Aerosolized nanoemulsion system encapsulating quercertin for lung cancer treatment

N.H. Arbain[1], C.L. Ngan[1], N. Salim[1], W.T. Wui[2] and M.B. Abdul Rahman[1*].
[1]Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia
[2]Non-Destructive Biomedical and Pharmaceutical Research Centre, iPROMISE, Universiti Teknologi MARA, 42300, Puncak Alam, Selangor, Malaysia
*Email: basya@upm.edu.my

***Abstract*—Formulation of aerosol nanoemulsion containing quercetin was carried out using low and high energy homogenizers. The effects of oils (1.0%–4.0% wt%), lecithin (1.0-2.0 wt%) and Tween 80 (0.5–1 wt%) on the droplet size of quercetin nanoemulsions were investigated. The optimum formulation for preparation of quercetin nanoemulsions having the desirable criteria was 1.0 % of oils, 2.0 % of lecithin and 1.0 % of Tween 80 and 95.95 % of aqueous phase. Under optimum formulation, the droplet size was 112.5 nm. Volume median diameter of nanoemulsion in this study was 4.991± 0.008 µm, Span value was 1.662 ± 0.029 and fine particle fraction (% FPF < 5.14 µm) was 55.19 ± 0.153. These results suggest that nanoemulsion -based palm oil in this study could be successful nanocarrier of quercetin as pulmonary delivery system for lung cancer treatment.**

Keywords— Aerosol, nanoemulsions, quercetin, lung cancer, pulmonary delivery.

I. INTRODUCTION

Cigarette smoking is known as the leading preventable cause of pulmonary diseases such as lung cancer and chronic obstructive pulmonary disease (COPD). Approximately 90% of lung cancer and 80% of COPD cases are linked to the cigarette smoking. Lung cancer is one of the leading causes of cancer-related death worldwide. Between 2010 and 2014, smoking caused nearly half a million premature deaths a year, more than 87% of all lung cancer deaths and 61% of all pulmonary deaths [1].

Due to the fact that the lung could provide a large absorptive surface area (up to 100 m^2), extremely thin absorptive mucosal membrane (0.1 – 0.2 µm) and rich in blood supply, growing attention has been given to the potential of a pulmonary route as a non-invasive administration for systemic delivery of therapeutic agents and that resulted in quick onset of action and rapid systemic absorption. By pulmonary route, the drugs can be administered by two techniques: aerosol inhalation (also used in intranasal applications) and intratracheal instillation. More uniform distribution we could achieve with greater extent of penetration into the peripheral or the alveolar region of the lung by applying aerosol technique.

Chemotherapeutics of cancer using natural molecules are becoming increasingly to inhibit or reverse carcinogenesis [2-3]. With several biological and pharmacological properties, quercetin is one of the most prevalent as well as thoroughly studied dietary flavonoids. Studies reported that quercetin could inhibit proliferation of multiple cancer cell types, including lung cancer, colon cancer, prostate carcinoma, and pancreatic tumor [4]. However, quercetin is poorly soluble in water, thus it has limited bioavailability upon oral administration [5]. To enhance the solubility of poorly soluble drugs through delivery systems, many approaches have been introduced such as nanoemulsions, solid lipid nanoparticles, and liposome encapsulation. Azuma *et al*. [6] reported that the absorption of quercetin was enhanced significantly by the combination of emulsifiers and lipids, which is affected by its solubility in the carriers used for the oral administration. The delivery systems such as nanoemulsions enhanced the bioavailability of poorly soluble that may be related to the better uptake of nanocarriers and the decreased of degradation and metabolism of drugs.

Nanoemulsions have the potential to deliver proteins as well as other new (or traditional) active drug compounds to the lungs because of their high solubilising and drug protection features. With the advantage of solution-like physicochemical properties of nanoemulsions, it is hypothesised that nanoemulsions perform as a solution when nebulized and will demonstrate improved aerosolisation performance. Although a number of studies have addressed the aforementioned concerns, the previously reported results are far from optimal, which necessitates further efforts in this area. Our study focused on development of aerosol nanoemulsions based palm oil esters for pulmonary drug delivery with quercetin as the model drug for lung cancer treatment and to identify the influence of the main components on the system. The physicochemical properties of prepared nanoemulsion were also determined.

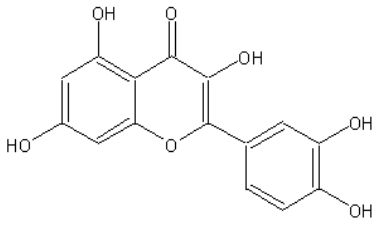

Fig. 1. Chemical structure of quercetin.

II. MATERIALS AND METHODS

A. *Materials*

Palm-based esters (POEs) were prepared in our laboratory through enzymatic alcoholysis of palm oil with oleyl alcohol using Lipozyme RM IM as the catalyst (Keng et., al. 2009). POEs were then purified with methanol (analytical grade, 99.9% purity) at ratio 1:2 (w/w), which was purchased from Merck (Germany). Quercetin (>90%), and ricinoleic acid were purchased from Sigma-Aldrich (Germany). Tween 80 (polyoxyethylene sorbitan monooleate) was purchased from Merck (Germany). Pure soy bean lecithin (Lipoid S75) was purchased from Lipoid GmbH (Ludwigshafen, Germany). Glycerol was purchased from JT Baker (Philipsburg, NJ, USA). All the oils and surfactants used are analytical grade materials. Water was deionized using a Milli-Q filtration system, EMD Millipore (Billerica, MA, USA).

B. *Methodology*

Quercetin (0.05 % w/w) was dissolved in oil phase (POEs:ricinoleic acid, 1:1 ratio, (1.0%–4.0% w/w) containing lecithin (1.0%–2.0% w/w). After quercetin was completely dissolved, Tween 80 (0.5%–1.0% w/w) was added into the oil phase. The oil phase was added dropwise into a beaker containing aqueous phase and stirred continuosly for 30 min using an overhead stirrer (IKA@ RW 20 Digital, Nara, Japan). The mixture was stirred at 300 rpm at 25°C. The emulsion obtained was further subjected to high shear homogenizer at 10, 000 rpm for 15 min. The formulation was analyzed with respect to the droplet size, volume median diameter (VMD), fine particle fraction (% FPF) and Span value.

C. *Characterizations*

The droplet size of the prepared nanoemulsions was measured using Zetasizer (Nano ZS, Malvern Instrument Ltd., UK) at 25 °C using dynamic light scattering technique, scattered at an angle 173 °. For the measurement, the samples were diluted with deionized water (1:200) and injected into the sample cell. Measurement was repeated for three times. The aerosol droplet size of optimum nanoemulsions was analyzed using a Malvern Spraytec laser diffraction size analyzer (Malvern Instruments Ltd., UK). Nanoemulsions (3 mL) were added into the nebulizer which was then placed perpendicular to the laser lens line of the instrument at a distance of approximately 30 cm from the laser beam. A vacuum of 15 L/min was applied so that the aerosol could be drawn through the beam for particle size analysis. The volume median diameter (VMD) was recorded to present the size of the aerosol droplets. The Span and fine particle fraction (FPF) were also determined to represent the size distribution (i.e. polydispersity) and fraction of particle that predicted to deposit in alveolar region of the lung. Span is calculated as (90% undersize-10% undersize)/VMD.

III. RESULTS AND DISCUSSION

A. *The influence of % composition on the droplet size*

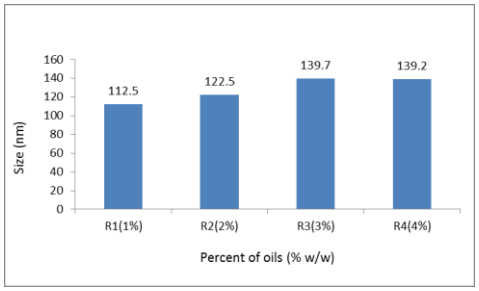

Fig. 2. The influence of % oils on the droplet size. The percent of oils: 1%, 2%, 3%, and 4% (wt %).

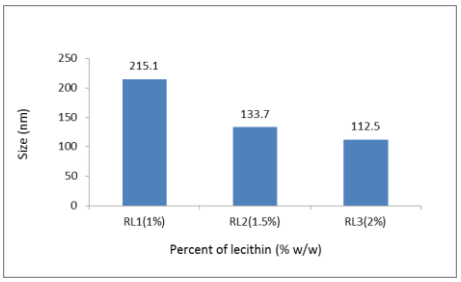

Fig. 3. The influence of % lecithin on the droplet size. The percent of lecithin: 1%, 1.5% and 2% (wt %).

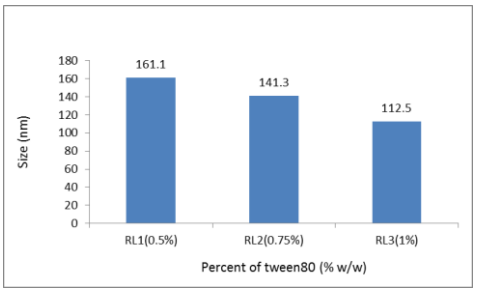

Fig. 4. The influence of % Tween 80 on the droplet size.The percent of Tween 80: 0.5%, 0.75% and 1.0% (wt %).

As shown in **Fig.2**, the droplet size of emulsion increases as the amount of oil (POEs:RC) increases. Increasing amount of oil phase resulted in increasing viscosity of the dispersed phase which causes an increase in flow resistance and restriction on the droplet break-up rate thus causing formation of larger particles [7-8]. On the other hand, as the amount of

surfactant (lecithin and Tween 80) increased as shown in **Fig. 3** and **Fig. 4**, the decreased in the particle size values was observed. This could be due to the presence of more emulsifier that can cover the new droplet surfaces forms during homogenization and interfacial tensions lowered between oil and water [7-8].

Li and Lu, [9] reported that the droplet size of a formulation should be in between 20-200 nm for it is classified as nanoemulsions which have uniform size and appear transparent or translucent and are more stable against aggregation, flocculation, coalescence and Ostwald ripening than with conventional emulsions because of the small droplet size. In addition, droplet size below about 500 nm increases the deposition in all regions of the lung because of the increasing diffusional mobility. Nanoemulsion in this study shows the smaller droplet size of 112.5 nm.

B. Determination of aerosol droplet size using laser diffraction

Table 1. Laser diffraction size analysis of the aerosols generated from quercetin nanoemulsion

Properties	Quercetin nanoemulsion
VMD (μm)	4.991± 0.008
FPF (%)	55.19 ± 0.153
Span	1.662 ± 0.029

Table 1 shows VMD of nanoemulsions are 4.991± 0.008 μm. Labiris *et al*. [10] reported that one of the most important factors for dose determination and lung distribution is the size of the inhaled particles which must have a diameter range in between 1 and 5 μm, so that the drug will be able to reach the peripheral airways and the alveolar region of the lung. Droplets greater than 5 μm in size are usually retained in the oral cavity and those smaller than 0.5 μm can be easily exhaled [11]. The Span value less than 2 indicates that the polydispersity of the aerosol droplets are reduced. The respirable fraction represents the fraction of droplets having a diameter less than 5 μm and deposit within deep lung areas. The respirable fraction should contain at least 50 % of the emitted dose. For example, the nebulisation of nanoemulsion-based formulation for pulmonary delivery of Budesonide by vibrating mesh nebulizers resulted in reasonably high FPF emitted of 53.1 % [12].

IV. CONCLUSIONS

The present study demonstrated that quercetin nanoemulsion was invented by emulsification process and some of major factors influencing the droplet size were determined. The optimal composition of quercetin nanoemulsion was revealed to be 1 % oils, 2 % lecithin, 1 % Tween 80 and 95.95 % of aqueous phase containing small droplet size (112.5 nm). Laser diffraction size analysis of the aerosols generated from quercetin nanoemulsion showed its suitability for efficient pulmonary delivery for lung cancer treatment.

REFERENCES

[1] World Health Organization Fact Sheet (WHOFS). Available at:http://www.who.int/mediacentre/factsheets/fs297/en/ (visited July 30,2016).

[2] D.A. Kennedy, J. Hart, D.Seely, "Cost effectiveness of natural health products: A systematic review of randomized clinical trials", *Evid-Based Complement Altern Med*., vol. 6(3), pp. 297–304, 2009.

[3] S.C. Gupta, S.H. Kim, S. Prasad, B.B. Aggarwal, "Regulation of survival, proliferation, invasion, angiogenesis, and metastasis of tumor cells through modulation of inflammatory pathways by nutraceuticals", *Cancer Metastasis Rev*., vol. 29(3), pp. 405–434, 2010.

[4] Y. Gao, Y. Wang, Y. Ma, A. Yu, F. Cai, W. Shao, & G. Zhai, "Formulation optimization and in situ absorption in rat intestinal tract of quercetin-loaded microemulsion", *Colloids and Surfaces. Biointerfaces*, vol. 71(2), pp. 306–14, 2009.

[5] A. Karadag, X. Yang, B. Ozcelik, Q. Huang, "Optimization Of Preparation Conditions For Quercetin Nanoemulsions Using Respon Surface Methodology", *Journal of Agricultural and Food Chemistry*, vol. 61, pp. 2130-2139, 2013.

[6] K. Azuma, K. Ippoushi, H. Ito,H. Higashio, "Combination of lipids and emulsifiers enhances the absorption of orally administered quercetin in rats", *J. Agric. Food Chem*., vol. 50, pp. 1706−12, 2002.

[7] H. R. F. Masoumi, M. Basri, W.S. Samiun, Z. Izadiyan, & C.J. Lim, "Enhancement of encapsulation efficiency of nanoemulsion-containing aripiprazole for the treatment of schizophrenia using mixture experimental design", *International Journal of Nanomedicine*, vol. 10, pp. 6469–6471, 2015.

[8] S. Samson, M. Basri, H. R. F. Masoumi, R.A. Karjiban, & E.A. Malek, "Design and development of a nanoemulsion system containing copper peptide by D- optimal mixture design and evaluation of its physicochemical properties", *RSC Advances*, vol. 6, pp. 17845–17856, 2016.

[9] P.H. Li, & W.C. Lu, "Effects of storage conditions on the physical stability of d-limonene nanoemulsion", *Food Hydrocolloids*, vol. 53, pp. 218– 224, 2014.

[10] N.R. Labiris, and M.B. Dolovich, "Pulmonary Drug Delivery Part I: Physiological Factors Affecting Therapeutic Effectiveness Of Aerosolized Medications", *Blackwell Publishing Ltd., British Journal Of Clinical Pharmacolology*, vol. 56(6), pp. 588–599, 2003.

[11] Bhavna, F.J. Ahmad, R.K. Khar, S. Sultana, A..Bhatnagar, "Techniques to develop and characterize nanosized formulation for salbutamol sulfate", *J. Mater. Sc.i Mater Med*., vol. 20, pp. S71–S76, 2008.

[12] A.Amani, P. York, H. Chrystyn, B.J. Clark, "Evaluation of a Nanoemulsion-Based Formulation for Respiratory Delivery of Budesonide by Nebulizers", *AAPS PharmSciTech*., vol. 11(3), pp. 1147- 1151, 2010.