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# ACRYLATED PALM OIL NANOPARTICLE SYNTHESIZED BY RADIATION-INDUCED PROCESS AS A CONTROLLED DRUG DELIVERY SYSTEM

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## *ABSTRACT*

*The acrylated palm oil (APO) nanoparticle is a potential product that can be used as carriers in medical field. The main focus of the present study was to study the potential of the APO nanoparticles for used in a controlled drug delivery system. The microemulsion system is used as a medium to incorporate an active substance such as Thymoquinone (TQ) into the APO polymeric micelle and then the radiation technique is used as a tool for the synthesis of TQ-loaded APO nanoparticle. The nano-size TQ-loaded APO particles resulted the particle size of less than 150 nm with spherical in shape. The TQ release profile was carried out in potassium buffer saline (PBS) solutions (pH 7.4) at 37$^{o}$C. And, the zero-order model has been used to determine the mechanism of the drug release from the corresponding nanoparticles, respectively. The TQ release was found to be sustained and controlled in pH 7.4. At pH 7.4, the release of TQ followed the zero-order model. The in-vitro drug release study showed a good prospect of the APO nanoparticle on being a potential drug carrier as there are toxic against colon cancer cells and not toxic towards normal cells. This suggested that the APO product produce using this radiation technique can be developed into different type of carrier systems for controlled drug release applications.*

**Keywords:** Acrylated palm oil, nanoparticles, polymeric micelle, radiation crosslinking, thymoquinone

## INTRODUCTION

Over the past few decades, technologies such as colloidal drug carrier systems, i.e. micellar solutions consisting of small particles of 10 - 400 nm diameters, called polymeric micelles, have been actively explored as delivery systems to provide more efficient drug absorption and enhanced bioaviability of pharmacologically active substance at relevant location, and at the same time, minimizing toxicity. For example, the solid colloidal nanoparticle under 1μm can be used for conjugating the drugs to improve circulating times in the bloodstream, targeting a specific organ, sustained release of drug to targeted location and reducing the side effects, which can be administered through oral, intravenous, pulmonary or ocular route.

Herbal medications have been used as therapeutic agents for the prevention and treatment of cancers. Recent studies demonstrated that Thymoquinone (TQ) from Nigella sativa herb shows encouraging chemopreventive as well as chemotherapeutic properties. For example, cell studies had found that TQ are cytotoxic towards cancer cells i.e. colon cancer, breast cancer and leukemic carcinoma cell lines. Overall, the TQ showed pharmacological actions such as antioxidant, antihistaminic, antibacterial, antihypertensive, hypoglycemic, antiinflammatory, antinociceptive, and immunopotentiating (Arslan et al., 2005).

Nanostructured lipid carriers show great promise for the topical, oral and parenteral administration of drugs. Abdelwahab et al. (2013) has developed TQ-loaded nanostructured lipid carriers and found the lipid carrier comprising of surfactant are potentially good colloidal drug carriers for TQ and promising vehicle for the oral delivery of TQ. The loading of TQ into nanostructured lipid carriers significantly improved the gastroprotective properties of this compound against the formation of stomach ulceration.

The focus of present work relates to an APO nanoparticle as a TQ carrier and controlled release of TQ for used in colon cancer treatment. Encapsulating TQ in APO micelle by gamma radiation-induced process can be devised for formation of TQ-loaded APO nanoparticles. These present study suggested that the ionizing radiation method can be utilized to prepare micro- and nano-size compound carriers (Tajau et al., 2011a, 2011b, 2013a, Ulanski et al., 1998; Ulanski and Rosiak, 1999). Thus, the development of APO nanoparticles as a delivery system is expected to be potential vehicle for targeting on site and controlled drug delivery.

## MATERIALS AND METHODS

### Preparation of TQ-loaded APO Nanoparticles

The acrylated palm oil was synthesized from a palm olein product by an acrylation process at Malaysian Nuclear Agency's laboratory in the radiation processing technology division (Tajau et al., 2013b). The polymeric micelles consist of 1.8% of an APO, 0.49% of a Pluronic F-127 (PF-127) supplied by Sigma Aldrich and aqueous solution was used for immobilization 0.1% of a TQ purchased from TCI, Japan. Then, the TQ-loaded APO micelle was subjected to radiation source to induce crosslinked polymeric structure of the TQ-loaded APO nanoparticle. The samples were irradiated at 1, 5, 10, 15 and 25 kGy using gamma radiation. The samples were then purified and dried using the freeze drying technique.

### Physiochemical Properties of TQ-loaded APO Nanoparticles

The formation of TQ-loaded APO micro micelle and nano particle were evaluated by the Dynamic Light Scattering (DLS), the Fourier Transform Infrared (FTIR) Spectroscopy and the Transmission Electron Microscopy (TEM) for characterization the size, the shape, the chemical structure and the irradiation effect of the micelle and the nano particle.

### TQ Release Profile

The ultraviolet-visible (UV-Vis) spectrophotometer was used to determine the TQ release from the corresponding nanoparticles in potassium buffer saline solutions (pH 7.4) at 37$^{o}$C while using the zero-order model to evaluate the TQ release mechanism. Approximately 0.01g of TQ/APO/PF-127 nanoparticle was use in this release study. The percentage of the thymoquinone release was calculated in accordance with the following equation (1).

$$\text{Drug release, milligram (mg)} = (m_t/m_\infty) \times 100 \quad (1)$$

Where; $m_t$ = the amount of drug release in time t, $m\infty$= the maximum release

## Cytoxicity Test

The in vitro tests are conducted using human colon carcinoma cell line (HT29) purchased from American Type Culture Collection (ATCC). The cells were allowed to grow to confluence in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum and antibiotics (100 mg/ml streptomycin, 100 □/ml penicillin), in a 5% carbon dioxide ($CO_2$) atmosphere at 37°C, respectively.

The viability of cells was determined with trypan blue reagent. Exponentially growing cells were harvested, counted with haemocytometer and diluted with the medium. Cell culture with the concentration of 1 x $10^5$ cells/well was prepared and was plated (100 □l/well) onto 96-well plates. The diluted ranges of samples were added to each well (0 - 2000 □g/ml for 3T3 cells and HT29 cells). The proliferative activity was determined using the (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) (MTT) assay. The incubation period used was 24, 48 and 72 hours. The spectrophotometric absorbance of the sample was measured using an ELISA reader at a wavelength of 540 nm. The cytotoxicity was recorded as the drug concentration causing 50% growth inhibition of the cells ($IC_{50}$ value) using the equation (2):

$$\% \text{ Cell viability} = \frac{\text{Sample absorbance (mean) x 100\%}}{\text{Control absorbance (mean)}} \quad (2)$$

After the determination of the percentage of cytotoxicity, graphs were plotted with the percentage of cytotoxicity against their respective concentrations.

## RESULTS AND DISCUSSION

### Physiochemical Properties of the TQ-loaded APO Nanoparticle

Figure 1 shows the molecular structure of the TQ. TQ is an organic compound and well known as a hydrophobic drug, where it is insoluble in an aqueous system. This makes its as non-polar compound, as well as APO that insoluble in aqueous medium. The interaction between these two compounds is called van der Waals forces where the non-polar solvent is used to dissolve the non-polar compound through the nature of inter molecules forces of attraction. This interaction is between the dipole-dipole attractions of the hydrogen atoms of APO hydroxyl group bonded the oxygen atom of TQ as shown in Fig. 2. Table 1 tabulated the particle size of the TQ-loaded APO particle in the the PF-127 system before and after the irradiation. Sizes of the particles are increases after loading with the TQ and decreasing when exposed to the irradiation source due to the inter-particle crosslinking of APO's molecules radical in micelle (Tajau et al., 2011a, 2011b, 2013a).

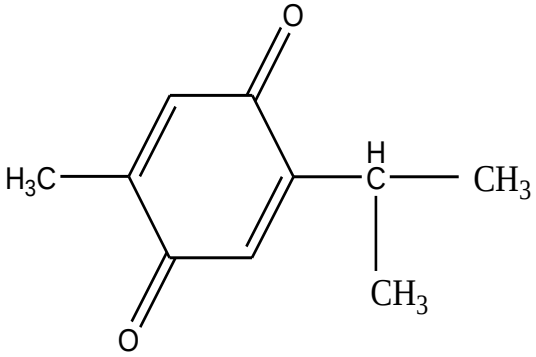



Figure 1: Molecular structure of the TQ

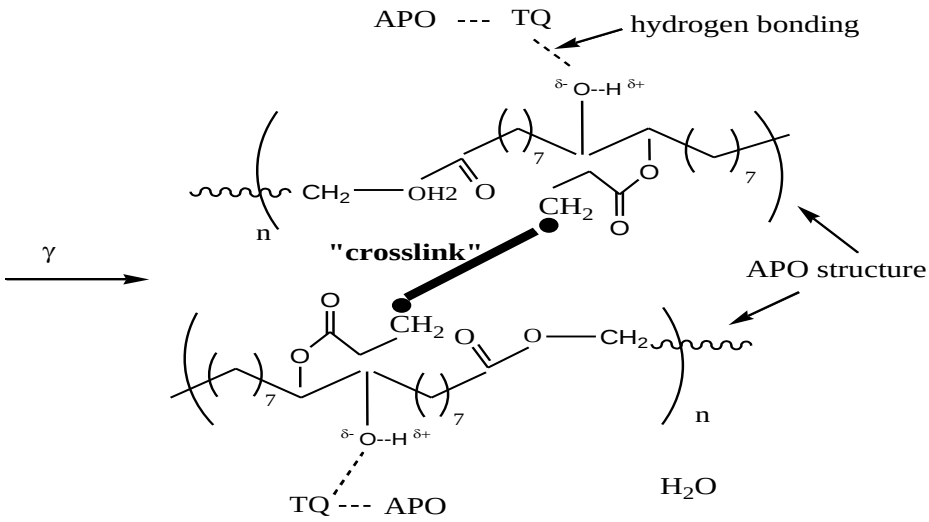



Figure 2: Radiation crosslinking reaction

Table 1: Particle size of the APO/PF-127 nanoparticles upon irradiation

| Dose (kGy) | TQ-free (nm) | TQ-loaded) (nm) |
|---|---|---|
| 0 | 219.45 | 234.14 |
| 1 | 132.36 | 216.67 |
| 5 | 132.35 | 210.74 |
| 10 | 130.65 | 142.77 |
| 15 | 133.05 | 145.83 |
| 25 | 141.80 | 149.66 |

Figure 3 shows the TEM images of the PF-127 micelle and Figure 4 ((a) – (c)) show the TQ-free and the TQ-loaded APO nanoparticle before and after exposure to the gamma irradiation, respectively. Overall, the nanoparticles are in spherical shape. The IR spectrum in Fig. 5 shows that the TQ is successfully incorporated into the APO/PF-127. Presence of TQ in the APO/PF-127 nanoparticle is indicating by the presence of the black solid particles of the TQ in the nanoparticle

core (Fig. 4c) and causes the reduction of the TQ band at peak 844 $cm^{-1}$ after irradiation i.e. 25 kGy, as shown in the IR spectrum (Fig. 5). At the dose of 25 kGy, TQ occupies the entire hydrophobic core of the APO nanoparticle (see Fig. 4c) where it consequently explained that the TQ was incorporated into the nanoparticle.

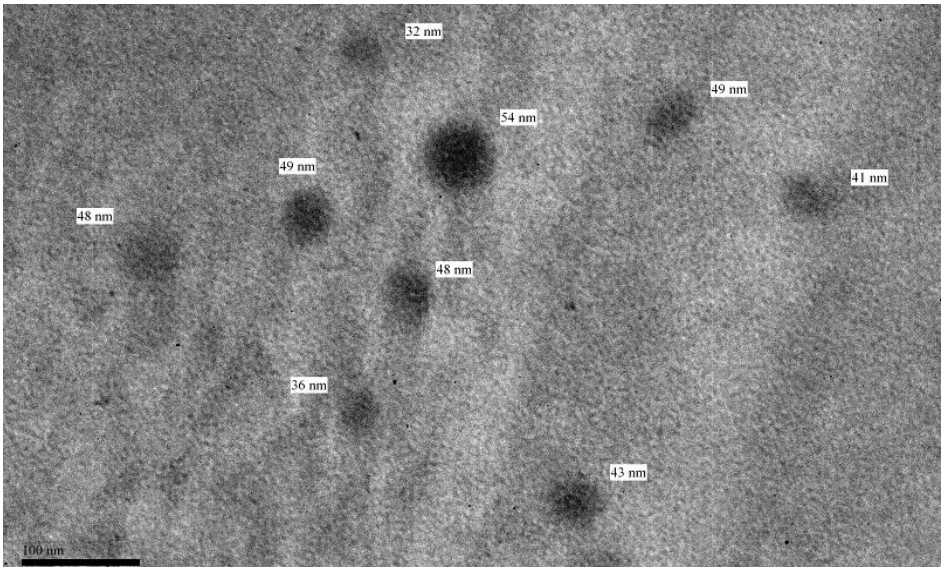
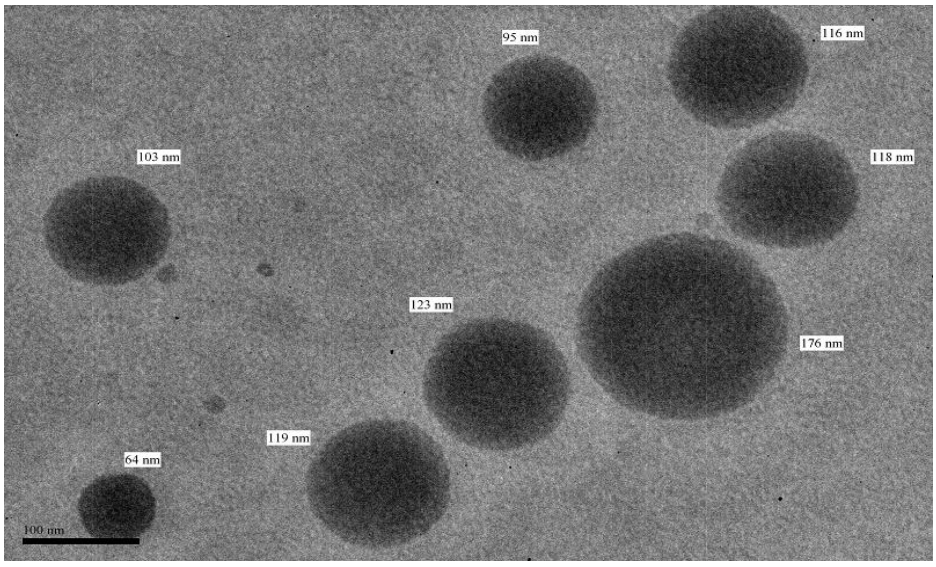
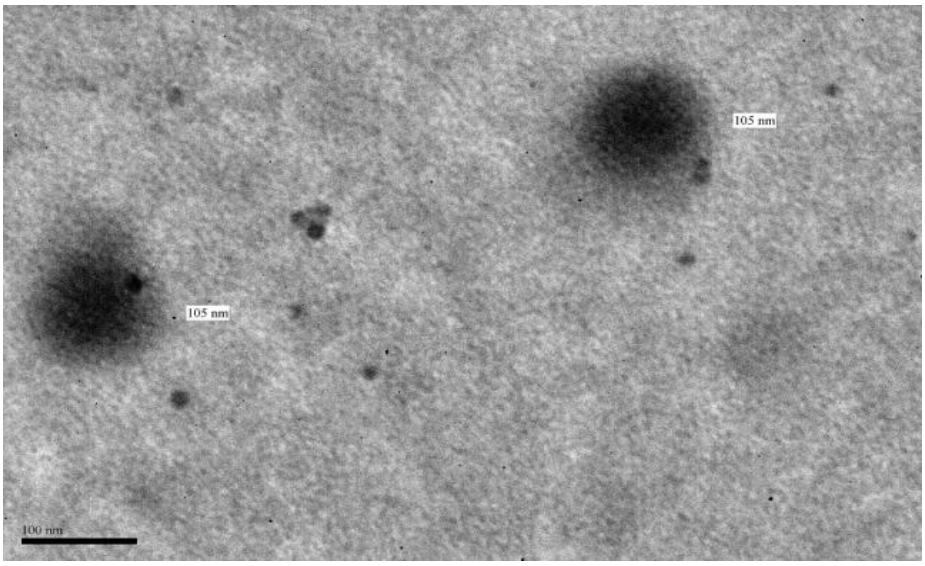


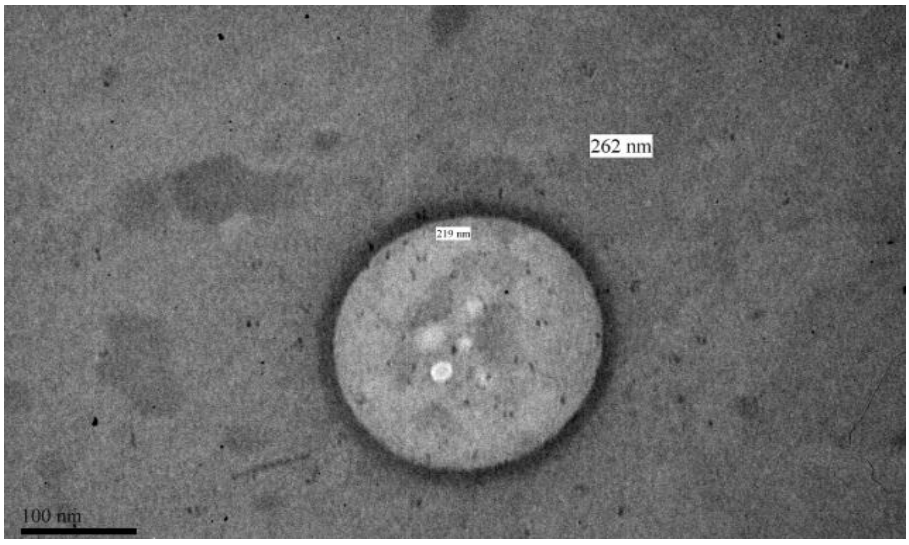

Figure 3: TEM image of the PF-127 micelle (80 kV, magnification 30000 times)




(a)APO/PF-127 particle, non-irradiated (80 kV, magnification 30000 times)




(b) APO/PF-127 particle at 25 kGy (80 kV, magnification 30000 times)




(c) TQ/APO/PF-127 particle at 25 kGy (100 kV, magnification 30000 times)

Figure 4: TEM image of the TQ-free and the TQ-loaded APO nanoparticles

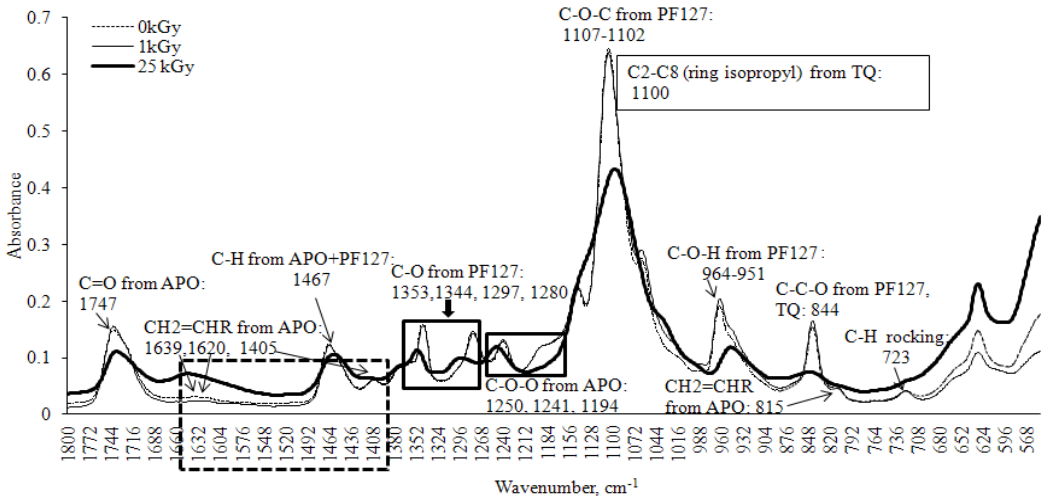



Figure 5: IR spectrum of TQ/APO/PF-127 nanoparticle

## TQ Release Profile

The size of the APO nanoparticle and the irradiation dose also play major role in controlling the release rate of the TQ from the APO/PF-127 nanoparticle. The present study revealed that such smaller particles i.e. 144 nm (irradiated at 15 kGy) was able retained the active substance for a longer period compare to that larger particle i.e. 211 nm (irradiated at 5 kGy) as shown in Fig. 6. Thus, the smaller particle (irradiated at 15 kGy) showed the compactness of a molecular structure networking. Furthermore, the linear of plot between percent cumulative TQ release and time suggests that batch with particle size 211 nm followed near to the zero-order kinetic, within early 60 minutes period (Fig. 7). Meanwhile, batch with the particle size 144 nm seemed unfilled the zero-order kinetic.

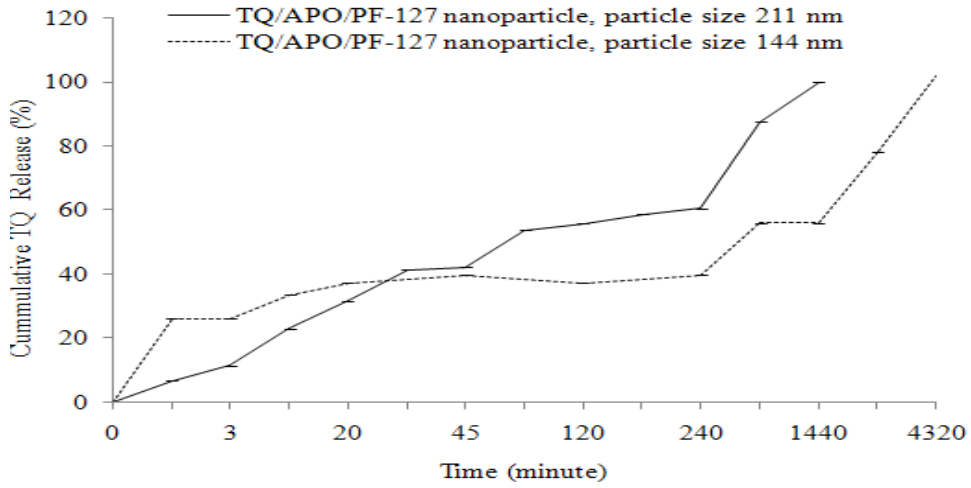



Figure 6: Cumulative percent release of TQ from the TQ/APO/PF-127 nanoparticles

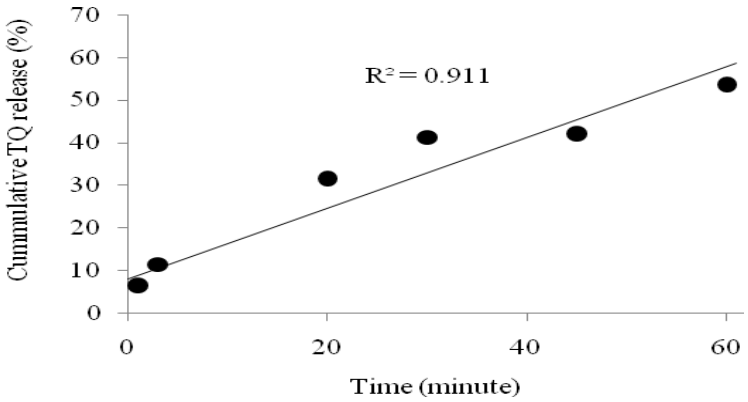
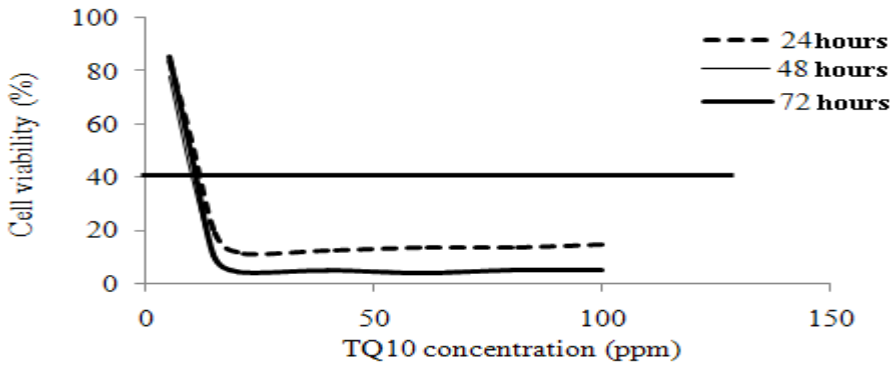


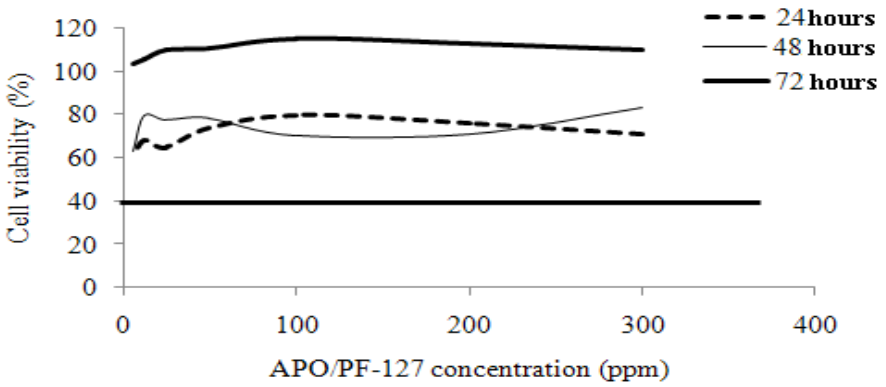

Figure 7: TQ release mechanism according to zero-order release model

**Cytotoxicity Test**

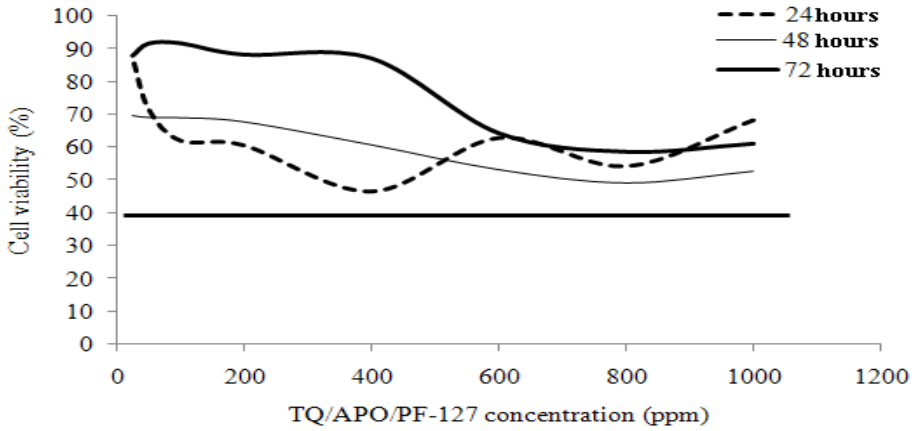
An active compound of TQ was widely known resulted toxicity against cancer cell (Abdelwahab et al., 2013). In this study, the TQ compound showed toxic againt HT29 at 24,48 and 72 hours with 10 ppm of IC50, as shown in Fig. 8a. The study also revealed that the APO nanoparticle carries consist of TQ showed toxicity against colon cancer cells (Fig. 8c, 8d) compared to the free-TQ-loaded APO nanoparticle (Fig. 8b), which is non-toxic against the colon cancer cells. Figures 8c and 8d illustrated the cytotoxicity results of cell viability of a complex composition of an APO/PF-127 containing the TQ against the HT29. As results, the complex composition comprising of TQ shows toxicity against HT29 at certain periods. For example, non-irradiated sample of TQ/APO/PF-127 shows toxicity against HT29: at 24 hours with 400 ppm of IC50 and at 48 hours with 600 ppm of IC50 (Fig. 8c). After 72 hours, the complex composition shows no toxicity against HT29 (Fig. 8c). Meanwhile, the irradiated sample of TQ/APO/PF-127 shows toxicity against HT29: at 24 hours with 400 ppm of IC50 (Fig. 8d). At 24 hours and 72 hours, the complex composition shows no toxicity against HT29 (Fig. 8d).



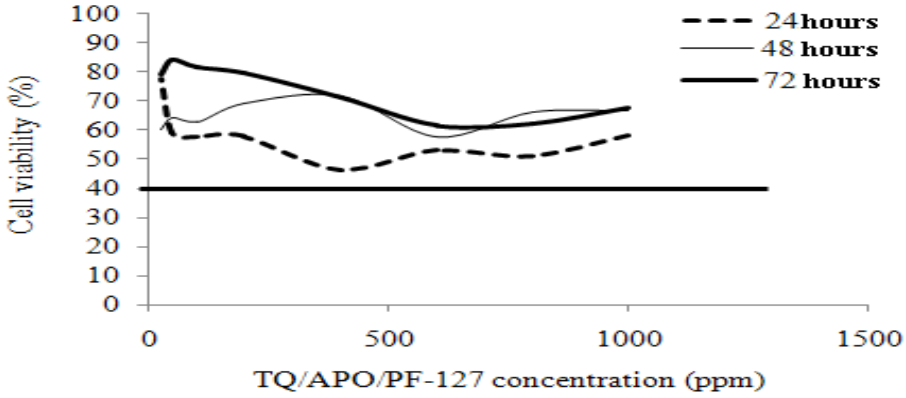

(a) TQ10 sample




(b) Free-TQ-loaded sample, non-irradiated sample




(c) Non-irradiated sample




(d) Irradiated sample, 5 kGy

Figure 8: Cell viability of TQ/APO/PF-127 nanoparticles on HT29

## CONCLUSIONS

This present study suggested that the complex composition of TQ/APO/PF-127 was a good carrier for slow release of hydrophobic drug/TQ. For this TQ/APO/ PF-127 nanoparticle, the study also revealed that this complex composition is toxic against colon cancer cells. Besides that, the present study demonstrated that the process of making this complex compound carrier by nuclear

technology, called radiation-induced process, is a safe and easy processes and can be devised for development of drug delivery particles.

## ACKNOWLEDGEMENT

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