

Supporting information:

DLS size distribution and Polydispersity Index (PDI Index) of insulin loaded CS/ALG and CS-g-PAMAM/ALG core-shell nanoparticles

The particle size distribution of freshly prepared insulin loaded CS/ALG and CS-g-PAMAM/ALG core-shell nanoparticle formulations were determined by photo correlation spectroscopy using a Malvern Zetasizer (Malvern Instruments Ltd., UK). Light scattering was monitored at a 90 ° angles and a temperature of 25 °C was maintained during the measurement. Every sample was diluted 100 times with distilled water before the measurement. The predetermined refractive index of the different formulae was incorporated into the computer software of the Zetasizer, which calculated the mean particle size, size distribution and polydispersity of the nanoparticles from intensity.

Polydispersity Index (PDI Index) is a parameter to define the particle size distribution, which is expressed as dimensionless number extrapolated from autocorrelation function in photon correlation spectroscopy. The value of Polydispersity Index may vary from 0.01 (mono dispersed particles) to 0.5-0.7, whereas, PDI Index value > 0.7 indicated broad particle size distribution of the formulation.

The particle size and particle size distribution are very critical factors for performance evaluation of nanoparticles. Significant variations are observed in drug loading, encapsulation efficiency, bioavailability and efficacy of the nanoformulations with wide particle size distribution. Formulation of nanoparticles of narrow size distribution (smaller particles) is a great challenge as the particles are internalized by the mechanism of endocytosis or through the tight junction of intestinal epithelial cells. The following Table 1 shows different formulation of insulin loaded CS/ALG and CS-g-PAMAM/ALG core-shell nanoparticles and their size distribution and PDI index. It is noticed that the particle size of CS-g-PAMAM/ALG core-shell nanoparticle at different weight ratios varies within the range of ~84-194 nm, whereas unmodified CS/ALG core-shell nanoparticle at different weight ratios are comparatively larger and vary between ~98-216 nm. However, in all the core-shell nanoparticle formulations a minority population of very smaller particles are observed. Again all the nanoparticles batches exhibit PDI index values of 0.25-0.65. So, it can be interpreted that the CS-g-PAMAM/ALG core-shell nanoparticles will be more preferred for intestinal internalization over unmodified CS/ALG core-shell nanoparticles in delivering insulin via oral route, due to its comparatively smaller size, particle size distribution and more stable particles showing mono dispersity.

Table 1: Insulin loaded CS/ALG and CS-g-PAMAM/ALG core-shell nanoparticles and their size distribution and PDI index

Nanoparticle Formulation code	CS or CS-g-PAMAM:ALG:INS ratio	Mean Particle size (nm) \pm SD	Size distribution	PDI Index \pm SD
CS/ALG/INS	0.25:3:1	143.26 \pm 22	9.3% (60-90 nm) 90.7% (140- 170 nm)	0.43 \pm 0.11
	0.5:3:1	119.84 \pm 19	15.36% (46-84 nm) 84.64% (115- 153 nm)	0.46 \pm .08
	01:03:01	98.69 \pm 12	12.5% (25-45 nm) 87.5% (100- 120 nm)	0.41 \pm 0.15
	02:03:01	157.12 \pm 28	19.1% (40-90 nm) 80.9% (150- 195 nm)	0.52 \pm .06
	03:03:01	216.11 \pm 27	6.33% (60-105 nm) 93.67% (190- 280 nm)	0.54 \pm 0.19
CS-g-PAMAM /ALG/INS	0.25:3:1	136.19 \pm 11	12.6% (40-55 nm) 87.4% (130- 145 nm)	0.39 \pm 0.14
	0.5:3:1	105.09 \pm 10	10.45% (30-50 nm) 89.55% (95- 110 nm)	0.28 \pm 0.1
	01:03:01	84.06 \pm 8	8.73% (25-40 nm) 91.27% (80- 99 nm)	0.25 \pm 0.15
	02:03:01	122.07 \pm 11	15.1% (65-80 nm) 84.9% (115- 140 nm)	0.41 \pm 0.08
	03:03:01	194.24 \pm 16	11.26% (60-90 nm) 88.74% (182- 215 nm)	0.51 \pm 0.04