Electronic Supplementary Information

For

β-Cyclodextrin–conjugated amino poly(glycerol methacrylate)s for efficient insulin delivery

Table S1 Molecular weights of PGMAs.

Polymer	Mn (KDa)	Mw (KDa)	Mw/Mn
L-PGMA	9.5	12.2	1.28
S5-PGMA	10.1	11.3	1.12
S21-PGMA	10.6	11.2	1.06

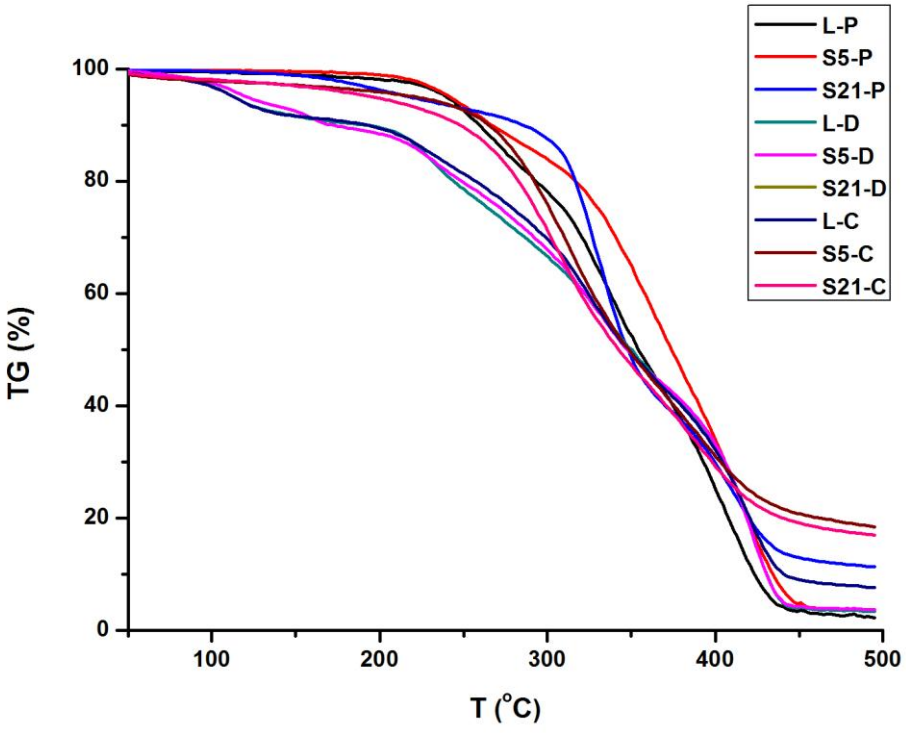


Figure S1. TGA curve of PGMAs, amino PGOHMAs and CD-PGOHMAs.

The effect of complexes forming conditions on their physichemical properties.

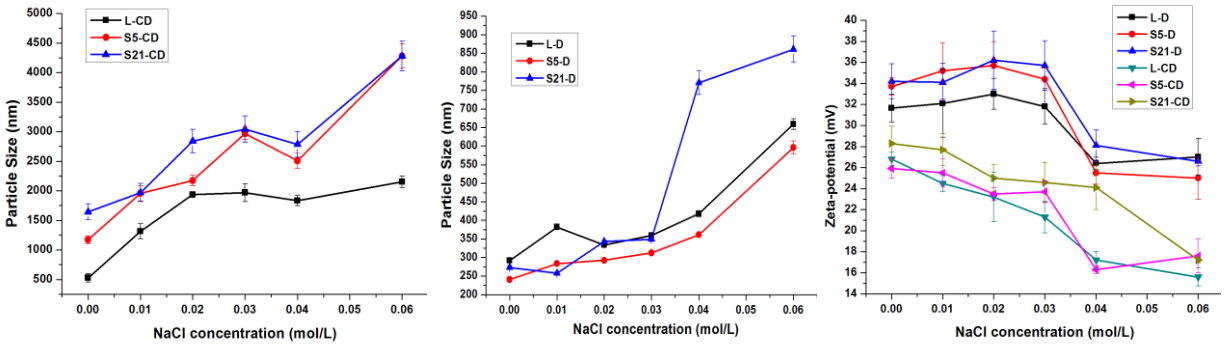
1. Effect of the ionic strength on the properties of the complexes.

The sizes of the insulin/polymer PECs formed at different NaCl concentrations were shown in Figure S2 (a) or (b). The initial pH value of insulin was adjusted to 7.4 using 0.01 M HCl and 0.01 M Tris base. The higher the salt concentrations, the weaker the electrostatic interactions between the oppositely charged electrolytes are. The increase of size observed in Figure S2 with the addition of NaCl was caused by the non-specific aggregation of polymers due to the charge screening effect. Higher salt concentrations enable rearrangement and flocculation of PECs through secondary aggregation.

Ins-CD-series (insulin/CD-DETA-PGOHMA) nanoparticles exhibited similar trend in particle size as D-series. While the size of CD-series were much larger than those of D-series. Not only electrostatic attraction, but also inclusion interaction existed in ins-CD complexes. [S1] Insulin is too bulky to be wholly included into CDs' cavities and its hydrophobic side chains can be only partially included into the CDs' cavity to form inclusion complexes. Therefore, the amino acid of insulin could be probably included in the hydrophobic cavities of CDs belonging to two or more CD-DETA-PGOHMA polymers, which may cause the physical cross-linking of CD-DETA-PGOHMA polymers and aggregation of the complexes. Another explanation could be that DETA-PGOHMA and insulin form a compact complex by electrostatic attraction. The electrostatic interaction is relatively weak between CD-DETA-PGOHMA and insulin due to the reduced charge density of

CD-substitution.

The influence of the physicochemical parameters on the polyelectrolyte complexes surface charge has been evaluated by zeta potential measurements. The zeta potential values at different NaCl concentrations were shown in Figure S2 (c). All the zeta potential values of PECs were positive. The zeta potential values ranged from 26 to 36 mV for D-series complexes, and varied from 16 to 28 mV for CD-series. The zeta potential value for D-series complexes was higher than that of CD-series because the amino density was higher in DETA-PGOHMAs than that in CD-DETA-PGOHMAs. Meanwhile, the zeta potential values decreased as NaCl concentration increased. The high ionic strength screened electrostatic interaction, resulting in a lowered zeta potential value.



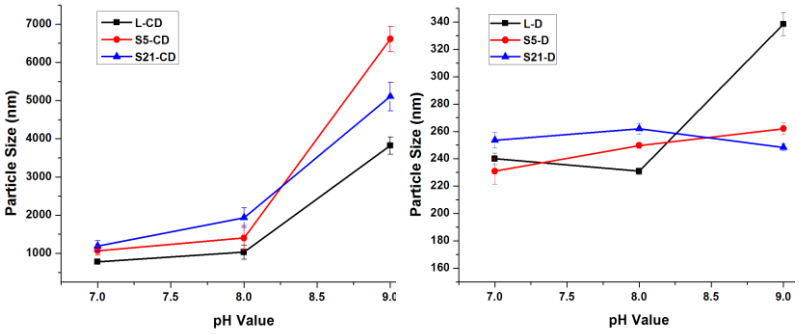
(a) (b) (c)

Figure S2. Effect of NaCl concentration on the particle size of CD-series complexes (a) and D-series complexes (b). Zeta potential of complexes at different NaCl concentration (c). Results are shown as mean ± standard deviation (n = 4).

2. Effect of the pH values of insulin solutions on the properties of the complexes.

The pH of the surrounding medium is another important factor for

polyelectrolytes with carboxyl and amino groups because it affects the degrees of ionization of polymer and the charge densities. [S2] The PECs were formed at equal volume of polymer (1 mg/mL) and insulin (1 mg/mL) solution. The initial pH value of insulin varied from 7 to 9. As can be seen in Figure S3, for CD-series, the particle size slightly increased from 780 nm to 1068 nm (ins-**L-CD** complex is shown as a typical example) when pH increased from 7 to 8, and became much bigger at pH 9. This was due to the variation of the segmental charge of amino and imino groups when pH changes. At pH = 7 (the initial pH value of the insulin solution), amino and imino groups are partly or completely charged, and the charged polymers repel to prevent particle from growing. As pH increased, the protonation degree of amino and imino groups in CD-DETA-PGOHMA decreased, thus the weakened repulsion led to the aggregation of the complex. At initial pH of 9, some particle precipitated, which may be caused by the presence of cloud point of CD-series polymer (cloud point of S5-CD pH = 7.25, S21-CD pH = 7.45), since the final pH values of ins-L-CD, ins-S5-CD and ins-S21-CD suspension were 7.50, 7.43 and 7.48, respectively. For D-series, the size of ins-L-D increased with increasing pH values, while the size of ins-S5-D & ins-S21-D kept the same. The charge density varied a little for the star-shaped polymers because of steric effect. In addition, the number of primary amine groups in D-series is much more than CD-series polymers. D-series showed considerably stronger buffer capacity. [S3] Variation of pH value has less impact on the size of D-series.



(a) (b)

Figure S3. Effect of initial pH value of insulin solution on the particle size of CD-series complexes (a) and D-series complexes (b). Results are shown as mean ± standard deviation (n = 4).

3. Effect of the polymer concentrations on the properties of the complexes

Because of the large particle size of CD-series complexes, the preparation condition of PECs was optimized by varying **L-CD** concentration. As shown in Figure S4, the diameter of ins-**L-CD** complexes decreases with increased **L-CD** concentration. Both electrostatic and inclusion interaction existed in the combination procedure. The probability of physical cross-linking between insulin and CD-DETA-PGOHMAs degraded sharply along with the decreased **L-CD** concentration, resulting in a decreased particle size.

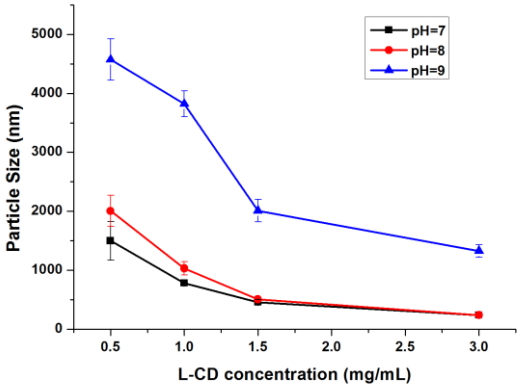


Figure S4. Effect of the polymer concentrations on the particle size of CD-series. Results are shown as mean ± standard deviation (n = 4).

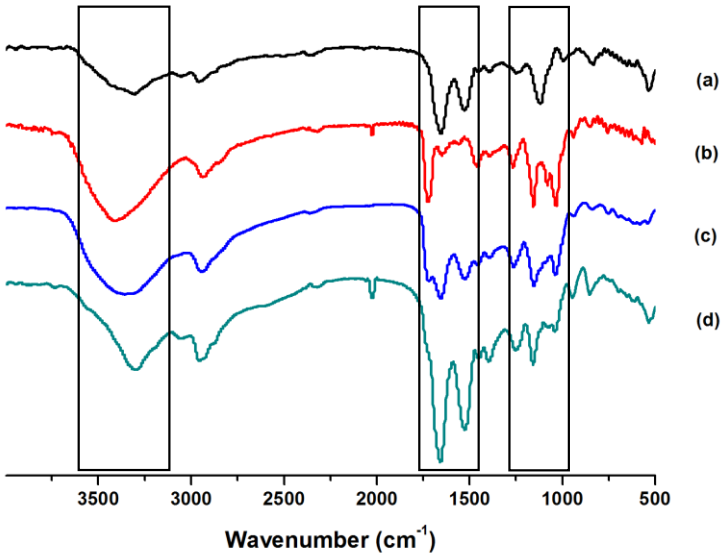


Figure S5. FT-IR spectra of (a) insulin (b) **L-CD** (c) **L-CD**/insulin physical mixture and (d) ins-**L-CD** complex.

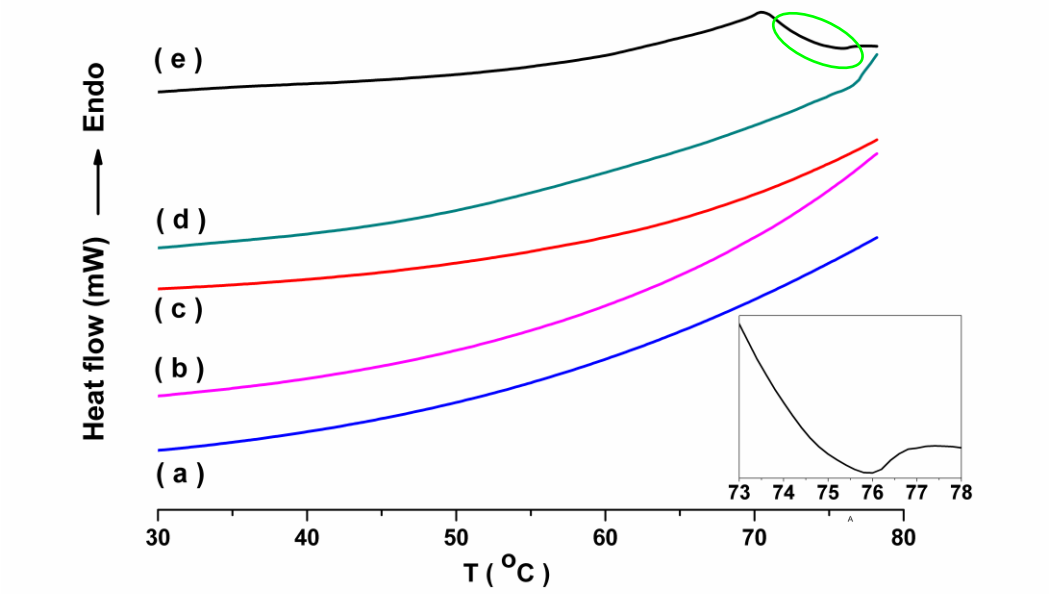


Figure S6. DSC thermograms of insulin solution (a), **L-D** solution (b), insulin/**L-D** solution (c), **L-CD** solution (d), insulin/**L-CD** solution (e).

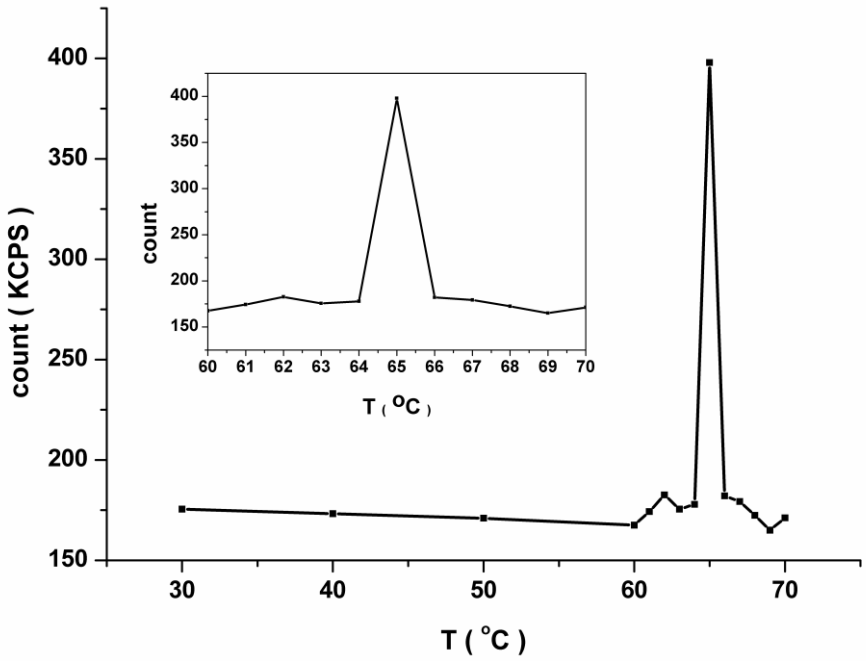


Figure S7. Melting temperature of insulin dissolved in 0.1 M tris (hydroxymethyl)-aminomethane buffer.

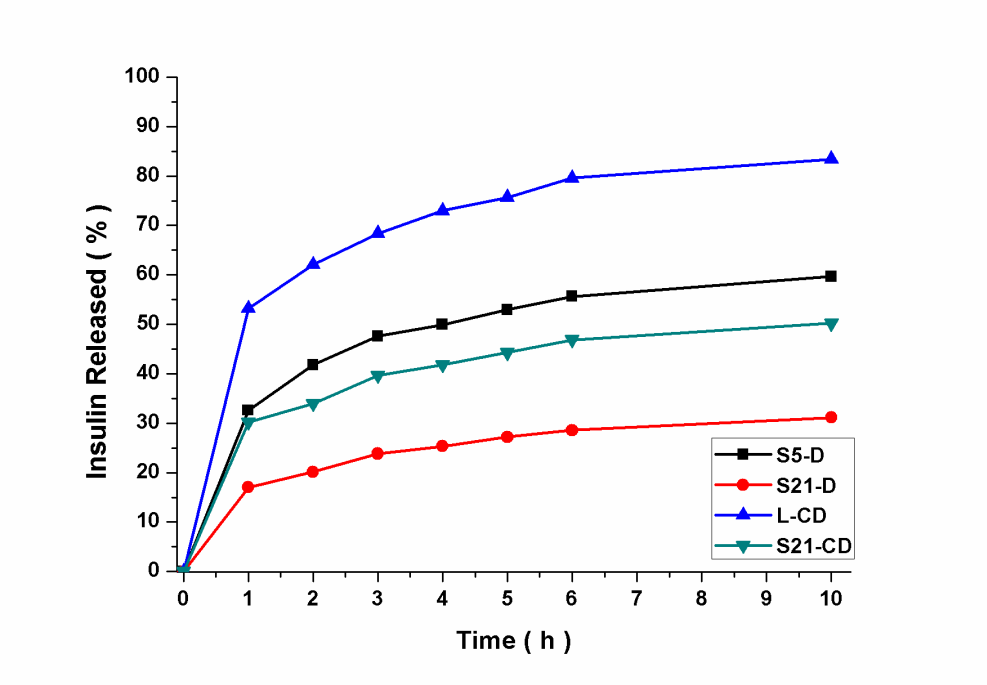


Figure S8. *In vitro* release of insulin from nanopaticles formed by DETA-PGOHMAs and CD-DETA-PGOHMAs with insulin at 25 ^{o}C in PBS.

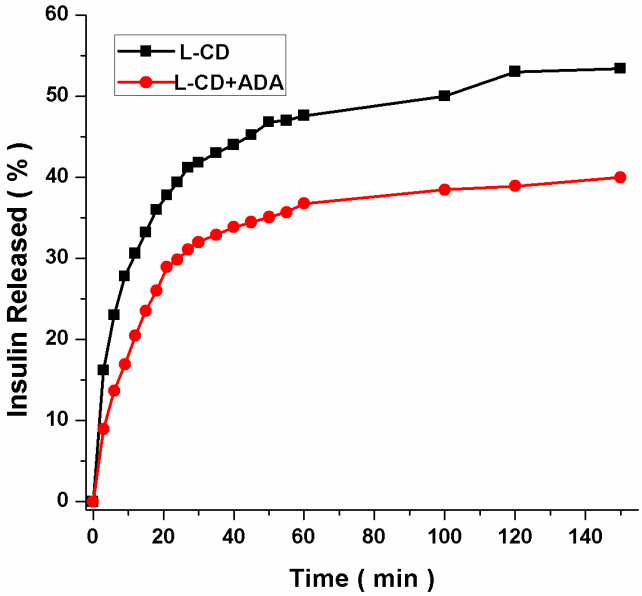
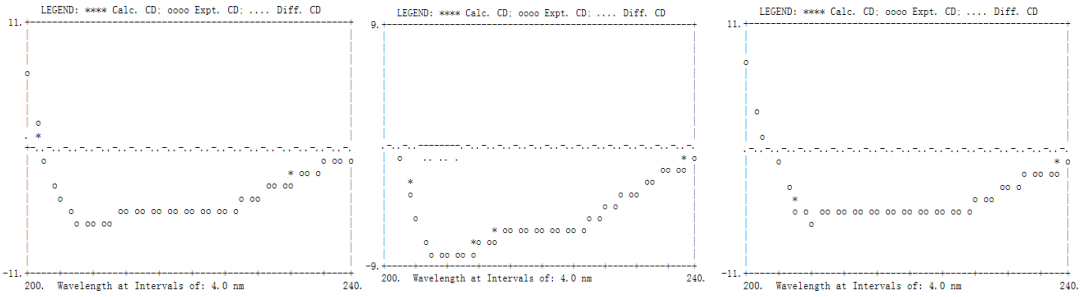


Figure S9. *In vitro* release of insulin from nanopaticles formed by **L-CD** with insulin with/without ADA in PBS.

Table S2. The secondary structure data of insulin

sample	H (r)	H (d)	S (r)	S (d)	Turn	Unrd
A	0.395	0.329	0.053	0.062	0.082	0.080
B	0.436	0.244	0.055	0.117	0.068	0.078
C	0.557	0.289	0.037	0.026	0.043	0.049

A) insulin; B) insulin released from **L-CD**-ins; C) insulin released from **L-D**-ins



(a) (b) (c)

Figure S10. Comparison of the experimental and fitted CD of insulin. (a) insulin; (b) insulin released from **L-CD**-ins; (c) insulin released from **L-D**-ins

Legend: **** Fitted CD; oooo Experimental data; .... Difference between fitted CD and experimental data.

References:

S1. L. Huang, J. Y. Xin, Y. C. Guo and J. S. Li, *J. Appl. Polym. Sci.* 2010, **115**, 1371.

S2. C. Márquez-Beltrán, L. Castañeda, M. Enciso-Aguilar, G. Paredes-Quijada, H. Acuña-Campa, A. Maldonado-Arce and J. F. Argillier, *Colloid. Polym. Sci.* 2013, **291**, 683.

S3. J. Suh, S. S. Hah and S. H. Lee, *Bioorg. Chem.* 1997, **25**, 63.