Electronic Supplementary Information

"The Reinforced Hydrogel for Drug Loading: Immobilization of Single-walled Carbon Nanotubes in Cross-linked Polymers via Multiple Interactions"

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Characterization

Ultra Sonication:

The probe sonicator Sonics VCX 750 (750 W, 20 KHz, 60% amplitude), USA and water-bath sonicator Equitron (42 KHz, 120 W), India were used for the dispersion of *f*-SWCNTs in water.

Spectral Characterization:

The FT-IR spectrum of the nanocomposite and nanogels were recorded on ABB MB3000 Fourier transform infrared spectrometer. The ^{1}H-NMR spectra were obtained on a Bruker AV 400 MHz NMR instrument using the D_2O as solvent. The Raman study was performed by using confocal Raman 11, nano Photon Corporation (Japan) instrument with wave length 532nm/ resolution 1.6 cm^{-1}. The UV-vis spectra were recorded on Shimadzu UV-2401 spectrometer using water as a solvent. The fluorescence spectrum was recorded in the Cary eclipse, Varian by exciting at λ_{exc} = 300 nm using water as solvent. The absorption and the fluorescence emission spectra of all the PVI and polymers gels are studied from their dilute solution (2 X 10^{-5} M) in water.

Morphology:

The dispersion and interaction of *f*-SWCNTs with polymer in nanogel was examined by high resolution transmission electron microscopy (HRTEM) and field emission scanning electron microscopy (FE-SEM). HRTEM images were acquired on JEOL JEM 2100 machine. The FE-SEM images were acquired on SIRION (Netherland). Optical microscopy images were acquired in Olympus BX50 microscope.[3]

Thermal measurement:

Thermogravimetric analysis (TGA) were done to study thermal decomposition behavior using TG Analyzer (TGA)-Model Q50, TA Instruments, with a heating rate of 10 °C/min from 30 °C to 800 °C under N_2 atmosphere. The Differential scanning calorimetry (DSC) analysis was performed using DSC-Model Q200, TA Instruments at a heating rate of 5 °C/min with N_2 flow

of 50 mL/min from -70 °C to 300 °C to analysis melting temperature (T_m) of nanocomposite and nanogels.

Rheology:

The rheological experiments were carried out in cone-plate geometry (CP 25-1) using Anton Paar 100 rheometer. Frequency sweep experiment was performed at a constant angular frequency (0.5 rad/s) and strain varied from 0.001 to 100% at 25 ºC.

Swelling ratio:

The swelling ratio of polymer gel (without CNTs) and nanogel samples were calculated according to following equation,

$$Swelling\ ratio(\%) = \frac{(W_s - W_d)}{(W_d)} \times 100$$

Where, W_s is the weight of the gel in swollen state at a given time, and W_d is the weight of the gel in the dry state.

Drug loading:

The riboflavin (vitamin B_2) drug was used for drug loading in polymer gel (without CNTs) and nanogel samples. The drug releasing kinetic was monitored by using UV-vis spectra (Shimadzu UV-2401 spectrometer) using water as a solvent.

MTT assay:

The reduction of live cell into the coloured formazon product was measured at 570 nm using synergy HT-1 micro reader.

Experimental

Preparation of poly(vinylimidazole) (PVI)

The PVI was prepared according to the reported method[1] by taking required amount of VIM in ethanol solvent (1ml), with 2,2'- azobis(isobutyronitrile) (AIBN) as initiator (0.01g), the reaction for an about 10 h, and purified by reprecipitation from methanol into acetone solvent. The preparation, purification, and identification of polymerization were carried out and reported. This PVI was used as such for further reaction. The TGA data of poly(vinyl imidazole) is in agreement with the reported values in the literature.[1]

Preparation of functionalized SWCNTs (*f*-SWCNTs)

Following a reported procedure,[2] the as-received SWCNTs were functionalized using sulfuric acid and nitric acid.[2] This functionalized SWCNTs was taken in a 100 ml beaker, mixed with distilled water and irradiated using probe sonicator (Sonics vibracell) at a power of 750 W, frequency 20 kHz and 60% amplitude for 20 min to afford well dispersed functionalized nanotubes in water. Raman and TGA analysis proves the functionalization of SWCNTs (*f*-SWCNTs) (ESI Fig. S11).

MTT assay

The biotoxicity effect of SWCNTs in nanogel samples (PSG1-4) was assessed by MTT assay. The reduction of live cell into the coloured formazon product was measured at 570 nm using synergy HT-1 micro reader. Here, 3T3-L1 cells were used and grown in Dulbecco's Modified Eagle Medium. All media were supplemented with 10% fetal bovine serum and 1% antibiotic and antimycotic solution and cultured in 5% CO_2 atmosphere at 37 ◦C. The $1x10^4$ 3T3-L1 cells were seeded in a flat-bottom 90 well plates. The different wet weight polymer gel (without CNTs) and nanogel samples (PSG1-4) of 10mg and 25 mg were introduced into the buffer solution (pH-7.4) along with the control. After 24 h incubation, the supernatant of each well was replaced with 100 µL MTT diluted in serum-free medium (0.5 mg/mL) and the plates were incubated at 37 ◦C for 4 h. Further, the formazon crystals were dissolved by adding 100 µL of DMSO to each well and mixed properly by pipetting up and down. The same has done for 48 h and 72 h duration and all the studies were done in triplicates and the values represent the

average from triplicate wells. The viability of non-treated control cells were arbitrarily defined as 100%. The optical density of each samples were compared with control and the graph was plotted. The results are expressed as mean ± standard error of the absorbance.[4] The cell viability was calculated using the following formula:

$$\%\,\text{Cell viability} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \times 100$$

Drug loading and releasing study

The riboflavin (vitamin B_2) and amitriptyline hydrochloride (AH) drugs were taken in water with concentration of 5mg in 10ml (w/v). Further, this drugs were preloaded to the polymer gel (without CNTs) and nanogel (PSG1) allowed for few minutes to reach the maximum percentage of swelling. The gels were washed repeat to remove the unloaded drug on the surface of gel. These gels were then placed in neutral (pH-7) and acid buffer medium (pH-4) to study their drug releasing property. The drug releasing kinetics was monitored by using UV-vis absorbance at 264 nm and 234 nm in different time interval by pipetting 200µl of drug from gel and makeup to 2ml.

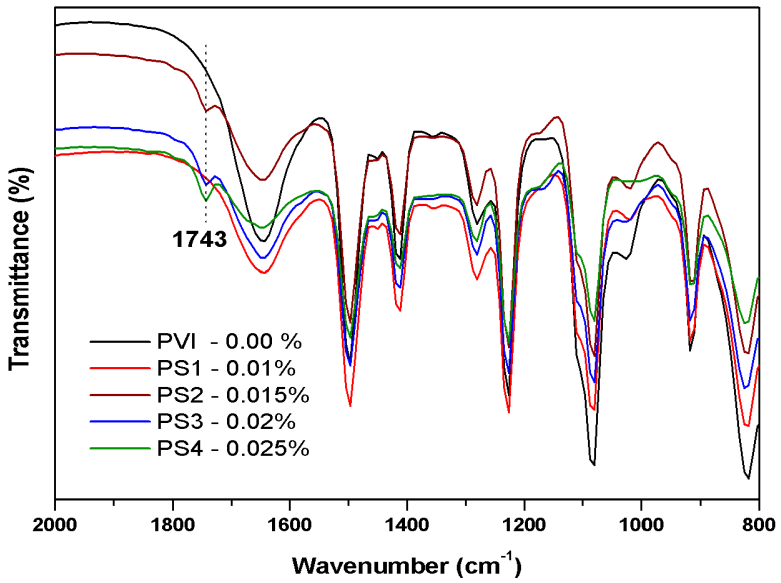


Fig. S1 FT-IR spectra (full range) of the polymer (PVI: black line) and nanocomposites (PS1, PS2, PS3, PS4) with various % of *f*-SWCNTs loading in the range 2000-800 cm^{-1}.

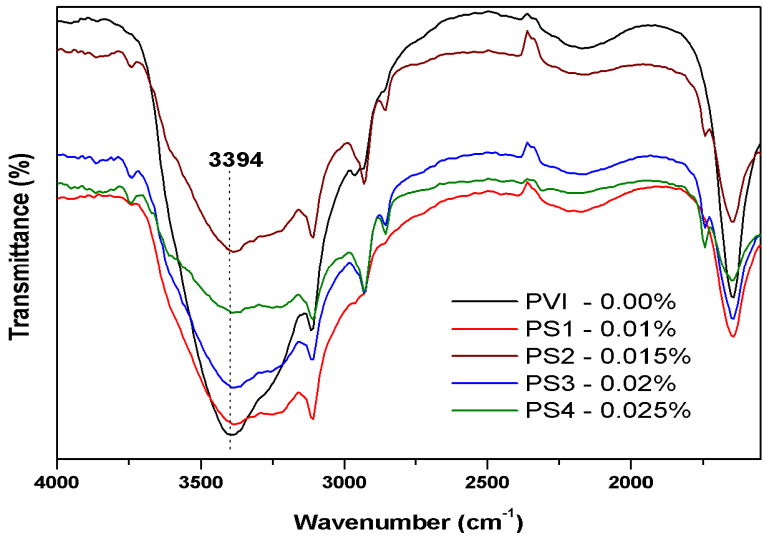


Fig. S2 FT-IR spectra (zoomed range: 1500-4000 cm^{-1}) of the polymer and nanocomposites with various % of *f*-SWCNTs loading in the range 4000-1800 cm^{-1}.

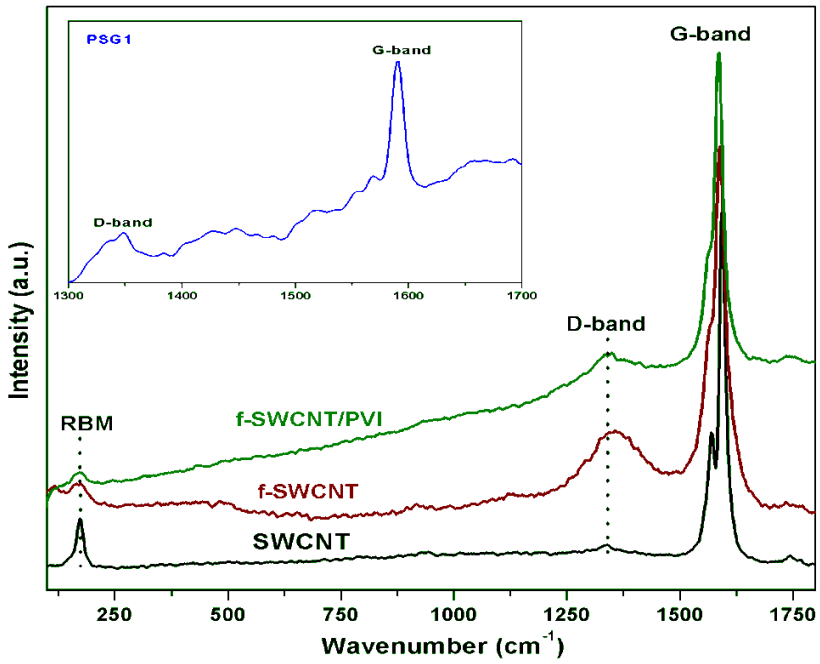


Fig. S3 Comparison of the SWCNTs, *f*-SWCNTs and *f*-SWCNTs/PVI by Raman spectra; Inset show the Raman spectrum of nanogel of PSG1.

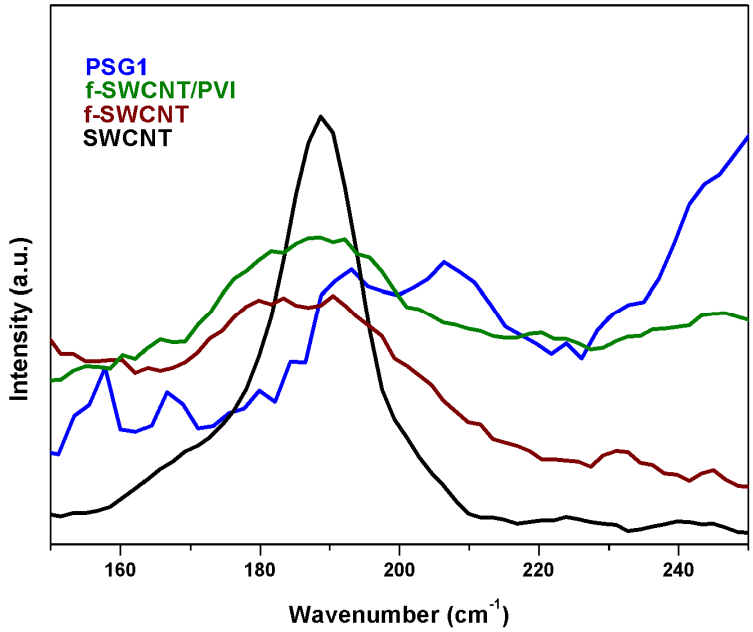


Fig. S4 Raman spectra of expanded RBM region (100-300 cm^{-1}) of the PSG1, *f*-SWCNTs/PVI, *f*-SWCNTs and control SWCNTs.

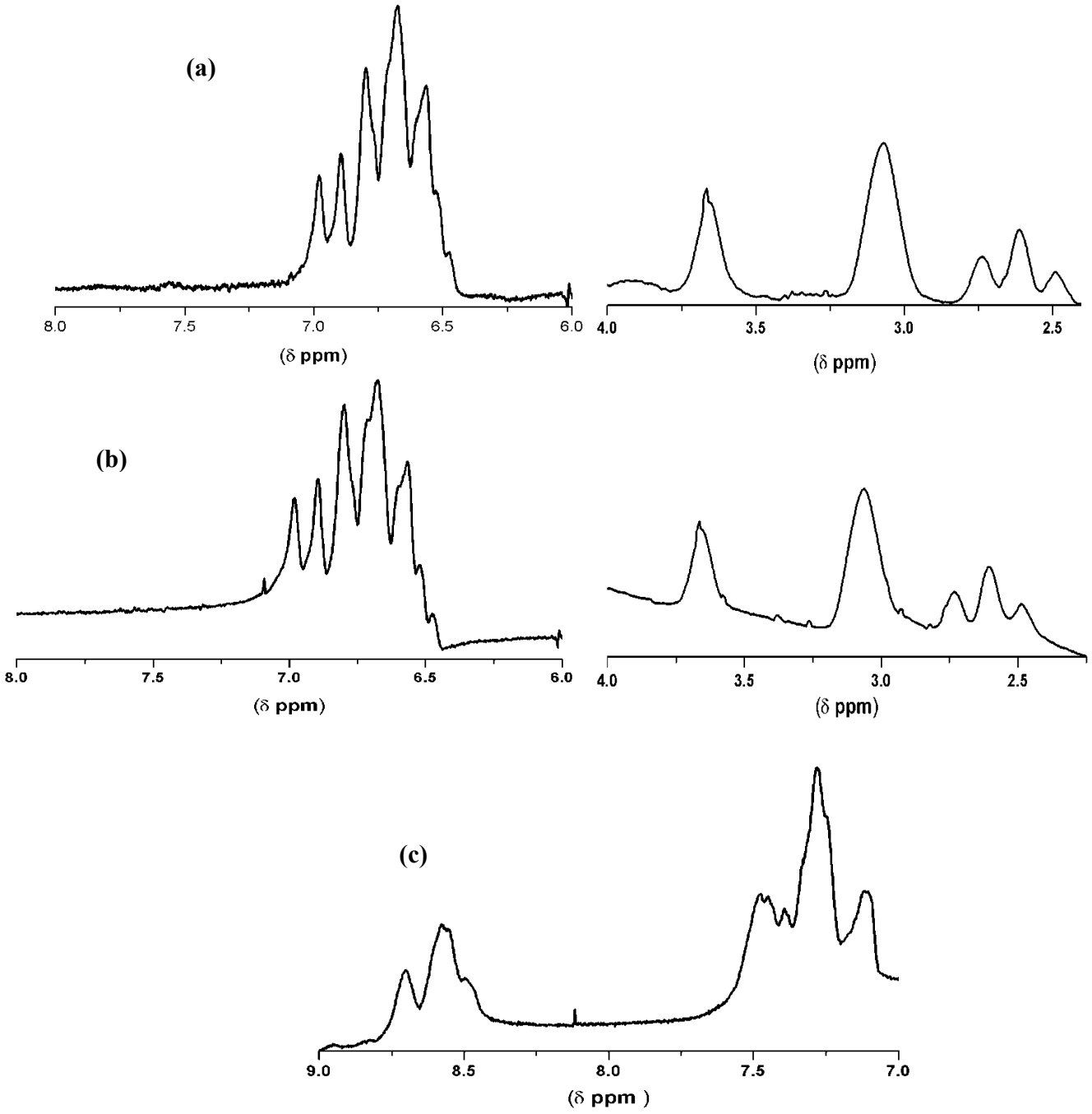


Fig. S5 ^{1}H-NMR spectra of (a) PVI (b) *f*-SWCNTs/PVI and (c) nanogel in D_2O solvent.

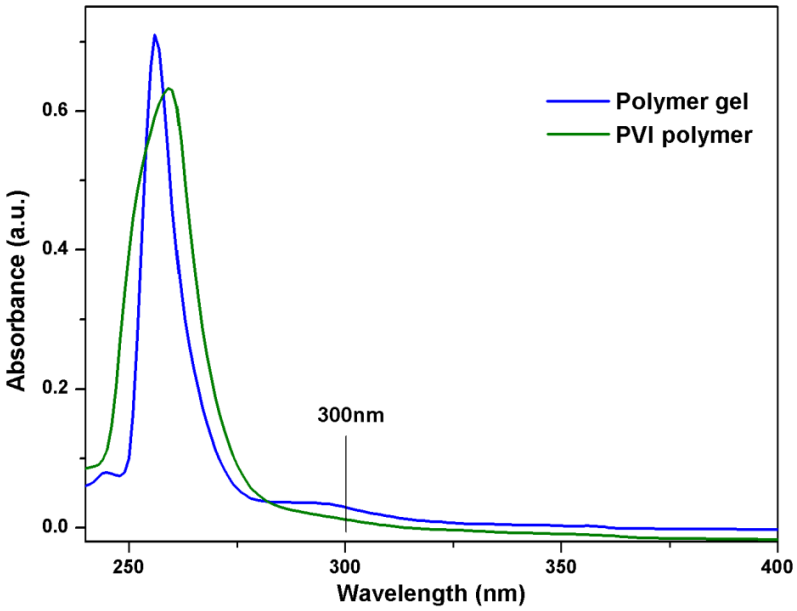


Fig. S6 UV-vis absorption spectra of polymer gel (without CNTs) and PVI polymer in aqueous solution with the concentration of 0.03% of w/v.

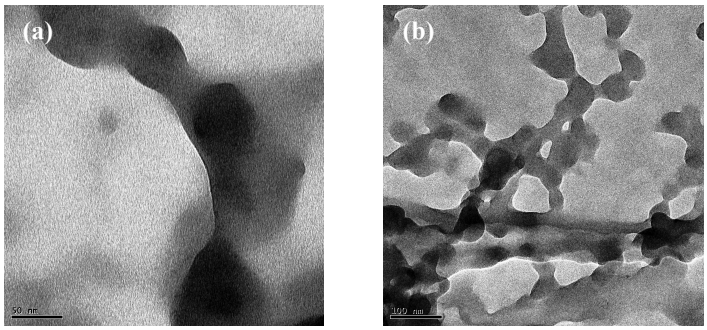


Fig. S7 HR-TEM images of nanogel (PSG2) (a) with high magnification (50 nm) and (b) with low magnification (100 nm).

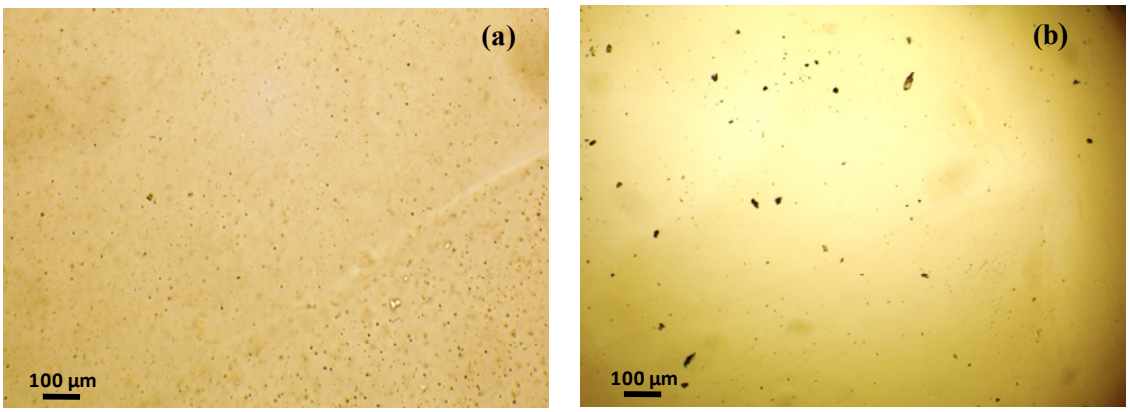


Fig. S8 Optical microscopic image of (a) CNTs in lower concentration (0.01%), (b) CNTs in higher concentration (0.025%).

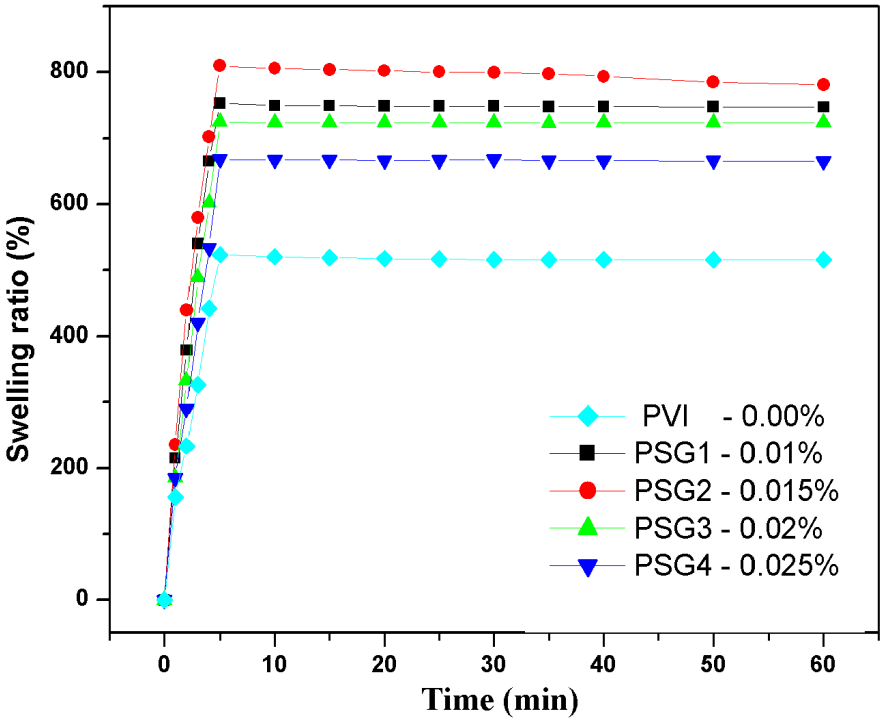


Fig. S9 Swelling ratio of polymer gel (PVI) and nanogels (PSG1-PSG4) with various % of *f*-SWCNTs loading in aqueous solution.

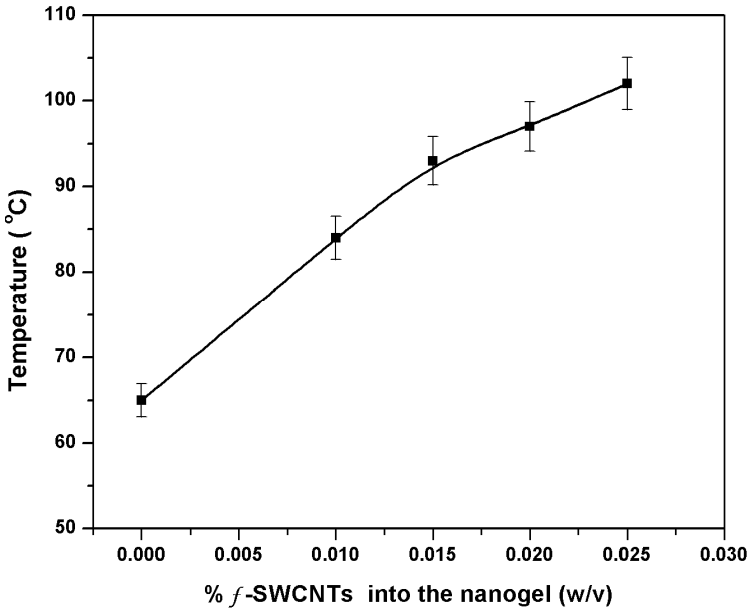


Fig. S10 Thermal stability of nanogels with various % of *f*-SWCNTs loading (w/v) in polymer matrix.

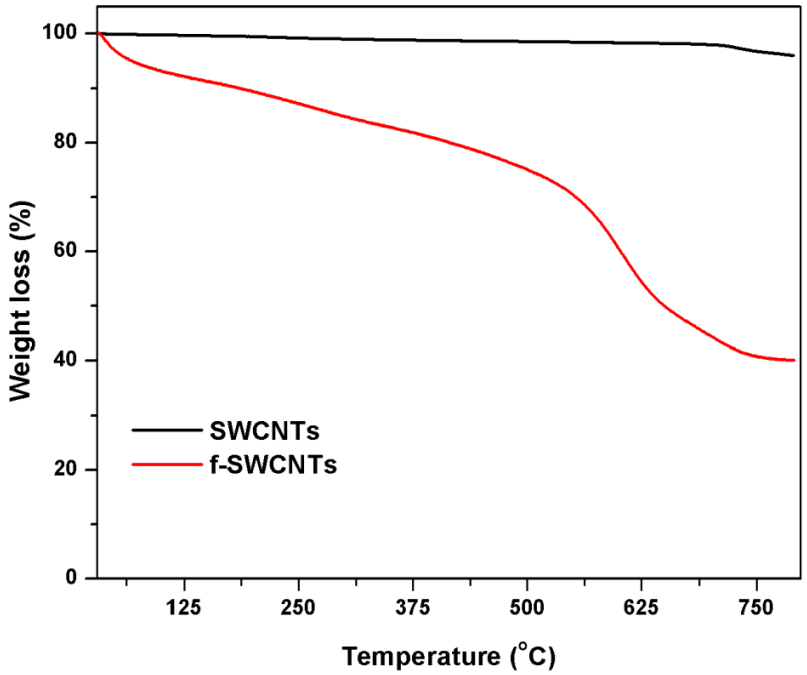
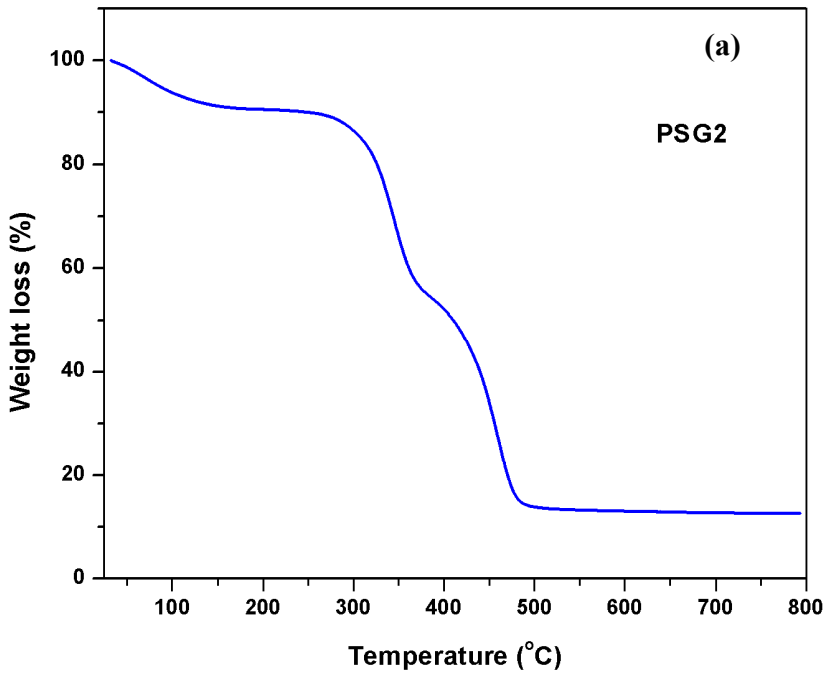
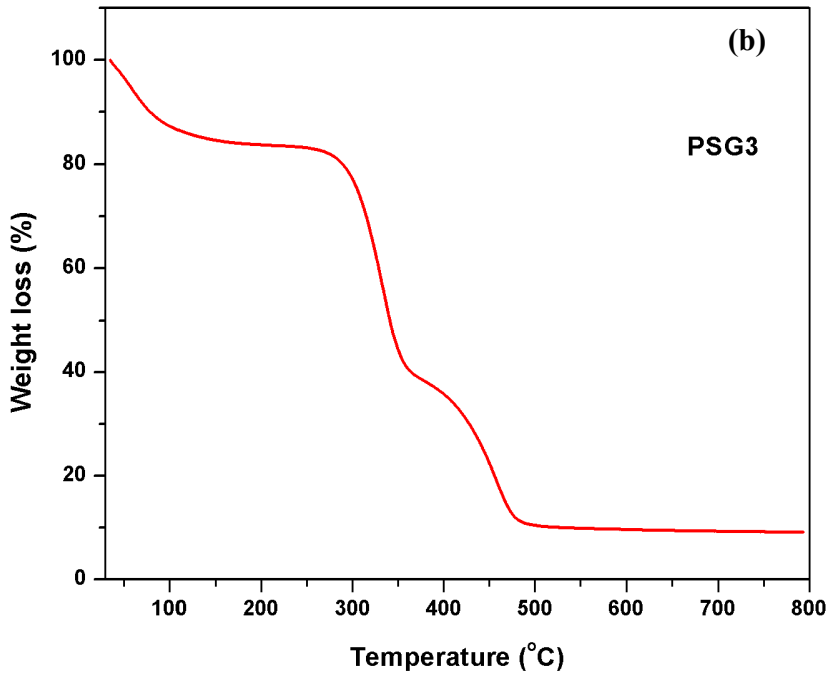


Fig. S11 TGA plots of pristine SWCNTs and acid functionalized *f*-SWCNTs.





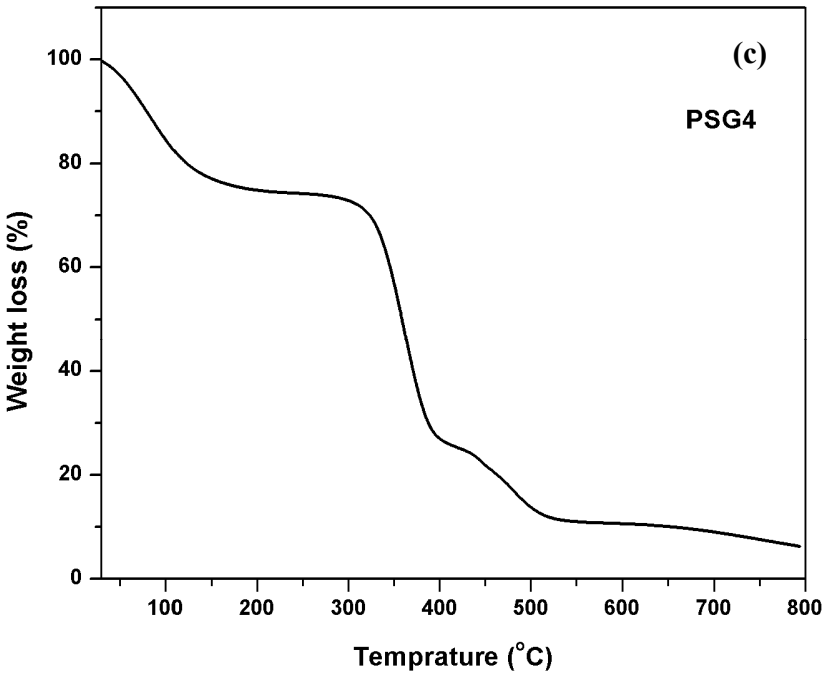


Fig. S12 TGA plots of (a) PSG2, (b) PSG3 and (c) PSG4.

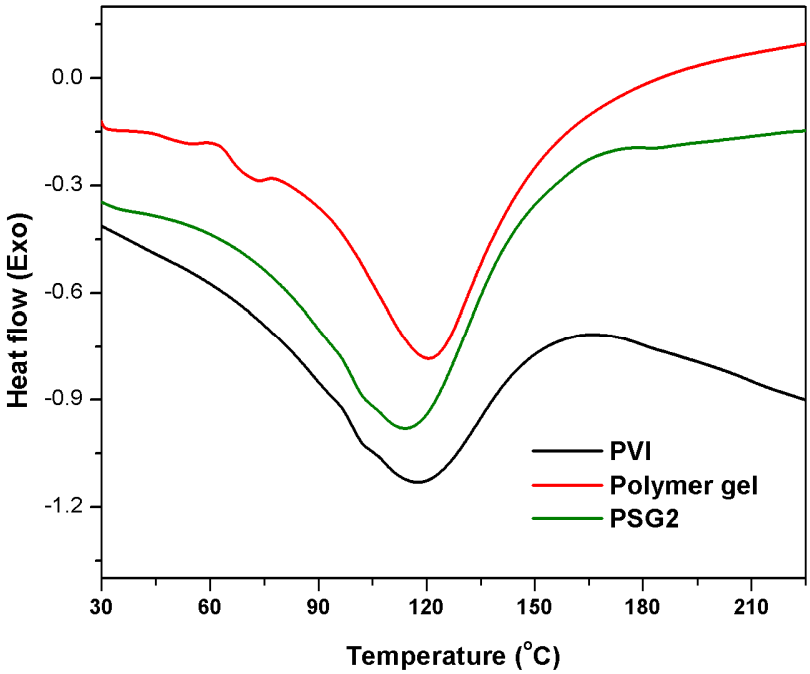


Fig. S13 DSC plots of PVI, polymer gel (without CNTs) and PSG2.

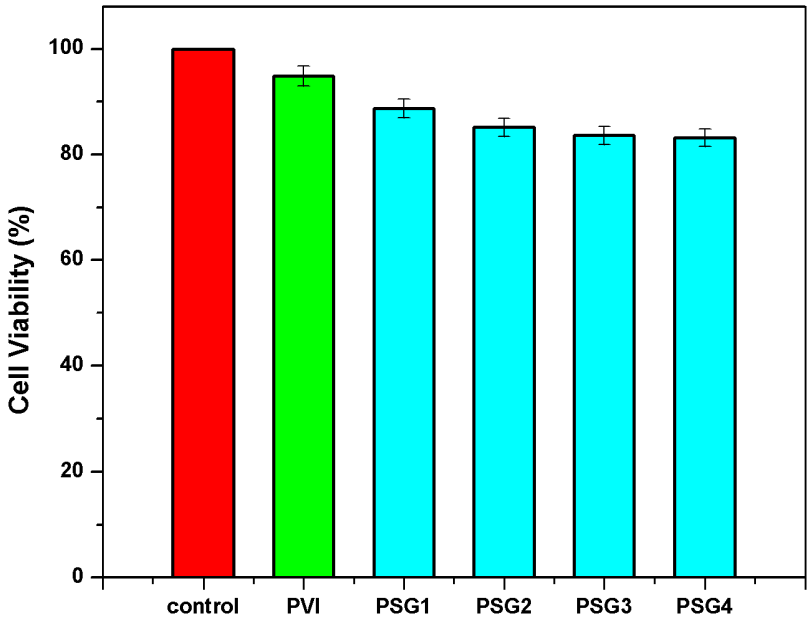


Fig. S14 MTT assay of PVI-polymer gel (without CNTs) and nanogels for biocompatibility in 48 h.

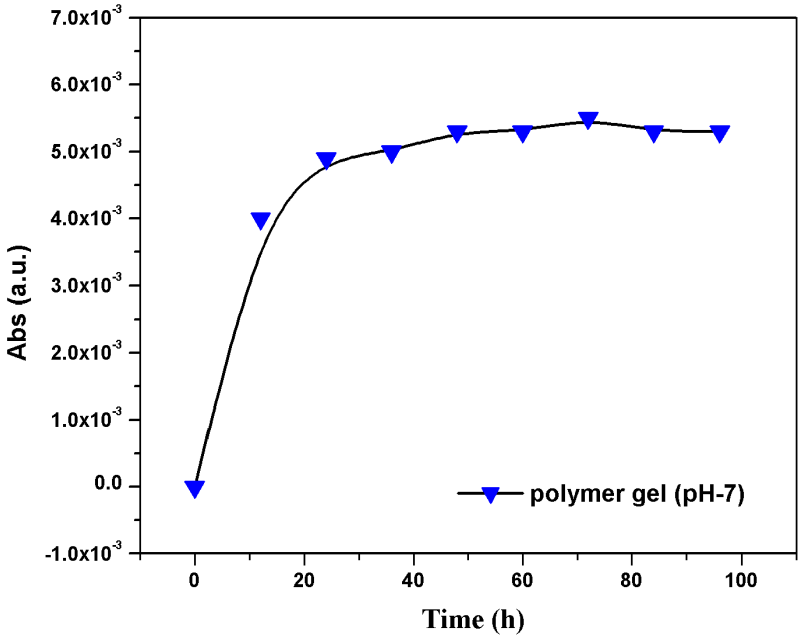


Fig. S15 UV-vis absorbance at 264 nm of the released riboflavin (vitamin B_2) from polymer gel (without CNTs) in different time interval in presence of neutral pH-7.

Table S1: Gelation behavior of polymer gel (without CNTs) and nanogel (PSG2) in different organic solvents.

Solvent	Polymer gel		Nanogel	
	Observations[a]	Gelation Time¶	Observations[a]	Gelation Time¶
Dimethylsulphoxide	**G**	30 min.	**G**	10 min.
Dimethylforamide	**G**	30 min.	**G**	10 min.
Dimethylacetamide	**G**	45 min.	**G**	45 min.
Benzene	NG	NA	NG	NA
Toluene	NG	NA	NG	NA
Ethylacetate	NG	NA	NG	NA
Chloroform	NG	NA	NG	NA
Methanol	**G**	7 min.	**G**	2 min.
Acetonitrile	NG	NA	NG	NA
Carbontetrachloride	NG	NA	NG	NA
Acetone	NG	NA	NG	NA
Tetrahydrofuran	NG	NA	NG	NA

[a] **G** = gel formation was observed, NG = No gel formation was observed, NA = Not applicable.

Table S2: Thermal data from DSC plot for PVI, polymer gel (without CNTs) and nanogel (PSG2).

Sample Code	T_m (^oC)[a]	ΔH (J/g)[b]	E_a (kJ/mol)[c]	Slope
PVI	115.80	248.60	102.65	-12347.79
Polymer gel	121.96	262.50	220.80	-26557.77
PSG2	114.15	247.60	147.86	-17784.88

[a]Melting temperature (T_m), [b]Enthalpy change (ΔH), [c]Activation energy (E_a).

Notes and Reference

¶ The gelation time of nanogels is shorter than the gelation time of polymer gel, due to the fact that the *f*-SWCNT have multiple interactions especially it has the ionic interaction on its surface. Hence the nanogels take longer time for gelation formation compared to polymer gels which has more free ions in the polymer matrix.

1 A. Srivastava, J. H. Waite, G. D. Stucky and A. Mikhailovsky, *Macromolecules,* 2009, **42**, 2168-2176.
2 J. Liu, A. G. Rinzler, H. Dai, J. H. Hafner, R. K. Bradley, P. J. Boul, A. Lu, T. Iverson, K. Shelimov, C. B. Huffman, F. Rodriguez- Macias, Y. S. Shon, T. R. Lee, D. T. Colbert and R. E. Smalley, *Science*, 1998, **280**, 1253-1256.
3 S. N. Jaisankar, N. J. Donna, and B. N. Christopher, *Polymer*, 2009, **50***,* 4775-4780.
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