2.3.9.3. Hepatocyte Cell Isolation

Within the cell culture hood, a cell scraper was used to gently disperse the cells into collagenase solution within a sterile Petri dish. The cell suspension was filtered through gauze into a second Petri dish, the suspension was dispensed into centrifugation tubes (50 ml tubes) in order to remove connective tissues and undigested tissue fragments. The cells were suspended in 40 ml collagenase solution and centrifuged at 100 x g for 3 min at 4 °C. The supernatant was aspirated, and gently cells were re-suspended in 40 ml cold William's complete Medium to wash cells then centrifugation was repeated. For the third time, the supernatant was aspirated and the cells were re-suspended with 40 ml William's complete Medium and centrifuged at 200 x g for 10 min at 4 °C. The cells were counted within the cell suspension using a hemocytometer.

2.3.9.4. Hepatocyte Culture

The cells were diluted with warm William's complete Medium to preferred concentration and plated at a desired volume on cell culture plates (96-wells) (Figure 2.8). The hepatocytes were cultured at 37 °C in a humidified atmosphere of 95% air and 5 % CO_2. The cells were recovered and grown at least overnight prior to experimentation then the cells were used for test within 24 h because this may help preserve the function of critical enzymes (Shen et al., 2012).