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Optimization of Alpinia galanga oil loaded self-nanoemulsifying drug delivery system using design of experiments for fish anesthesia

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Ethanol used for enhancing water miscibility of the essential oils for fish anesthesia provides undesirable side effects to the fish. The aim of this study was to develop a water dispersible formulation of Alpinia galanga oil (AGO) self-nanoemulsifying drug delivery systems (SNEDDS) in order to minimize the amount of ethanol in the formulation and to investigate the effects of the AGO and AGO-SNEDDS for fish anesthesia. Response surface methodology was used to investigate how excipients affect the droplet size on AGO-SNEDDS formation. The fish anesthetic activity of AGO-SNEDDS with different droplet sizes was evaluated by the time it took for zebrafish (Danio rerio) to go into surgical anesthesia stage which fish stopped swimming activity, showed loss of equilibrium and responsiveness and subsequent recovery. The predicted contour plots of droplet size indicated that cremophor RH 40 provided smaller droplet size than tween 80. The goodness of model fitting ($R2>0.89$), prediction power ($Q2>0.72$) and the droplet size values between prediction and real measurement showed similar values (% error <10%). Therefore, these models had a good prediction power. Cremophor RH 40, miglyol 812:capmul MCM EP=1:1 and AGO concentrations showed the most influential variables affecting the droplet size. The droplet size plays an important role in fish anesthesia. The larger droplet required longer time to take fish to enter surgical anesthesia stage. SNEDDS3 with a droplet size around 200 nm sedated the fish into the anesthetic stage within 270 sec, significantly slower than SNEDDS1 and SNEDDS2 (218 and 212 sec) with droplet sizes around 60 and 110 nm ($p<0.03$). All formulations had significantly increased anesthetic activity compared to AGO in an ethanolic solution. In conclusion, the SNEDDS are promising nano delivery systems of AGO for anesthetic use in zebrafish.

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