2.2.2.2. Preparation of the oily phase

The oily phase was composed of oleic acid, Labrasol® (PEG 8 caprylic/capric glycerides) and Plurol Oleique® (polyglyceryl-6-dioleate) as Surfactant/Cosurfactant (SCOS), and diacylglycerol (DAGs).

A mixture of 4 gm of Labrasol® - Plurol Oleique® at (1:1) ratio, 0.2 gm of diacylglycerol and 15.8 gm of Oleic acid was accurately weighed into screw-capped glass tube. The compounds were vortexed at 40 HTZ by using vortex mixer for 30 seconds.

2.2.2.3. Preparation of the insulin-loaded nanoparticles dispersion system

The aqueous phase and an oily phase were mixed together to prepare the nanoparticle dispersion system. By using micropipette, the aqueous phase (400 µl) was added to 20 gm of oily phase then vortexed for 30 seconds. The insulin-loaded nanoparticles formula was held for 15 minutes to equilibrate before measuring their particle size.

2.2.2.4. Characterization of the insulin-loaded nanoparticles dispersion system

The particle size distribution of the nanoparticles prepared with the surfactant system was assessed by photon correlation spectroscopy, using a Malvern Zetasizer Nano-ZS series (Malvern Instruments, UK). Collective 13 readings were performed three times on a sample at 25°C with detection angle of 173°. The instrument built-in software calculated the average and the standard deviation of particle size measurements for each percent of loading.