Electronic Supplementary Material (ESI) for Materials Advances.

In vivo Potential of Polymeric N-Acryloyl-Glycine Nanoparticles with Anti-inflammatory Activities for Wound Healing

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Figure S1: Apparatus for measurement of the spreadability of nanoformulation

Animal Protocol and Ethics: All the animal studies were examined and approved (Approval No. IIT(BHU)/IAEC/2022/078, dated 03/05/2022) by the Institutional Animal Ethical Committee established at Department of Pharmaceutical Engineering and Technology, Indian Institute of Technology (BHU), Varanasi (Registration no. 2123/GO/Re/S/21/CPCSEA). The use of animals in this work was carried out in compliance with the IEAC's recommendations and guidelines. In a nutshell, the male Wistar rats (wt: 125-150 gm, age: 12-14 weeks) were purchased from Institute

of Medical Sciences, BHU and Central Drug Research Institute, Lucknow. They were given unrestricted access to the laboratory by keeping them in cage and put on fed to commercial pellet meal and unlimited amounts of water. Prior to the start of the experiment, the animals have to spend 1 week becoming acclimatize to the laboratory environment. One day before to the commencement of the experiment, all rats had their skin cleansed with water and shaved with the help of an electric razor. The skin was then disinfected with a povidone-iodine solution or with 70% v/v ethanol solution, and kept apart in animal cages. The animals were given a once-over for general health on the day of the experiment, right before the application of test material, and the skin was inspected for any anomalies. Only when there was no visible evidence of prior skin irritation were experiments carried out. Pentobarbital sodium (50 mg/kg) was injected intraperitoneally to anaesthetize rats. The absence of pedal and corneal responses confirmed anaesthesia. At 0^{th}, 12^{th}-, and 24^{th}h following surgery, tramadol hydrochloride (20 mg/kg) was administered intraperitoneally as an analgesic.

Table S1: Table showing the ingredients (in percentage) used for the preparation of oleaginous ointment base

S. No	Ingredients	Weight (%)
1.	Glycerine	5
2.	Paraffin Wax	10
3.	Liquid Paraffin	15
4.	Stearyl Alcohol	25
5.	White soft Paraffin	45
6.	Triethanolamine (98%)	q.s.

Draize Scoring System[1, 2]

Table S2: Dermal irritability grading system according to Draize

Value	Erythema development	Value	Oedema development
0	No erythema	0	No oedema
1	Extremely minor erythema	1	Extremely minor oedema

2	Mild erythema (well defined margins)	2	Mild oedema (well defined margins)
3	Moderate severe erythema (Specified colour and erythema region)	3	Moderate severe oedema (Specified colour and oedema region)
4	Maximum possible erythema	4	Maximum possible oedema

Erythema and edema were graded on a scale of 0–4, with 0 denoting no symptoms and 4 denoting severe ones. The average irritation score per time point was calculated for each animal by summing the cutaneous response scored at 1 h, 24 h, 48 h, and 72 h following the removal of the test substance and dividing the result by four group. The results for PNAG nanoformulation induced skin irritation were compared with the basic formulation and with a positive control. The dermal irritation index (PDII) was calculated using the following equation.

$$PDII = \frac{PDI}{4} \qquad 1$$

Table S3: Qualitative characterization of base formulation and PNAG-nanoformulation on the basis of observational parameters

S. No.	Quality Parameter	Required quality	Formulation base	PNAG-nanoformulation
1	Colour	Same as API's colour or white/ off white	Colourless/White	Colourless/White
2	Appearance	Homogenous	Homogenous	Homogenous
3	Consistency/ Homogeneity	Good	Good	Good
4	Phase Separation	No	No	No
5	Odour	Same as API's odour/ Odourless	Odourless	Odourless

Table S2: in-vivo wound healing results shown in terms of relative area of wound

Days	Formulation base (Mean ±SD)	PNAG Nanoformulation (Mean ±SD)
0	100.00 ±2.80	100.00 ±0.97
1	104.79 ±2.74	105.08 ±1.62
3	96.45 ±2.38	95.12 ±0.71
5	92.96 ±2.47	90.19 ±3.25
7	83.15 ±4.28	72.63 ±5.44
9	70.91 ±4.81	25.33 ±0.34
11	67.17 ±7.17	4.49 ±0.88
13	55.47 ±3.83	2.93 ±0.43
14	30.74 ±3.11	0.00 ±0.1

NMR

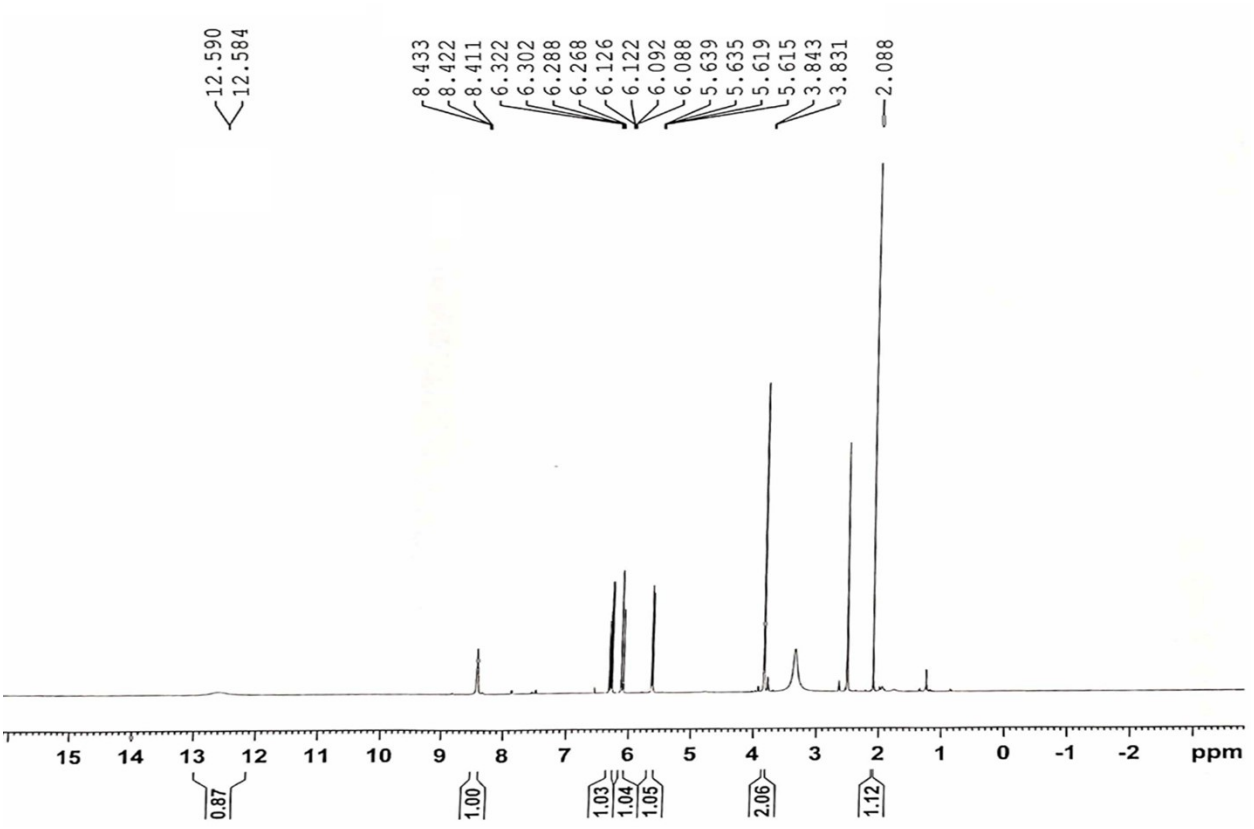


Figure S2: H[1]-NMR spectrum of N-acryloyl-glycine (NAG), monomer recorded in DMSO deuterated (500 MHz)

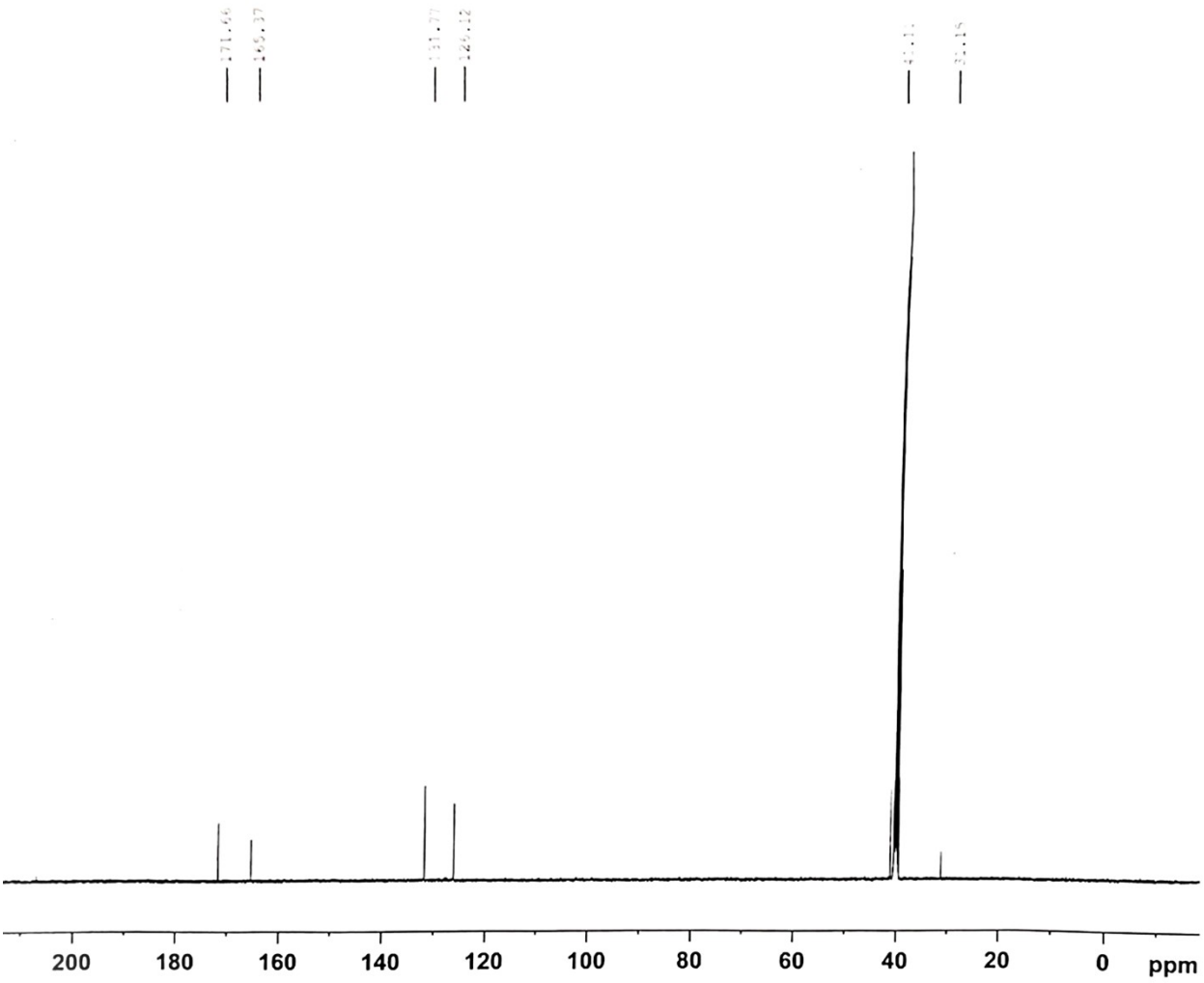


Figure S3: C^{13}-NMR spectrum of N-acryloyl-glycine (NAG), monomer recorded in DMSO deuterated (500 MHz)

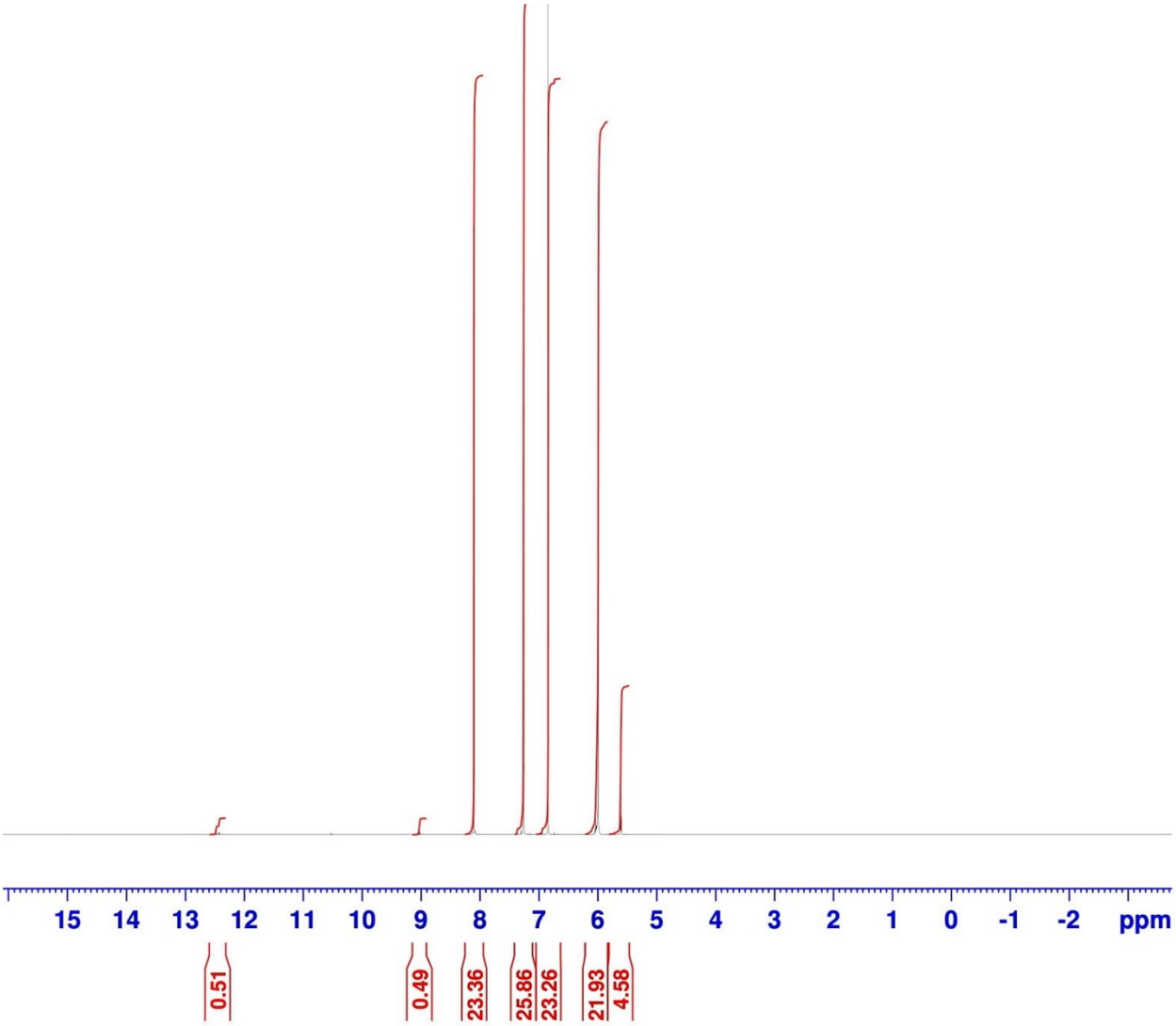


Figure S4: H^1-NMR spectrum of Poly-N-acryloyl-glycine (PNAG), monomer recorded in $CDCl_3$ deuterated (500 MHz)

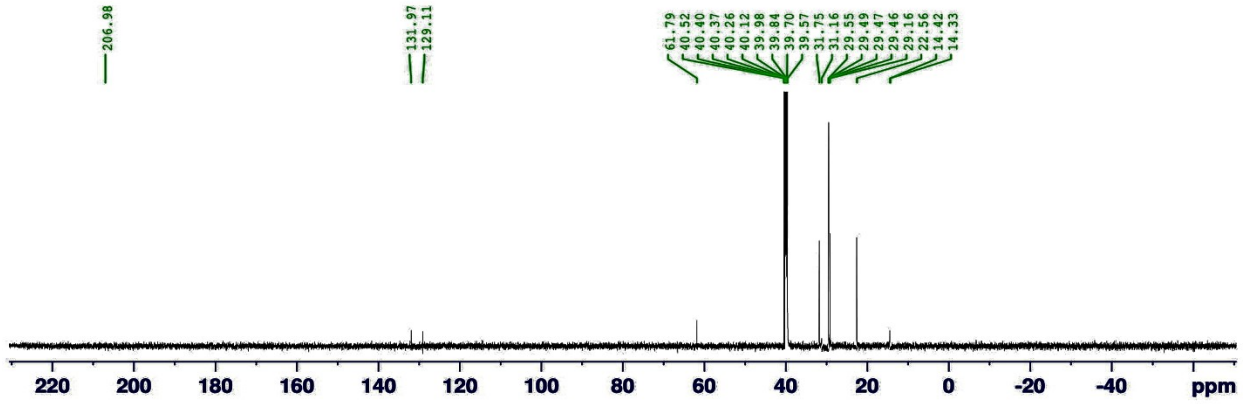


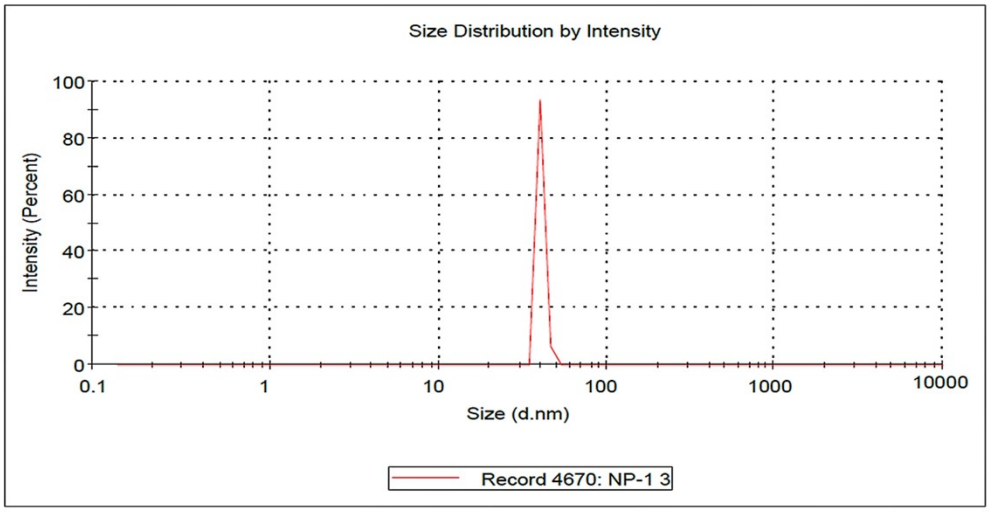
Figure S5: C^{13}-NMR spectrum of Poly-N-acryloyl-glycine (PNAG), monomer recorded in $CDCl_3$ deuterated (500 MHz)

Particle size and zeta potential

Results

		Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 5756	Peak 1:	40.2	100.0	15.53
PdI: 1.000	Peak 2:	0.000	0.0	0.000
Intercept: 1.06	Peak 3:	0.000	0.0	0.000

Result quality : Refer to quality report



		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -21.9	Peak 1:	-21.9	100.0	5.61
Zeta Deviation (mV): 5.61	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0561	Peak 3:	0.00	0.0	0.00

Result quality : Good

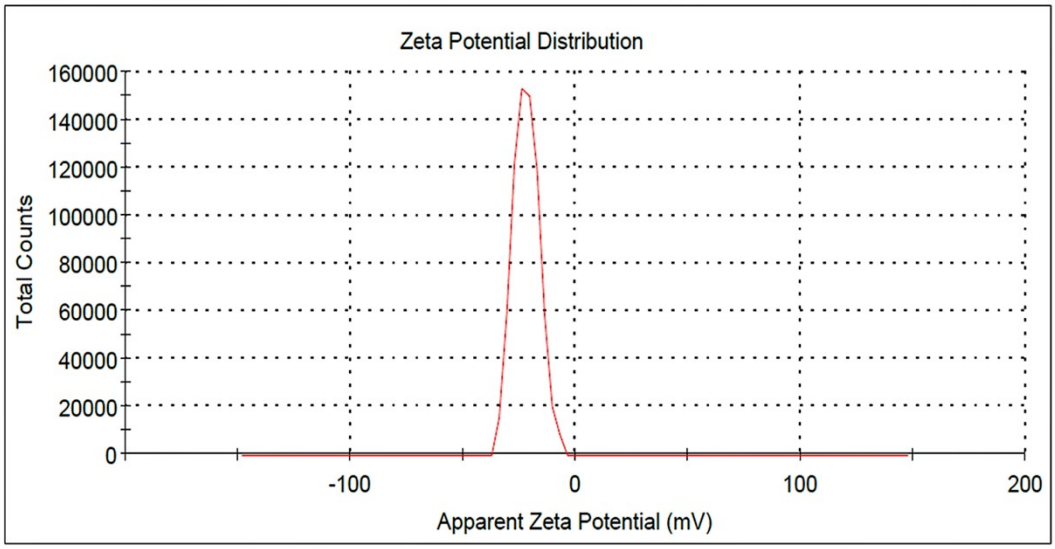


Figure S6: PNAG NPs particle size and zeta potential recorded by dispersing in PBS (pH 7.4)

1. Draize, J. H., Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exp. Ther.* 1944, *82*, 377-390.
2. Djerrou, Z.; Djaalab, H.; Riachi, F.; Serakta, M.; Chettoum, A.; Maameri, Z.; Boutobza, B.; Hamdi-Pacha, Y., Irritantcy potential and sub acute dermal toxicity study of Pistacia lentiscus fatty oil as a topical traditional remedy. *African journal of traditional, complementary, and alternative medicines : AJTCAM* 2013, *10* (3), 480-9.