Understanding of the Impact of the Molecular Crowding Environment on the Glyoxal Mediated Glycation of Hemoglobin

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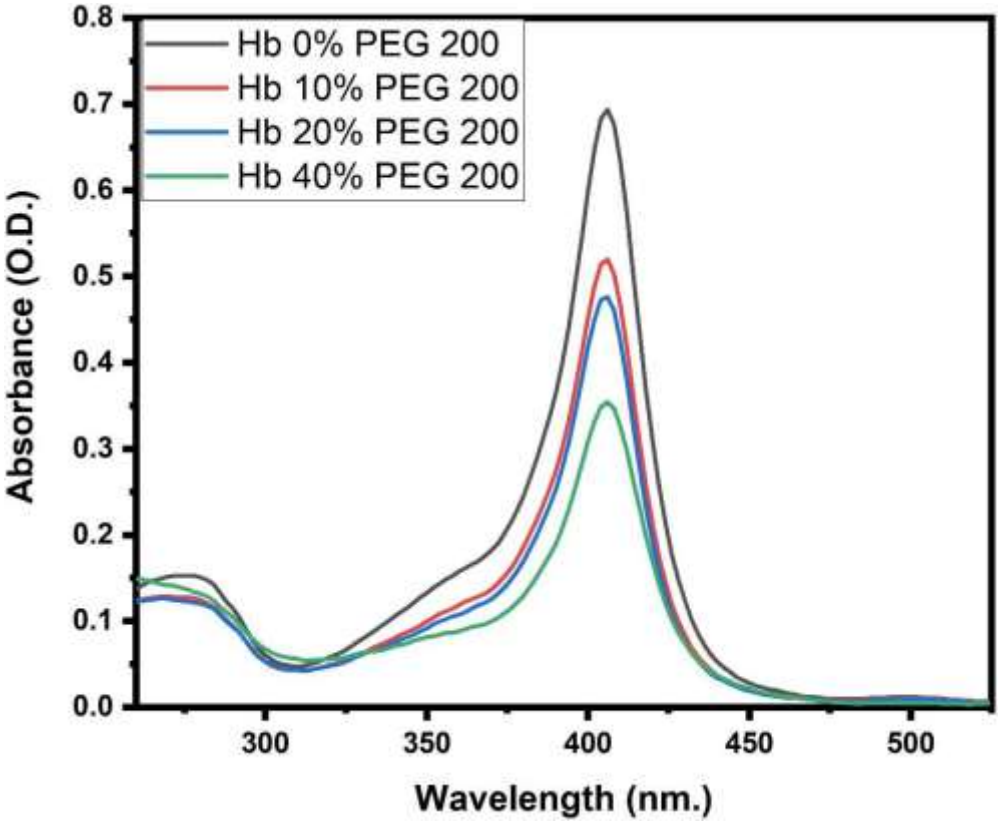

Fig. S1 UV-Vis absorption spectra of Hb incubated with different percentages of PEG 200.

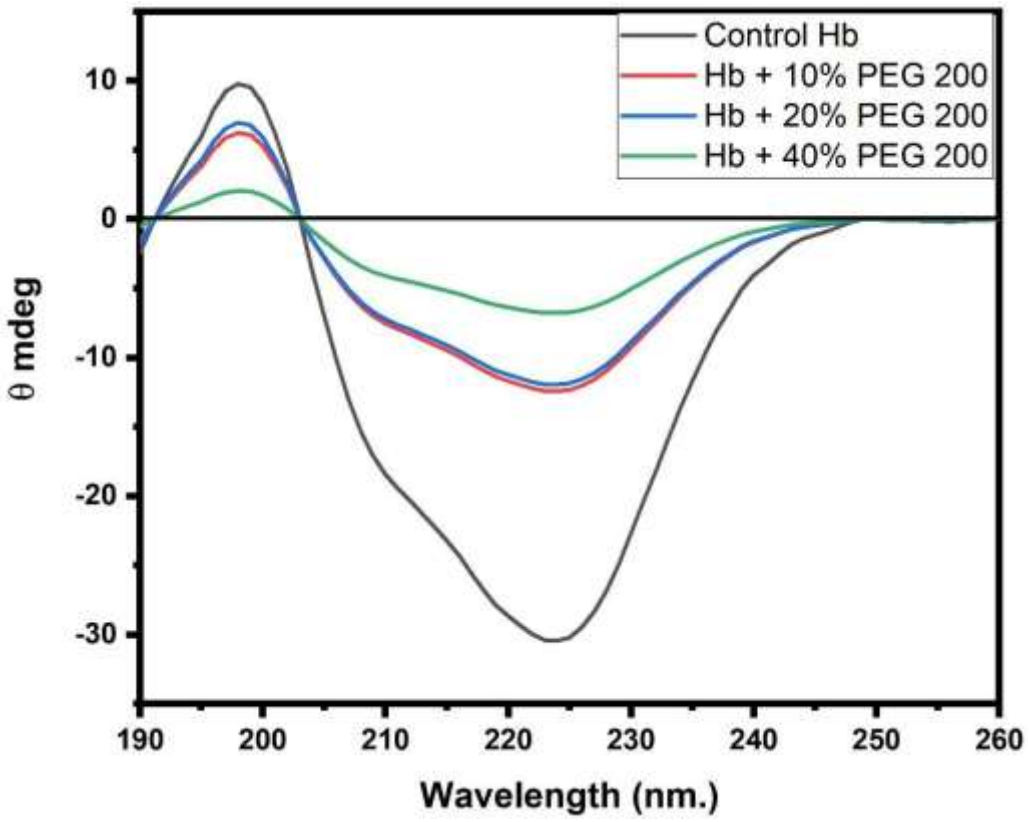

Fig. S2 CD spectra of Hb incubated with different percentages of PEG 200.

Table. S1 Table of percentages of α-helix of different Hb samples.

Samples	**Helix (%)**
Control Hb	49.77
Hb + 10% PEG 200	15.7
Hb + 20% PEG 200	14.79
Hb + 40% PEG 200	5.04

Dynamic Light Scattering-

Dynamic light scattering experiment was performed using Nano-ZS (Malvern) instrument. Before the experiment, all samples were filtered using 0.2 μm filter. The 10 μM samples were used for the experiment using a cuvette of 1.5 ml with the path length 1 cm. The operating method was the DTS software that came with the instrument to record each run average for 10 seconds. In each case, a specific Stokes radius (Rh) was calculated, and the results were represented as a size distribution based on Rh numbers.[1]

Assessment of reduction of radius of glycated Hb in the crowded environment

The change in the molecular radius of different Hb samples were investigated using dynamic light scattering (DLS) method to quantify size of the protein. The molecular radius of the native Hb was 3.739 nm. The increase in the radius of glycated Hb indicated the structural alteration (aggregation) of Hb after glycation.[1] The radius of glycated Hb in the presence of different percentages of PEG 200 was getting decreased with increase in the percentages of PEG 200. These results indicated that as the crowding in the media was increased the free volume in the media was decreased. Hence, the Hb in the presence of PEG200 attained more compact structure.

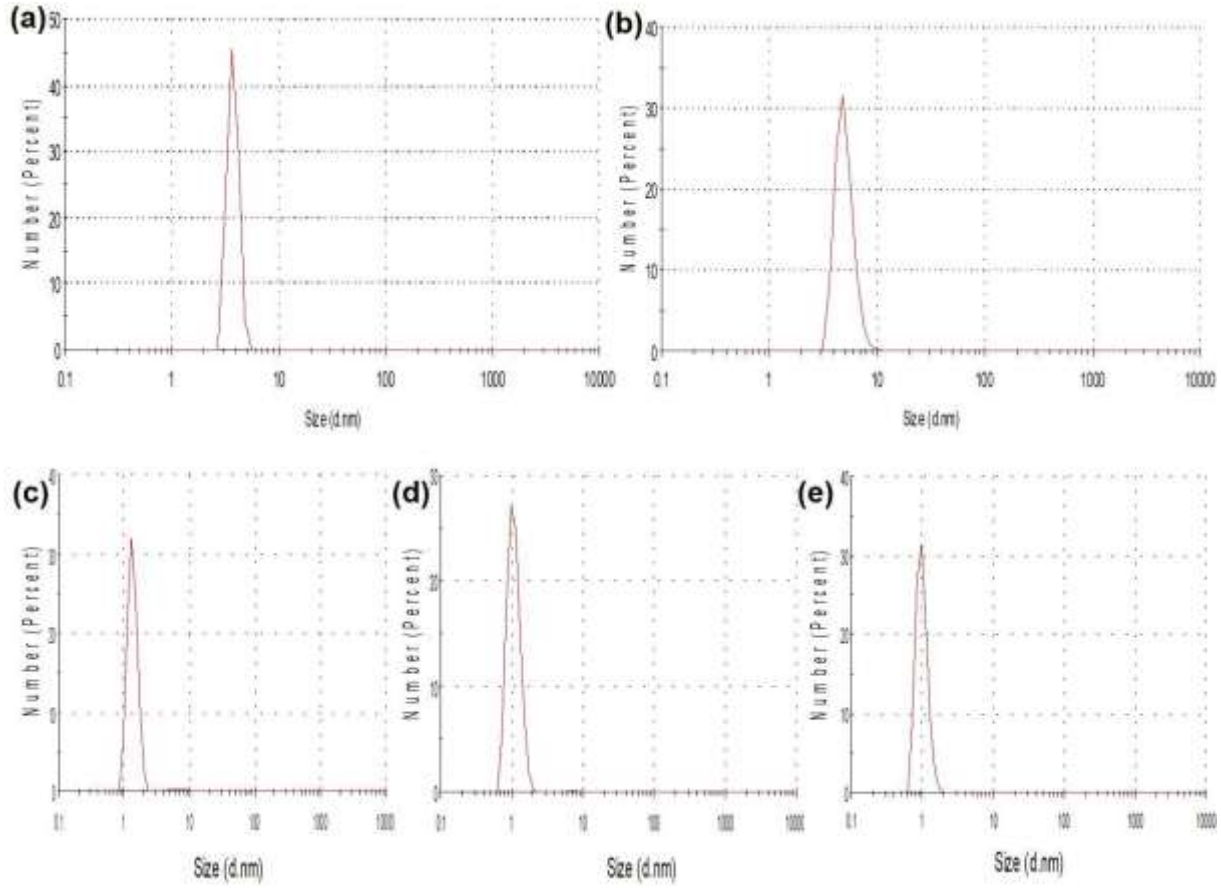

Fig. S3 Dynamic light scattering results of different Hb samples. (a) Control Hb, (b) Glycated Hb, (c) Glycated Hb + 10% PEG 200, (d) Glycated Hb + 20% PEG 200, (e) Glycated Hb + 40% PEG 200.

Table S2. DLS analysis results of different Hb samples.

Samples	Rh (nm)
Control Hb	3.739
Glycated Hb	5.115
Glycated Hb + 10% PEG 200	1.392
Glycated Hb + 20% PEG 200	1.052
Glycated Hb + 40% PEG 200	0.979

Reference

1 A. Ghosh, S. Banerjee, A. Mitra, M. Muralidharan, B. Roy, R. Banerjee, A. K. Mandal and I. B. Chatterjee, Interaction of p-benzoquinone with hemoglobin in smoker's blood causes alteration of structure and loss of oxygen binding capacity, *Toxicol. Reports*, 2016, **3**, 295–305.