deprotected nanogels, 2 chitosan (positive control) 3 protected nanogels, and 4 dextran (negative control).

Equation S1

$$Maleimide\ content\ (mmol/g) = \frac{Ci(mmol) - Cf(mmol)}{Wn\ (g)}$$

where F_i is the amount of cysteine HCL added to the nanogels suspension, C_f is the remaining amount of cysteine HCL after reacted with the maleimide presented on the nanogels, and W_n is the total mass of the nanogels (g)

Equation S2

$$Loading\ efficiency\ (\%) = \frac{(Fi - Ff) \times 100}{Fi}$$

where F_i is the initial mass of fluorescein sodium added to the nanogels suspension, and F_f is the final mass of fluorescein sodium in the supernatant after centrifugation.

Equation S3

$$Loading\ capacity\ (\%) = \frac{(Fi - Ff)}{Wf + Wn}$$

where F_i is the initial mass of fluorescein sodium added to the nanogels suspension, W_f is the total mass of fluorescein sodium in the nanogels (mg) and W_n is the total mass of the nanogels (g).