Electronic Supplementary Material (ESI) for RSC Advances.

Differential Influence of Additives on the Various Stages of Insulin Aggregation

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Electronic Supporting Information

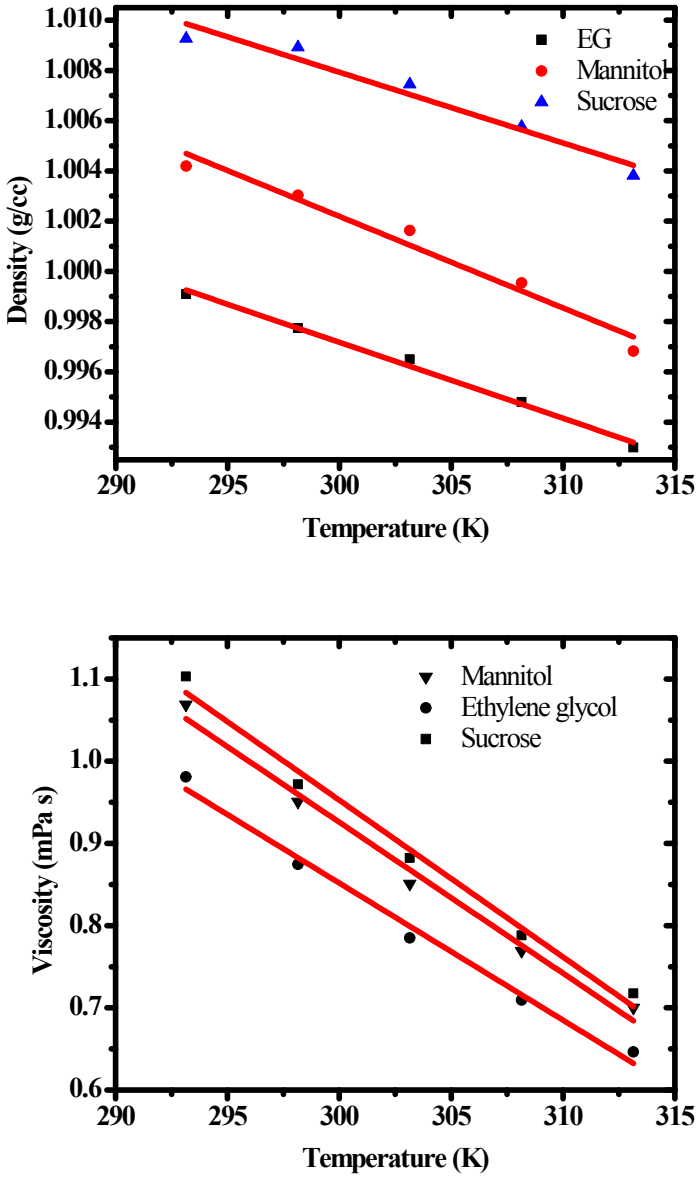


Fig. S1 Variation of densities and viscosities with temperature

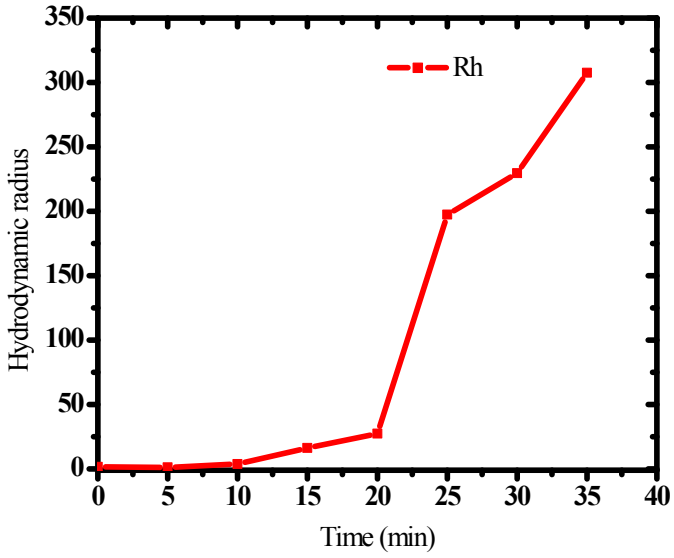


Fig. S2 Aggregation of insulin monitored by Dynamic Light Scattering

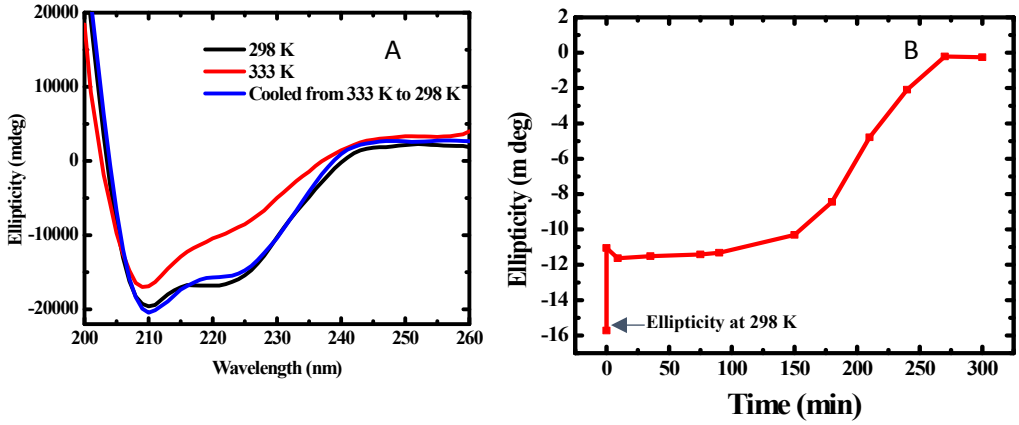
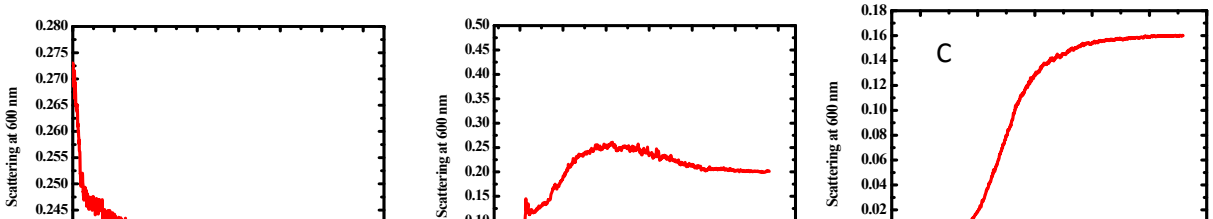


Fig. S3 Reversibility in the aggregation of insulin (A) The first unfolding step in the aggregation scheme is reversible . (B) The ellipticity at 333 K is less than that at 298 K, representing the phenomenon of unfolding. But the helicity at 333 K is retained for quite some time before aggregation can start, suggesting that unfolding is faster than association.



A B

Fig. S4 (A) Aggregation of insulin was taken to completion. The solution was cooled, and then again put at 333 K. No aggregation was observed, suggesting that aggregation is irreversible. (B) Aggregation was stopped in the middle of the growth phase, solution was cooled , and the temperature was again raised to 333 K (C) Aggregation was stopped in the lag phase, solution was cooled , and the temperature was again raised to 333 K

Table 1. Parameters of aggregation of insulin at 333 K at pH 4.0

	t_{lag}(min)	k_{app}(min^{-1})	A_{max}
No additive	84.6±2.6	0.076±0.006	0.13±0.02
Mannitol	126.0±3.1	0.083±0.005	0.11±0.06
Ethylene glycol	175.9±3.2	0.084±0.008	0.062±0.009
Sucrose	127.7±2.8	0.085±0.012	0.11±0.05

Table 2. Parameters of aggregation of insulin at 333 K at pH 7.0

	t_{lag} (min)	k_{app} (min^{-1})	A_{max}
No additive	73.3±1.5	0.0408±0.005	0.36±0.04
Mannitol	82.6±2.4	0.0503±.006	0.25±.06
Ethylene glycol	167.1±4.1	0.026±0.004	0.078±.006
Sucrose	83.6±2.6	0.062±.005	0.11±.03