Electronic Supporting Information

Self-assembled capsules of Poly N-glycidyl histidine ether-tannic acid for inhibition of biofilm formation in urinary catheter

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1. Experimental Procedure

1.1 Materials

L -histidine hydrochloride monohydrayte, epichlorohydrin, polyvinyl pyrolidone (PVP) were purchased from Sigma Aldrich. Tannic acid was purchased from Alfa Aesar (Ward Hill, USA). All solvents were analytical grade from Merck (India).

1.2 Instrumentation

1.2.1 Ultraviolet-Visible (UV-Vis) Spectroscopy

The UV-vis absorbance spectra were recorded on a Shimadzu UV-1601 spectrophotometer against solvent blank reference in the wavelength range of 300-600 nm. In all experiments, solutions were taken in quartz cuvette of 1-cm path length.

1.2.2 Fourier Transform Infrared (FTIR) Spectroscopy

For IR experiment, samples were dissolved in chloroform and placed onto a KBr pellet and dried. The dried specimen was recorded on Shimadzu 8400 FT-IR spectrophotometer. Absorbance spectra were obtained from 4000 to 400 cm^{-1} with a 4 cm^{-1} resolution, background spectra were also collected and subtracted.

1.2.3 Nuclear Magnetic Resonance (NMR)

The ^{1}H spectra were taken on a Bruker DPX400 (400 MHz for ^{1}H) in $CDCl_3$ using tetramethylsilane (TMS) as an internal standard. Both cases 2 mg/mL concentrated solutions were prepared. All signals were referenced to TMS to within 0.1 ppm.

1.2.4 MALDI-TOF-MS.

To obtain MALDI mass spectra, a Voyager time-of-flight mass spectrometer (Applied Biosystem, USA), equipped with 337 nm N_2 laser was used and operated in accelerating voltage 20 kV. The spectra were recorded in the positive ion linear mode. Reproducibility of the spectrum was checked four times from separately spotted samples.

1.2.5 Thermogravimetric analysis (TGA)

The thermo oxidative stability of histidine and GHEP polymer were carried out using a TGA Q50 thermal analyzer of TA Instruments, USA. All the experiments were performed by heating the sample at a rate of 20 °C/min, in nitrogen atmosphere, from room temperature to 600 °C.

1.2.6 Dynamic light scattering (DLS) measurements

The dynamic light scattering experiment was performed with an argon ion laser system (DLS, Malvern Instruments, Series 4700) at 25 °C. The concentration of colloidal solution was 1 mg/ mL for analysis. Scattering angle of 90° was used for the DLS measurements of particle size, and the results are reported as the average and standard deviation from more than twenty DLS measurements over a time period sufficient to reach equilibrium. Also, zeta potential of all samples in deionized water (1 mg/mL) was measured by same instrument.

1.2.7 Catheter coating procedure

A urinary catheter, uncoated natural rubber latex catheter (16 Fr, Rusch, USA), was used after being washed with ethanol for 30 min to remove any additives or low-molecular weight components. A composite of GHPE-TA/PVP was prepared for catheter coating; desired amounts of GHPE-TA hybrid material (1 mg) and PVP lubricant were dispersed in DMF (1mg/ mL). Then catheters were coated with composite solutions by dipping technique in solution for 10-min. The blend coated catheters were placed in a vaccum oven for 1 day at room temperature to remove any residual solvent.

1.2.8 Contact angle experiment

The static water contact angles for each sample were measured by Rame-Hart geniometer, Model No. 250 at room temperature. Measurements for five samples were reported as the average degree ± standard deviation.

1.2.9 Scanning electron microscopy (SEM) and Atomic Force Microscopy (AFM)

To record the FE-SEM and AFM images of samples, 5-10 µL of each solution was directly spotted on the glass cover slip as a drop-caste method. Samples were dried and fixed onto a graphite stub and kept in an auto sputter coater (E5200, Bio-Rad) under low vacuum for gold coating up to 120 seconds. Surface morphology was studied by using AFM and scanning electron microscope (JEOL JSM 5800) with an accelerated voltage between 5-20 kV.

1.2.10 Transmission electron microscopy (TEM)

The TEM was performed in JEOL JEM 2100 instrument. A small portion of self- assembled samples were drop cast on a Cu-grid. After drying the grid at ambient temperature, the samples were directly imaged under TEM.

1.2.11 Degradation of coated material : 15 mg of GHEP-TA/ PVP composite was incubated in 1 mL urine for 2 days, 5 days, 10 days and 15 days separately and dried over vacuum oven under 60 - 80 °C for 12 hours. The degradation of all samples was studied by thermogravimetric analysis from 50 °C to 700 °C with a heating range 10 °C/min in TA instrument (model Q50).

1.2.12 Adhesion stability of coated polymer on catheter surface:

A small piece of catheter tube were incubated in PVP/Capsules composites mixture in DMF solution for 2 hours, dried under vacuum oven for 24 hours under heating 70-80 °C. Then weight of coated material on the catheter surface was calculated by gravimetrically. The release study was performed by incubated coated catheter by immersion in 3 mL urine after 2 days, 5 days, 10 days and 15 days respectively. One blank test was also performed to determine the total concentration of coated material onto catheter surface.

1.2.13 Biofilm formation on urinary latex catheter

Biofilm formation of a clinically isolated uropathogen, *E. coli,*[1] was grown in 6 well polystyrene flat bottomed well plates and each well contains 3 mL of Trypticase Soy Broth (TSB) with 0.5% glucose, with an inoculum dose of 10^8CFU/mL and incubated for 48 h at 37°C. The latex urinary catheter was cut into small pieces of 2 cm^2 and sink into individual well. Catheters containing inoculated wells are allowed for growth of 7 days and each 48h interval the old medium was

replaced with fresh medium. After 7 days of bacterial growth, the catheters were removed from culture medium washed thrice gently with PBS (1X) buffer and visulalized under FE-SEM. Simultaneously, both coated and uncoated biofilm containing catheters are also assayed with crystal violet stain for quantifying the amount of remaining adhered biofilm[2]. Moreover, the grown biofilms on the surface of catheter are also removed by vortexed and sonicated for 2 min, respectively, with 4 mL of PBS containing 0.1% Tween-80 to detach bacteria adhering to the surfaces, following Flemming et *al*.[3]. The number of adherent bacteria was quantitatively determined by colony counts and expressed as the number of colony-forming units (CFU) per square centimeter. All independent assays were performed four times. Two well are used as positive control without catheter for monitoring the growth of bacteria and two wells as negative control without inoclum (only medium) to maintain the sterility.

1.2.14 Cytotoxicity assay

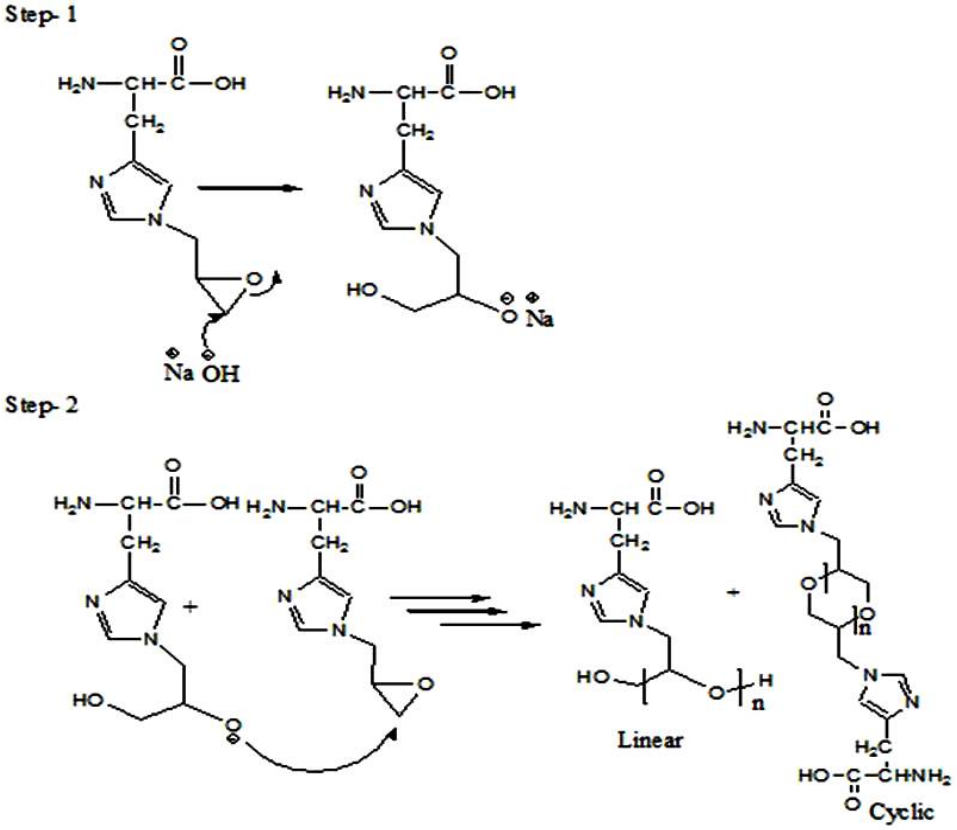
MTT [(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2Htetrazolium)], assay was performed to determine cell cytotoxicity following the method described earlier by Mandal et *al*.[4]. 3T3, primary mouse embryonic fibroblast (2.0×10^3) cells were seeded in 100 µL complete DMEM medium per well in 96 well plates. Plates were incubated at 37 ^{0}C in 5% CO_2 for 24 hours for cell attachment. Cells were treated with individual compounds with variable concentration from 5-100 µg/mL and incubated at 37^{0}C in 5% CO_2 for 48h. Three wells were used in the 96 well plates for each derivative and repeated three times. For the MTT assay, thiazolyl blue tetrazolium bromide solution (100µL; 1 mg/mL) in incomplete medium was added and this mixture incubated for 4 hours. After that, 100 µL of dimethylsulphoxide (DMSO) was added and the plates were rotate for 5 minutes. Optical density was recorded at 550 nm with DMSO as the blank. Percentage of cell viability was plotted against concentrations of derivatives. Cells treated without any compound used as control.

1.2.15 Synthesis procedure of N-glycidyl histidine ether polymer (GHEP)

L- histidine hydrochloride monohydrayte (1.05 gm, 0.005 molar) was dissolved in 150 mL ethanol at pH 8.0 by addition of NaOH (0.006 molar) at 40 °C temperature for 5 hours and then epichlorohydrin (2 mL) added drop-wise in mixture. The pH of the solution was further

increased from 8 to 12 and the reaction mixture was refluxed for 24 hours at 55 °C temperature. A solid reddish resinous product was obtained and isolated by filtration. The solid product was dissolved in water and neutralized by dilute HCl, then precipitated in methanol. The product was dried under vacuum oven over a night. Thus resinous product was formed (Scheme S1,a & b) and characterized by the UV-vis, FTIR, ^{1}HNMR and MALDI-TOF-MS spectroscopic study.

Scheme



S1. Mechanistic basis of polymer formation. The initiation reaction is thought to proceed via hydroxyl nucleophilic attack at the terminal epoxy group resulting in anionic ring opening reaction in step 1. The formation of anionic nucleophile is further reacting with another epoxy group of N-glycidyl histidine which propagates the ring opening polymerization[5] reaction and finally gives mixture of oligomeric products as linear and cyclic which is formed by the elimination of water molecule from terminal hydroxyl group, presented by the chemical reaction in scheme S1,a.

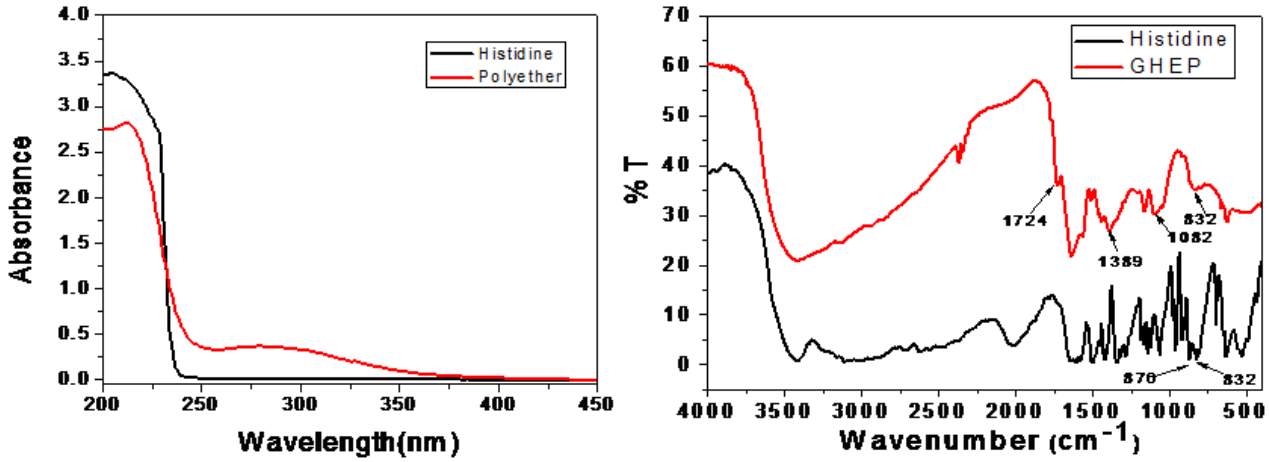
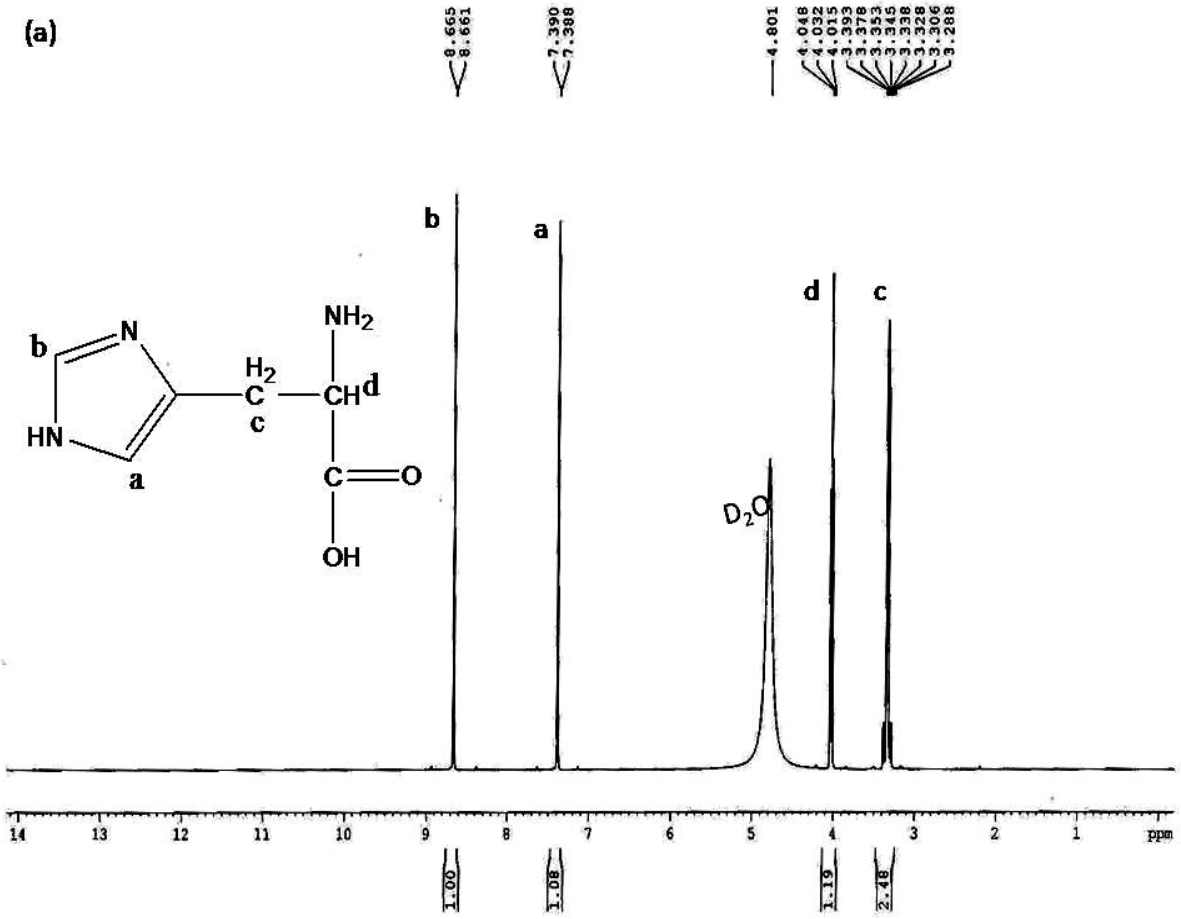


Fig. S1. Spectroscopic characterization of GHEP polymer. (a) UV spectrum of (i) histidine (ii) GHEP; (b) FT-IR spectrum of (i) histidine (ii) GHEP.



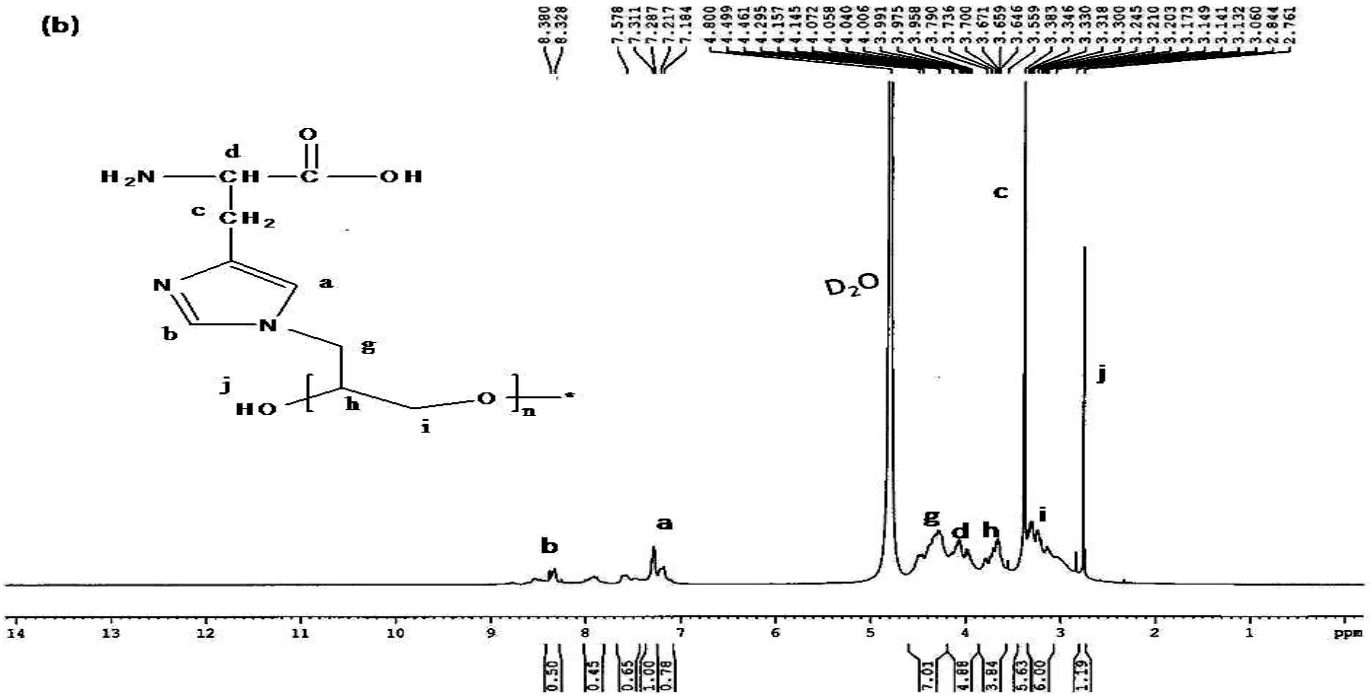


Fig S2. The [1]HNMR spectra of (a) histidine and (b) GHEP polymer.

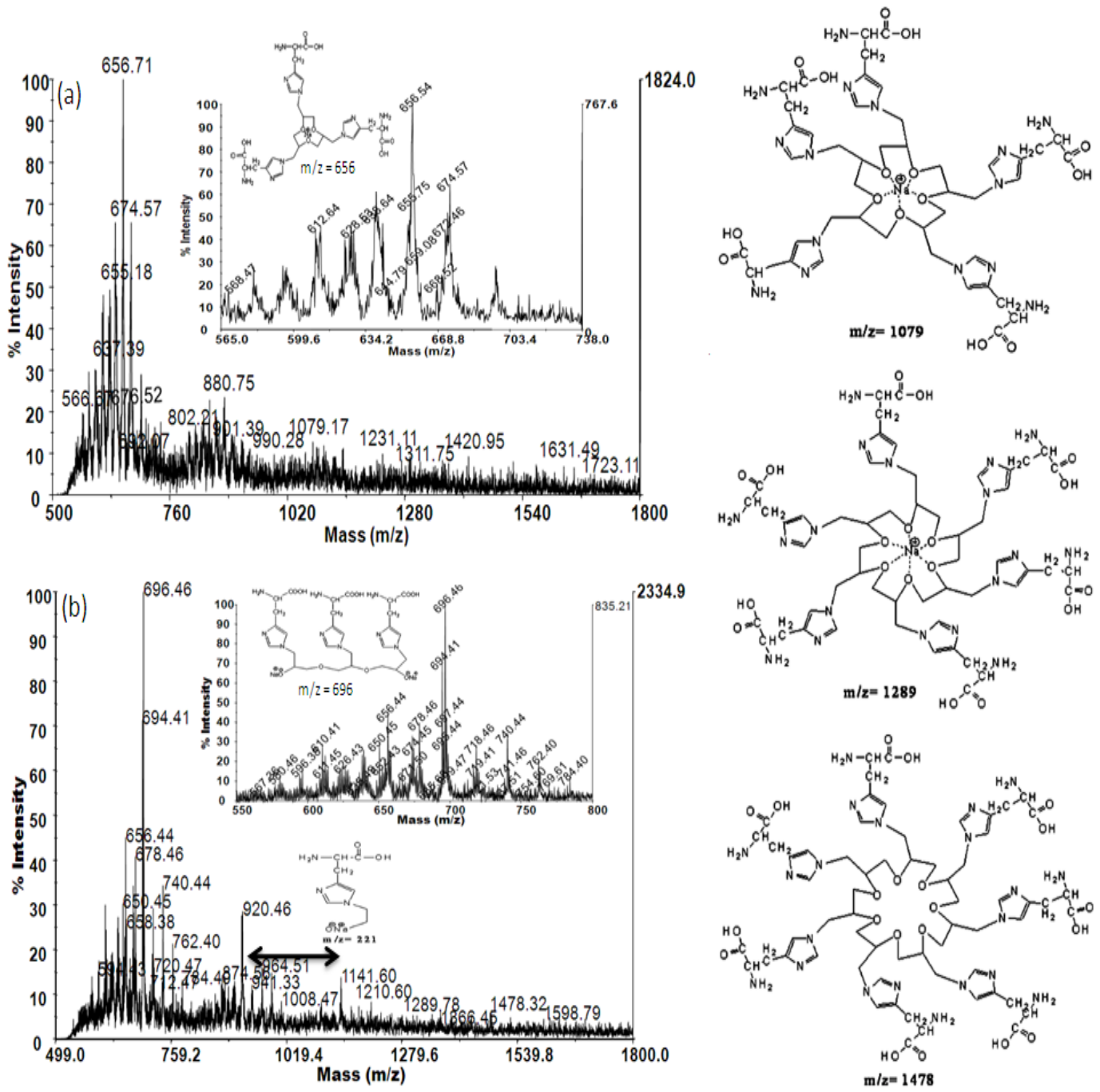


Fig. S3. MALDI TOF-MS analysis of the synthesized polymer represents their linear (a) and cyclic structures (b).

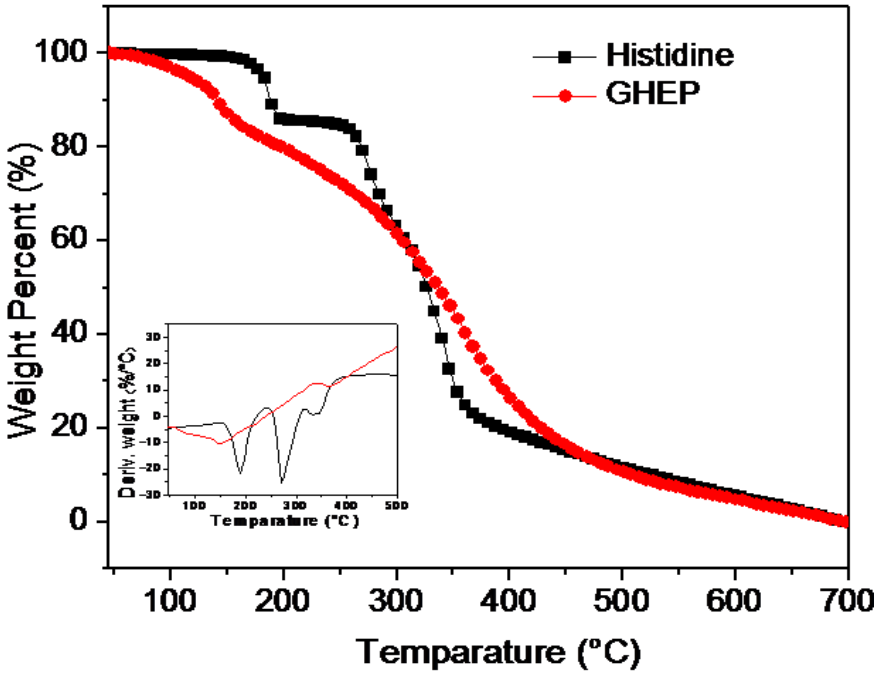


Fig S4. TGA and derivative of TGA (inset) thermograms of histidine and GHEP.

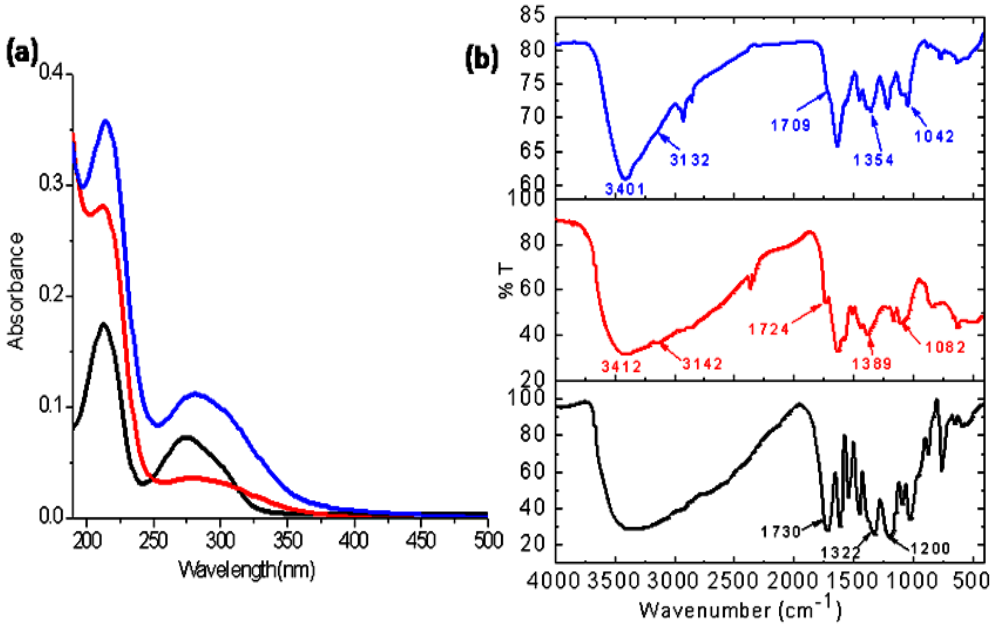


Fig S5. Spectroscopic characterization of GHEP-TA capsules. UV-vis spectra obtained from N-glycidyl histidine ether polymer self-assembled with tannic acid (blue color), only tannic acid (black colour); GHEP (red colour) and their assembled capsule (red colour). FTIR spectra obtained from N-glycidyl histidine ether polymer self-assembled with tannic acid (b), only tannic acid (black colour); GHEP (red colour) and their self-assembled capsule (red colour).

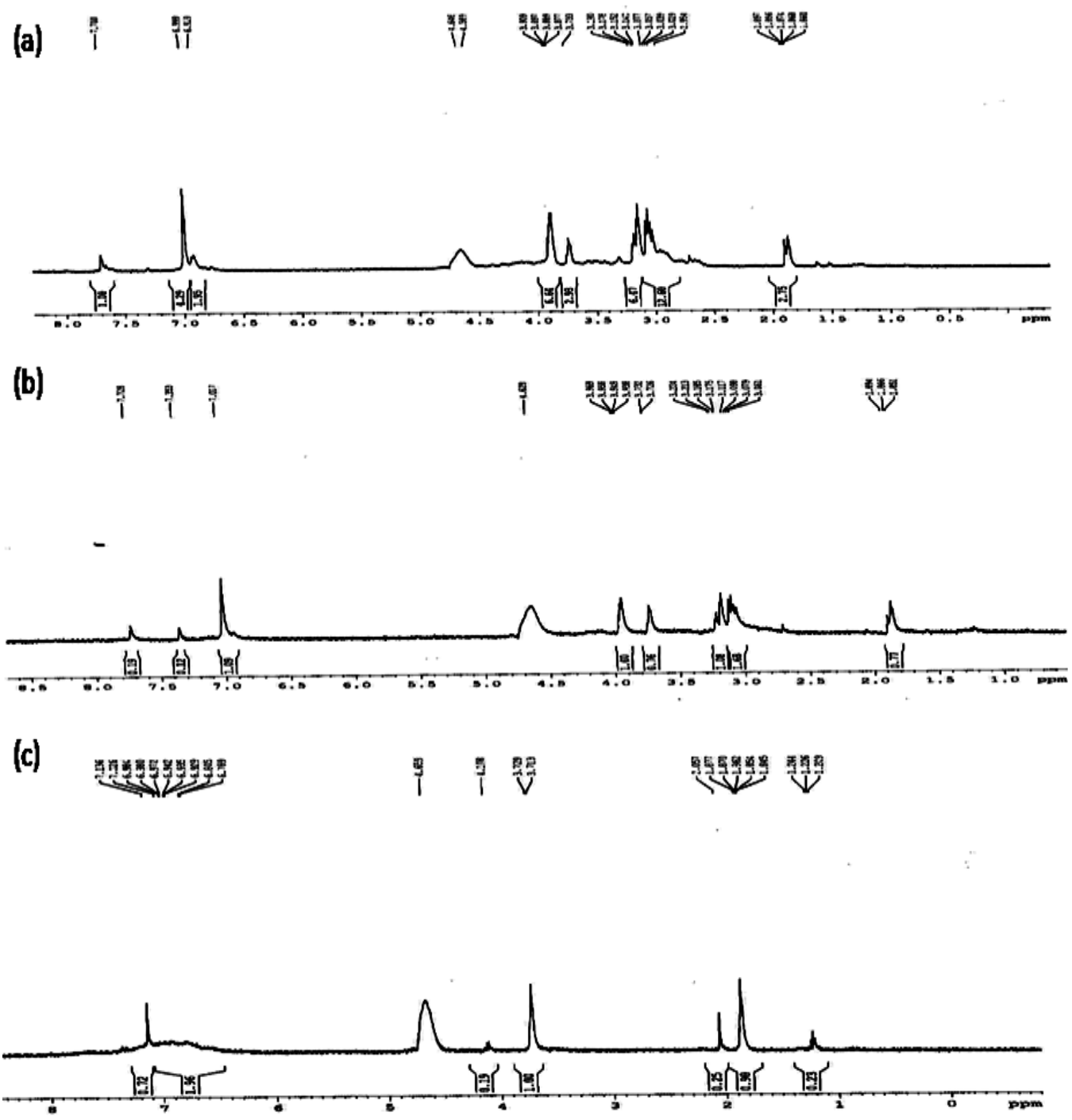


Fig S6. The [1]HNMR spectra of (a) pure tannic acid (b) before assembly and (c) after assembly of tannic acid and GHEP polymer.

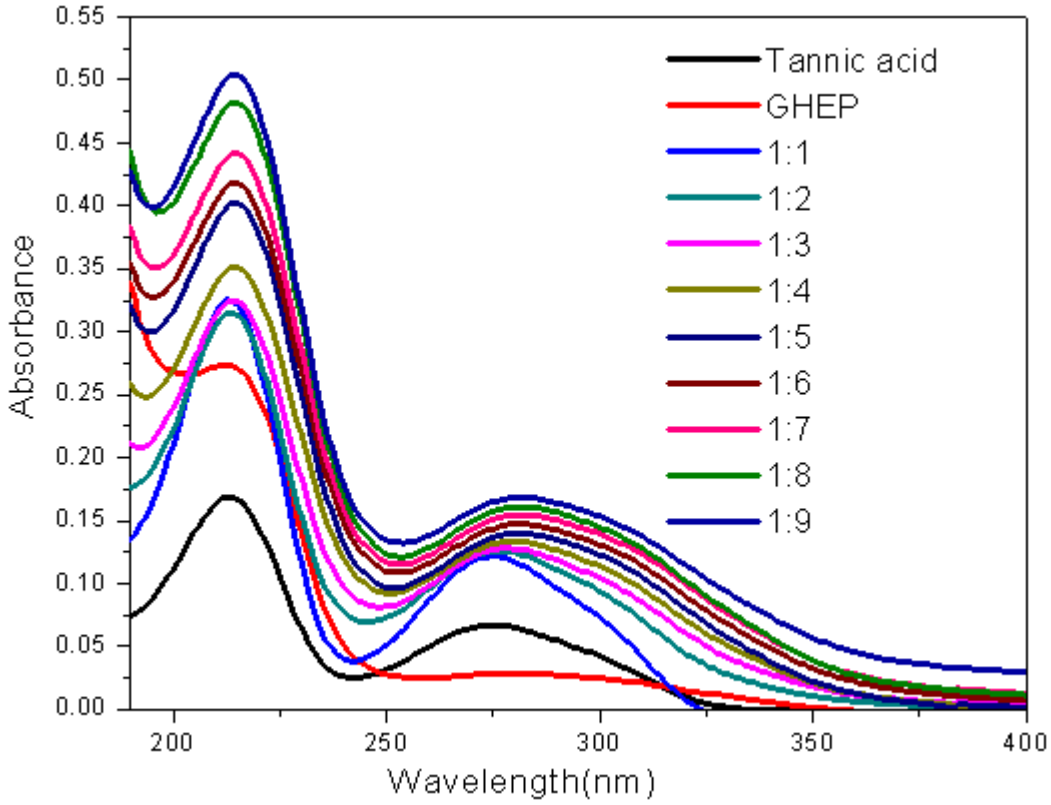


Fig S7. Optimization of tannic acid : GHEP polymer ratio (mg/mL) for the preparation of self-assembled micro-capsules.

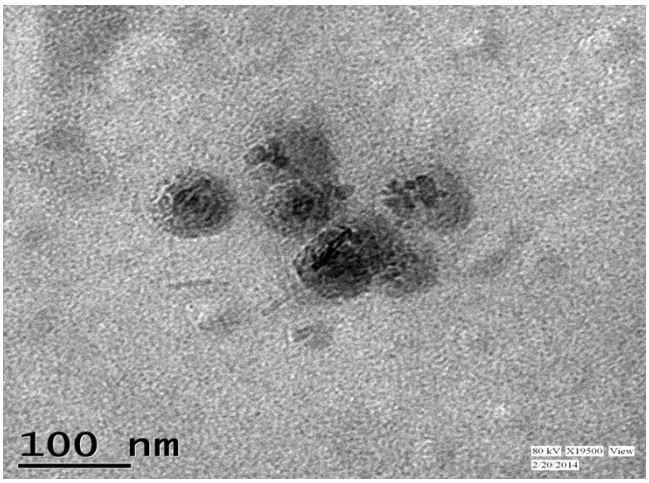


Fig S8. TEM image of GHEP-TA capsules.

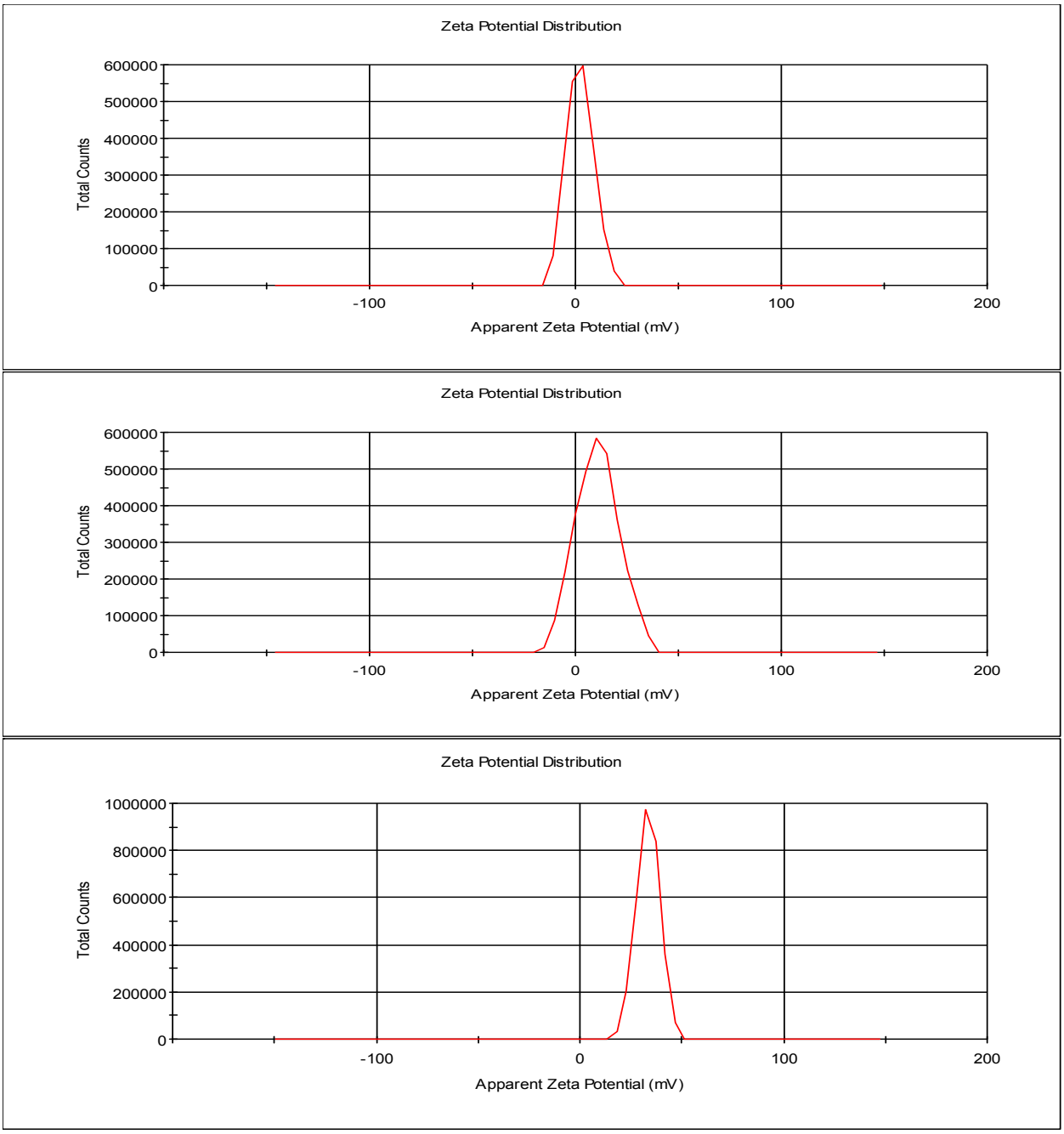


Fig S9. Zeta potential of (a) GHEP polymer (b) tannic acid and (c) GHEP-TA capsules in aqueous medium.

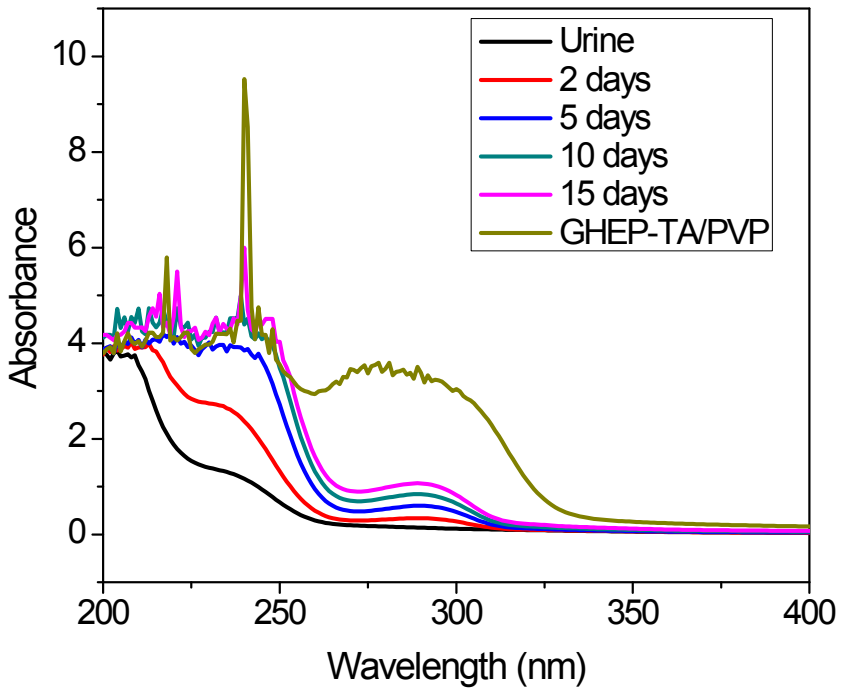


Fig. S10. UV-Visible absorbance spectra of GHEP-TA/PVP composites adhesion stability on catheter tube surface in urine with different time periods.

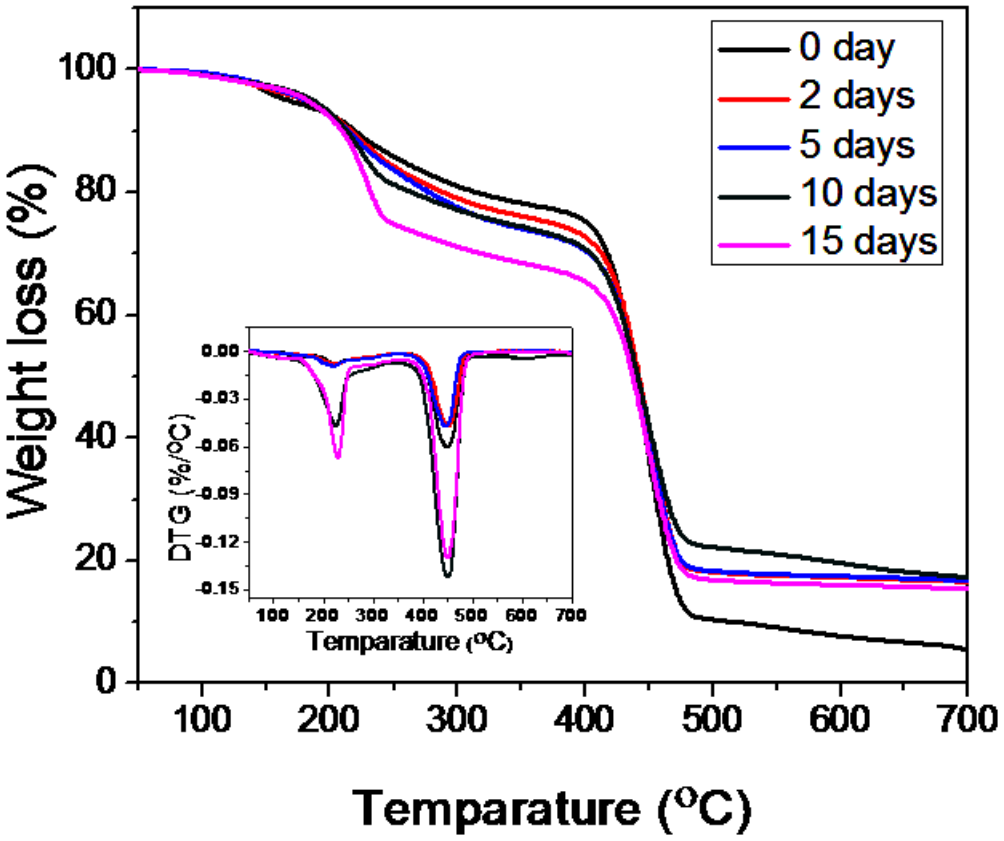


Fig. S11. TGA and derivative of TGA (inset) thermograms of GHEP-TA/PVP composite polymer for degradation after incubation in urine with different time periods.

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