Supplementary Information

Stabilization of Chitosan-Based Polyelectrolyte Nanoparticle Cargo Delivery Biomaterials by a Multiple Ionic Cross-Linking Strategy

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1. Experimental section

1.1. Materials

Low molecular weight CS with MW of 50-190 kDa, degree of deacetylation ≥75 %, and degree of substitution=1.26 was provided by Sigma Chemical Co. (St. Louis, MO, USA). Degree of substitution was calculated using Eq. (1) where W is the weight of the substituent group, Ws is the net increase in CS weight caused by introduction of one substituent group per unit, and Y is the percentage of substituent (Hu, Thalangamaarachchige, Acharya, & Abidi, 2018). The average molecular weight of monomer repeat unit was calculated as 164 Da, (Falco, Falkman, Risbo, Cárdenas, & Medronho, 2017) and percentage of substituent was 25% according to the manufacturer specification sheet.

$$\text{Eq. (1)} \qquad \text{Degree of substitution} = \frac{164Y}{100W - YWs}$$

2. Result and discussion

2.1. Evaluation of z-average size and ζ-potential of PENs

Table S1. Z-average size, ζ- potential and PDI of PENs at different concentrations of DS and PEI.

			aPENs			bPENs		
			z-average size	**PDI**	**ζ- potential**	**z-average size**	**PDI**	**ζ-potential**
Concentration of DS	**(mg/mL)**	**0.116**	342.53±5.41***	0.48±0.01	18.30±2.67	249.00±2.61^{ns}	0.39±0.02	17.00±1.71
		0.133	**268.80±4.00*** **	**0.40±0.01**	**18.73±1.41**	**246.97±0.25** **	**0.35±0.038**	**17.53±0.63**
		0.15	277.37±3.40*	0.42±0.01	20.50±1.83	273.60±4.26**	0.33±0.04	18.17±1.46
		0.167	384.70±19.16**	0.64±0.12	19.17±2.15	280.93±3.49**	0.41±0.01	17.50±0.85
Concentration of PEI	**(mg/mL)**	**1.16**	254.73±1.42***	0.42±0.03	34.10±2.19	**258.13±4.85*** **	**0.25±0.01**	**34.23±3.01**
		1.33	**206.40±2.08*** **	**0.29±0.01**	**36.40±3.77**	300.60±0.61**	0.24±0.01	35.43±2.63
		1.5	214.10±0.43*	0.29±0.01	33.83±2.70	319.00±1.93**	0.21±0.02	34.70±1.32
		1.67	227.30±3.22**	0.31±0.01	37.67±2.94	323.13±4.15***	0.24±0.01	36.83±2.63

The confidence level was set to 95%. A *p* value of <0.05 shows significant change and labelled as * (more * symbols indicate higher significance). P-value of PENs was compared to P-value of 0.133 mg/mL DS; 1.33 and 1.16 mg/mL PEI in aPENs and bPENs formulations, respectively.

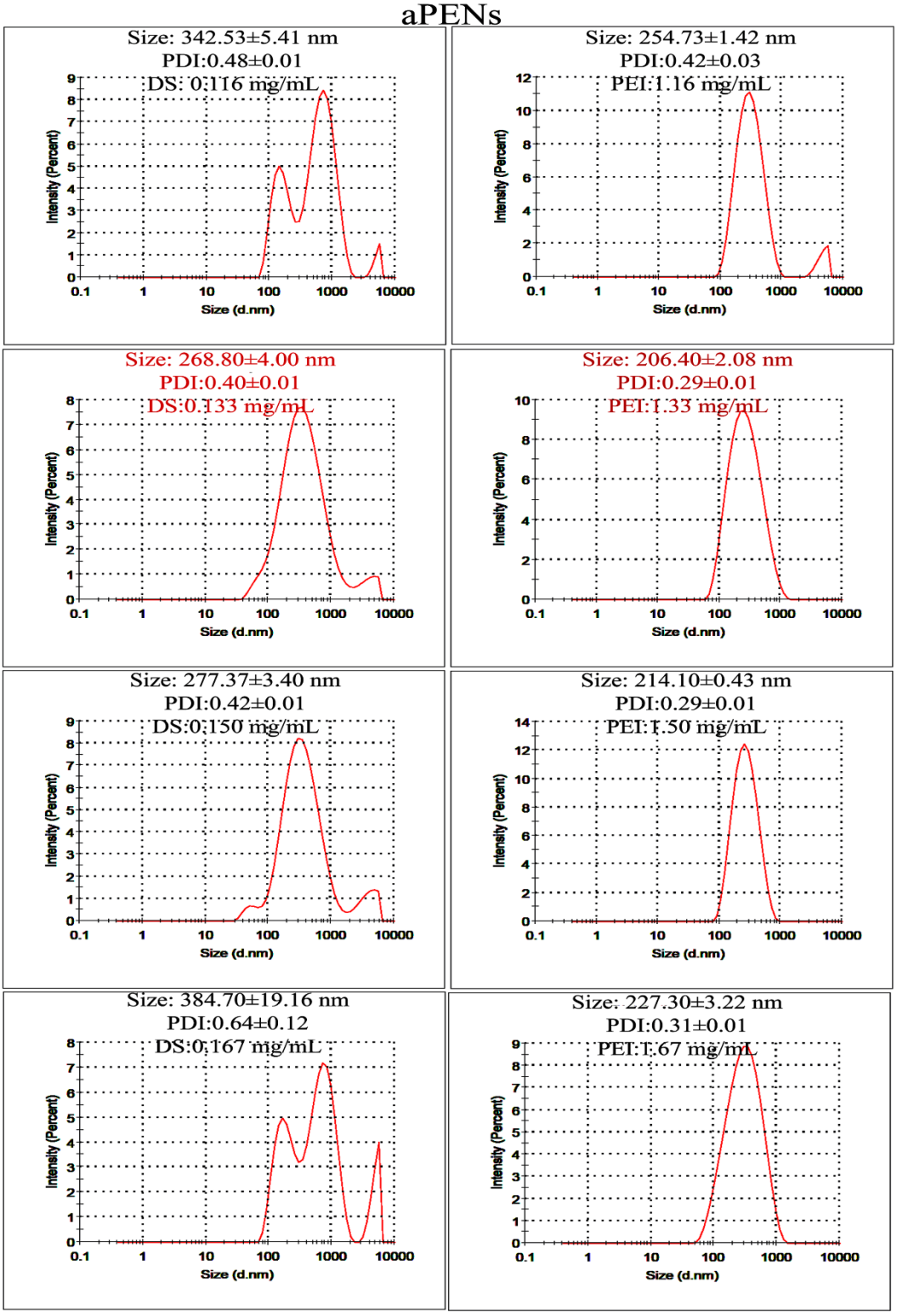

Fig S2. Z-average size and PDI of aPENs at different concentrations of DS and PEI.

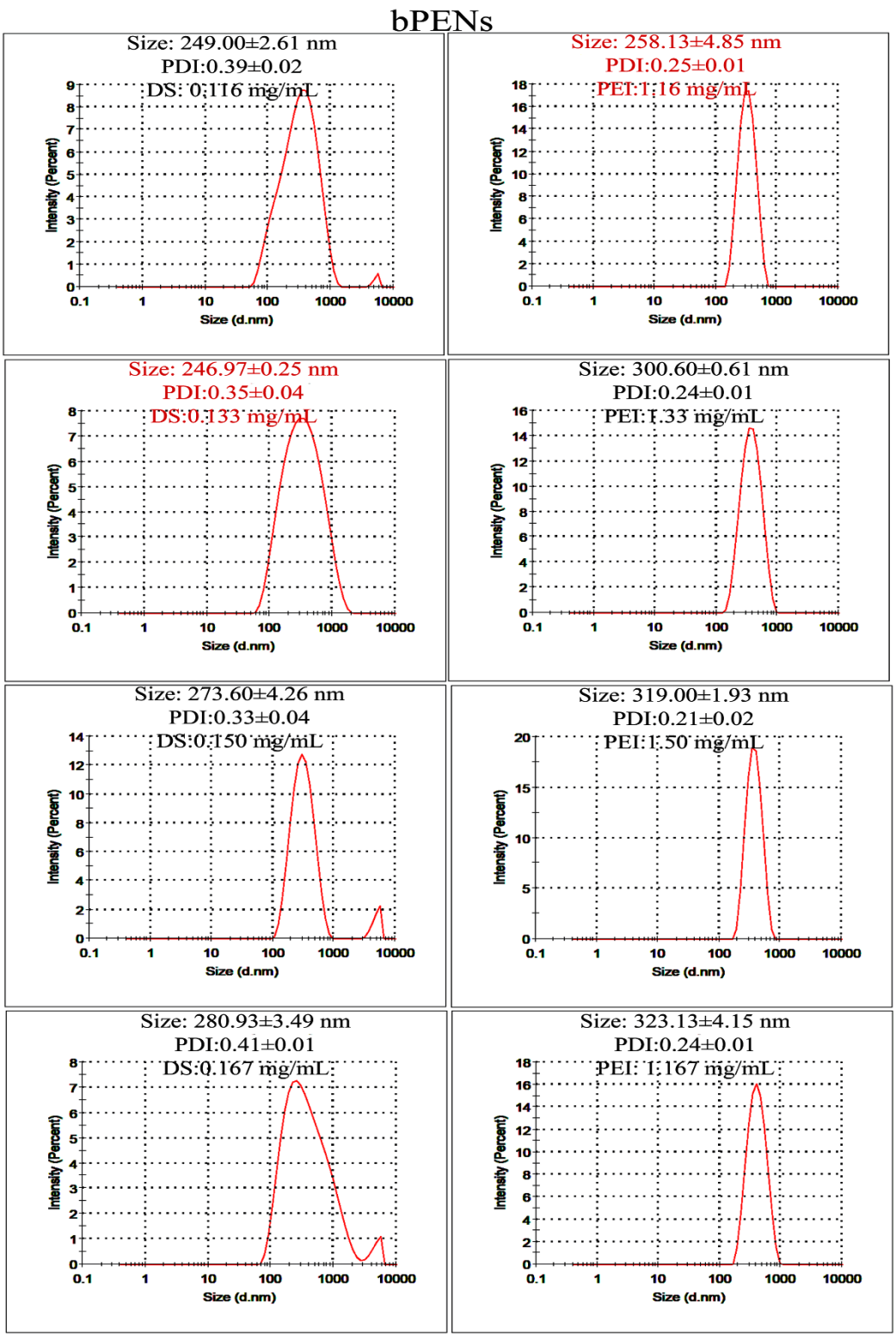

Fig S3. Z-average size and PDI of bPENs at different concentrations of DS and PEI.

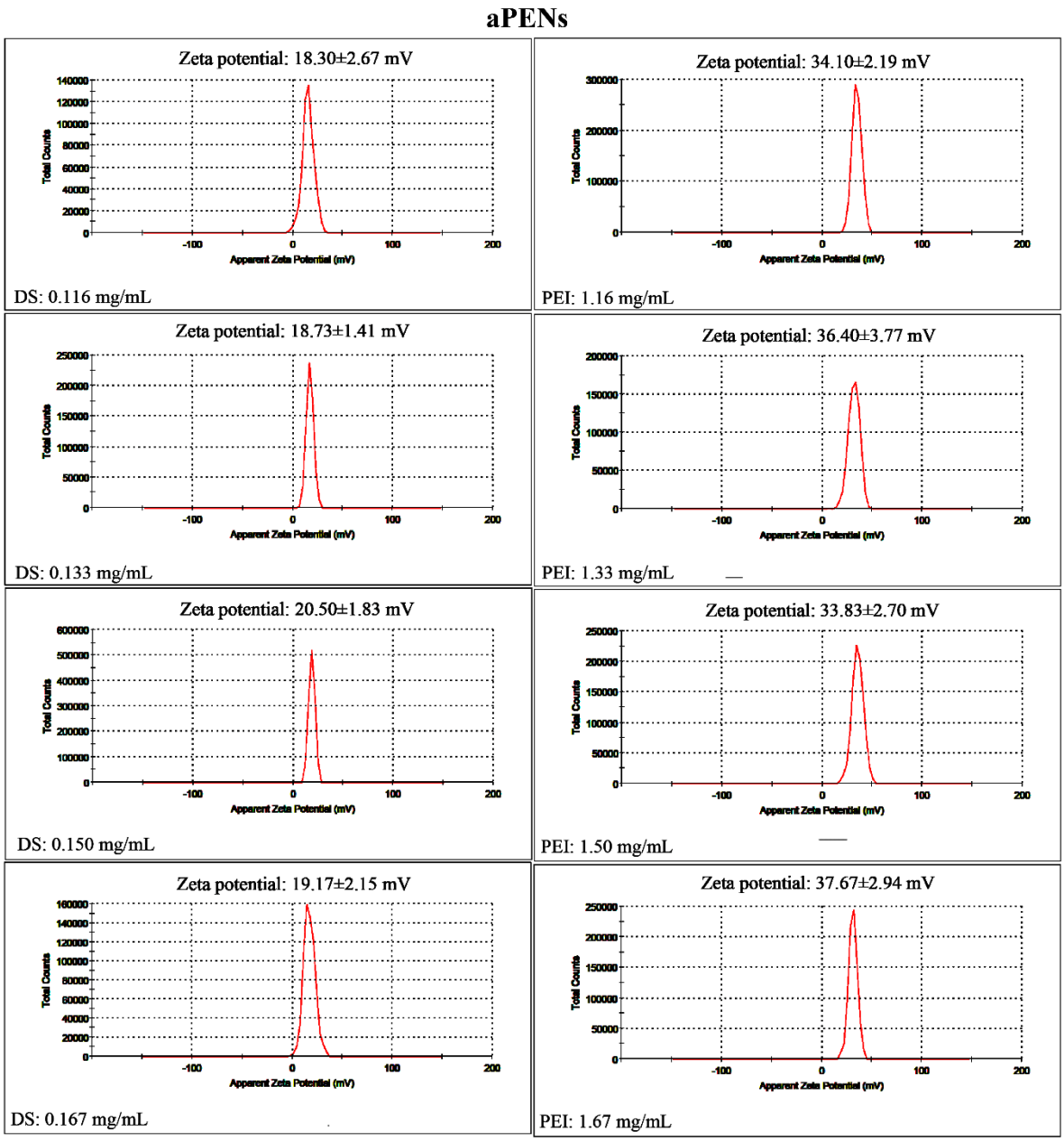

Fig S4. ζ- potential of aPENs at different concentrations of DS and PEI.

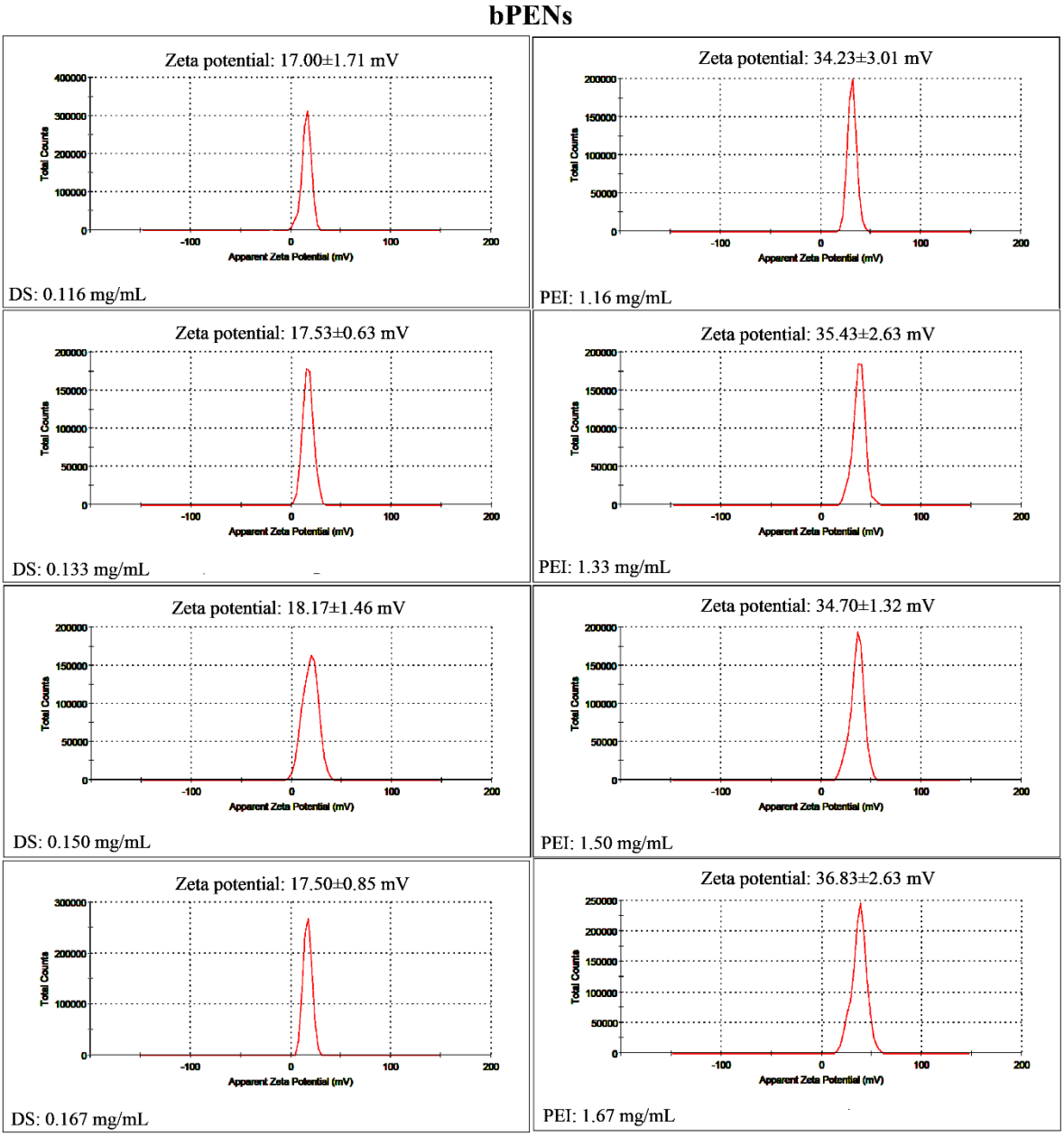

Fig S5. ζ- potential of bPENs at different concentrations of DS and PEI.

2.2. Evaluation of colloidal stability of PENs

Table S2. Comparison of PENs' z-average size by DLS at day1 and day 60 in different media including water, PBS 1 and 10mM.

	aPENs			bPENs		
	Day1	Day60	P-value	Day1	Day60	P-value
Water		352.43±3.35*	0.0002		284.80±4.41$^{\#}$	0.0004
PBS 1mM	176.31±1.77*	239.47±3.04*	0.003	219.42±2.28$^{\#}$	267.7±6.17$^{\#}$	0.0015
PBS 10mM		245.5±5.54*	0.0016		243.83±8.70	0.1644

n = 3, Mean ± Standard Deviation, *$^{\#}$ p value<0.05

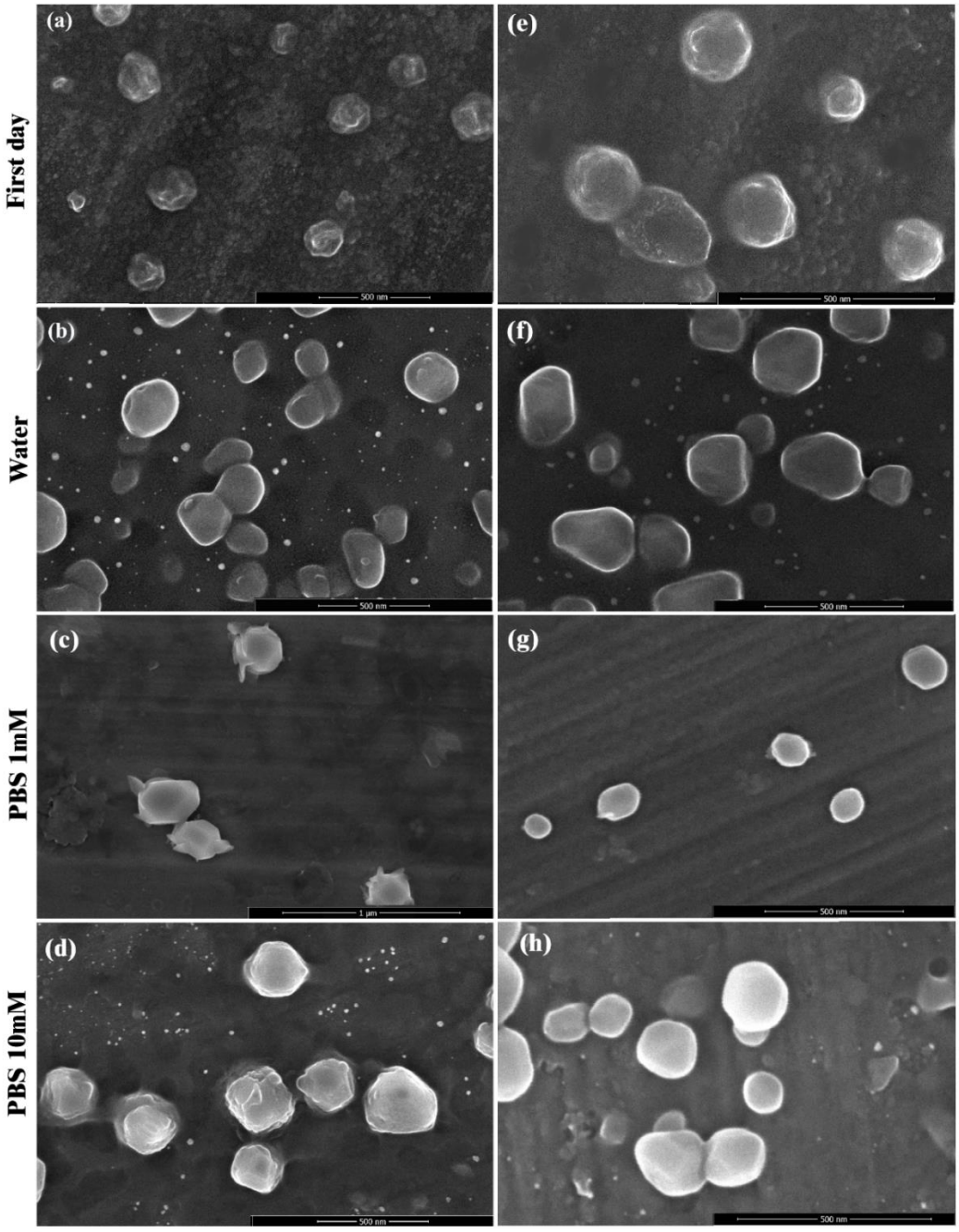

Fig. S1. Comparing SEM images of aPENs and bPENs after storage in water and PBS (1 and 10mM) for 2 weeks. Size of aPENs showed significant increase in size from 157.97±47.84 (a) to 196.78±35.61 (b), 244.87±20.30 (c) and 236.19±29.59 (d) after storage in water, PBS 1 and 10mM, respectively. Nonetheless, bPENs demonstrated significant increase from 170.55±40.49 (e) to 188.49±58.64 in water (f), and thereafter significant decrease to 124.66±16.42 (g) and 134.28±33.75 (h) in PBS 1 and 10 mM, respectively.

2.3. Thermogravimetric analysis

Table S3. Total percentage loss of weight in different compounds

Compounds	Total loss of mass (%)
CS	69.25
TPP	5.75
DS	61.27
PEI	97.69
aPENs	78.97
bPENs	84.77

2.4. In vitro release studies in two media with different pHs

Table S4. The percentage of cumulative release rate of PENs composed of single layers (TPP) and multilayer structures (TPP/DS and TPP/DS/PEI) after 72h at two different pH values of media (3 and 7.4).

	aPENs			bPENs		
	TPP	TPP/DS	TPP/DS/PEI	TPP	TPP/DS	TPP/DS/PEI
pH3	90.61±5.25*$^{\#}$	44.49±3.75*	57.69±2.66$^{\#}$	74.82±1.66§	45.17±2.82 §	64.38±1.80
pH7.4	64.32±5.85$^{\&}$	43.23±2.27$^{\&\text{ɤ}}$	59.90±3.02$^{\text{ɤ}}$	57.44±2.76	49.90±2.13	60.67±7.27

n = 3, Mean ± Standard Deviation, $^{\#*\S\&\text{ɤ}}$ *p* value<0.05

References

Falco, C. Y., Falkman, P., Risbo, J., Cárdenas, M., & Medronho, B. (2017). Chitosan-dextran sulfate hydrogels as a potential carrier for probiotics. *Carbohydrate Polymers*, 172, 175-183.

Hu, Y., Thalangamaarachchige, V. D., Acharya, S., & Abidi, N. (2018). Role of low-concentration acetic acid in promoting cellulose dissolution. *Cellulose*, 25(8), 4389-4405.