**Pluronic F127-micelles improve the stability and potentiate the anti-cancer stem cell efficacy of citral in breast cancer**

**Materials and methods**

*Drug loading and release*

Drug loading capacity (DL%) and encapsulation efficiency (EE%) were calculated according to Equation 1 and 2 respectively by indirect quantification of the free drug in the supernatant after CLM filtration with centrifugation using 30 KDa ultracentrifugal devices (Amicon, Merk Millipore, Ireland) at 6000 rpm for 5 min. The filtrate (with unloaded citral) was quantified spectrophotometrically using 2,4-dinitrophenylhydrazine reagent according to Praveen *et al*. [1]. The size and surface charge of citral-loaded micelles were measured using Zetasizer NanoS (Malvern Instruments, UK). The morphology of CLM was assessed by transmission electron microscopy (TEM) using a JEOL 1400 (JEOL Ltd., Japan) at 120 kV.

Drug release of CLM was evaluated in PBS pH 7.2, PBS pH 6.5 and PBS containing 50% of fetal bovine serum (FBS, Invitrogen). A certain volume of CLM was incubated with an equal volume of each one of the previously prepared media, at 37°C during several time-points (1, 6, 12, 24, 48 and 72 h). To each time-point, the released citral was separated and quantified as described above.

 **Equation 1**

 **Equation 2**

**References**

1 Sai Praveen P, Anupama B, Jagathi V, Devala Rao G. Spectrophotometric determination of Tolperisone using 2, 4-dinitrophenylhydrazine reagent. *Int. J. Res. Pharm. Sci.* 3(1), 317-320 (2010).