Article

Journal of
Biomedical Nanotechnology
Vol. 17, 1453–1458, 2021
www.aspbs.com/jbn

Characterization and Biological Evaluation of Solid Dispersed Nanoparticles of Synthetic Epoksilignan Targeting Pancreatic Cancer Cell as an Anticancer Agent

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MMEO (3′-methoxy-3′,4″(methylenedioxy)-2,5-epoksilignan-4′ol-6-on) is a derivative of DMEO (3′-methoxy-3″,4″(methylenedioxy)-2,5-epoksilignan-4′,6-diol) synthesized through demethylation using dimethylsulfoxide-acetic anhydride reagent. MMEO inhibits Hedgehog signaling at a concentration of 4.1 μM. The current study aimed to formulate MMEO as solid dispersed nanoparticles and determine their physicochemical properties and inhibitory activities. XRD (X-ray diffraction) analysis showed that the crystalline particles of the pure compound MMEO was smaller than MMEO nanoparticles. Image J software showed that at concentrations of 25 mg/mL and 50 mg/mL, the average nanoparticle sizes were 852.26 nm and 178.65 nm, respectively. Therefore, the MMEO solid dispersion system with the PEG 4000 polymer increases the solubility of MMEO. The higher the concentration of PEG 4000 the greater the solubility of MMEO. Treating pancreatic cancer cell lines with MMEO silenced the smoothened function by downregulating mRNA Ptch expression. This study suggests that MMEO may inhibit pancreatic cancer disease.

KEYWORDS: *Glioma Inhibitors, Solid Dispersions, Nanoparticles, MMEO, DMEO.*

INTRODUCTION

Glioma is a general term for the group of tumors that grow in glial cells. About 4 out of 10 cases of brain cancer are gliomas. Glioma (GLI) is one of the transcriptional targets in the Hedgehog pathway (Hh) [1]. Research has shown that activated mutations of Smo induce the nuclear translocation of transcriptional GLI factors. One of the potential anti-cancer compounds to inhibit gliomas is MMEO (3′-methoxy-3″,4″(methylenedioxy)-2,5-epoksilignan-4′-ol-6-on). MMEO is a derivative of DMEO (3′-methoxy-3″,4″(methylenedioxy)-2,5-epoksilignan-4′,6-diol) which is synthesized through demethylation using dimethylsulfoxide-acetic anhydride reagent. MMEO has been shown to inhibit Hh signaling at a concentration of 4.1 μM [2].

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Received: 27 March 2021
Accepted: 23 June 2021

MMEO is poorly soluble in water, resulting in a low dissolution rate and absorption in the gastrointestinal tract [1]. MMEO solubility can be increased via various methods, including a solid dispersion system. Solid dispersion system contain active ingredients in an inert, solid-state carrier or matrix [3].

Sekiguchi and Obi [3] were the first to use solid dispersion systems for improving the solubility and absorption of oral drugs with poor solubility in water; they dispersed sulfathiazole in physiological water, which had better oral absorption than sulfathiazole without dispersion. There are several ways to formulate solid dispersions, including melting, dissolution, and mixed melting-solvent methods. PEG (*polyethylene glycol*) 4000 is one of the most common polymers used in the formulation of solid dispersions, especially by the fusion method. PEG 4000 has a molecular weight of 3000–4800 and a melting point of 50°–58 °C [4]. Yang et al. [5] improved the solubility of repaglinide by dispersing it with PEG 4000 using the fusion method.

Nanoparticle drug delivery systems can improve the bioavailability of drugs. Nanoparticles have a diameter of <100 nm, this may lead to higher absorption efficiency and greater permeation, thereby prolonging the time of drug molecule release; enabling precise drug targeting because of their ability to easily pass or penetrate the blood-brain barrier, the branching paths in the pulmonary system, and epithelial tissue; reducing the toxicity of the drug; as well as improving the distribution of drugs [6]. Other advantages of using nanoparticles include their ability to deliver drug molecules directly into cells and their capacity to target tumors in healthy tissue because of the discontinuous nature of the tumor [6].

The aim of this research was to improve the solubility of synthetic MMEO using a solid dispersion system and hydrophilic PEG 4000 polymer. We also used precipitated ultrasonication to produce smaller and more homogeneous particles. Then we characterized the solid dispersion system using X-ray diffraction, thermal, DSC, FT-IR spectroscopy, microscopic, SEM and UV spectrophotometry analyses.

EXPERIMENTAL DETAILS

A solid dispersion was prepared by mixing MMEO and PEG 4000 at ratios of 1:1, 1:3 and 1:5 w/w. The physical mixture of MMEO-PEG 4000 (1:1, 1:3 and 1:5 w/w) were perfectly melted as gels. Then, samples were stirred until homogeneous and then cooled. The result was a solid dispersion of MMEO-PEG 4000, which was then crushed. Furthermore, the solid dispersion was stored in a desiccator before being tested. MMEO nanoparticles were also prepared at concentrations of 25 mg/mL and 50 mg/mL using precipitated ultrasonication. MMEO compounds were dissolved in DMSO at concentrations of 25 mg/mL and 50 mg/mL. Polyvinyl alcohol (PVA) was dissolved in water at a concentration of 0.5%. Then, the MMEO solution was combined with PVA solution at a ratio of 1:10 under constant stirring for 3 hours. The suspension formed then it was ultrasonicated at 15° for 30 minutes. The suspension was then lyophylized using a freeze dryer [7].

A solubility test was conducted with the MMEO solid dispersion powder-PEG 4000. As for each comparison, the mixture was combined with 5 mL of distilled water and then stirred for 24 hours at a 37 °C. Furthermore, it was centrifuged at 1000 rpm for 5 minutes. Then the concentration and the absorbance of the filtrate were measured using UV-vis spectrophotometry at a wavelength of 264 nm [5]. Thermal Analysis was done using a Differential Scanning Calorimeter. Temperature was lowered from 50 °C to 35 °C to 10 °C per minute. The MMEO-solid dispersion PEG 4000 powder can be determined from the data thermogram [6].

The crystallinity of nanoparticles was analyzed by X-ray diffraction. Samples were placed in a glass sample holder. Samples were scanned with CUK radiation at 30 mA and 40 kV from the points 50 to 650. The X-ray diffraction pattern of the solid dispersion MMEO-PEG 4000 powder was analyzed at 27 °C using an analytical PAN diffractometer. Measurement conditions were as follows: the target was the Cu, Kα Filter, voltage was 40 kV at a current of 40 mA, the analysis was done at an angle of 5–35 2θ. Samples were placed on the glass and leveled to prevent particle from moving during sample preparation [8].

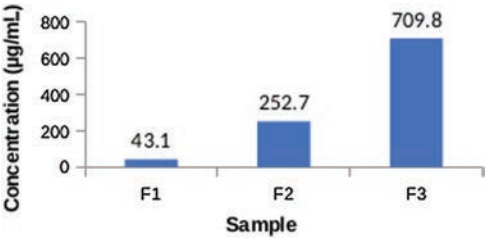

Figure 1. Solubility MMEO:F1 = Solid dispersion by comparison MMEO:PEG 4000 (1:1 w/w) F2 = Solid dispersion by comparison MMEO:PEG 4000 (1:3 w/w) F3 = Solid dispersion by comparison MMEO:PEG 4000 (1:5 w/w).

The interaction between the active substance and excipients was analyzed by FTIR spectroscopy. The powder sample was dispersed in KBr pellets at hydrostatic pressure. Then the percent transmittance was measured from 400 to 4000 cm [9].

MMEO solid dispersion 4000 powder was placed on a sample holder made of aluminum and coated with gold at a thickness of 10 nm. The samples were then observed over a wide range of magnification with the SEM tool. The voltage was set to 30 kV, and the current was 12 mA. Samples were dried in a gold cover and then placed in

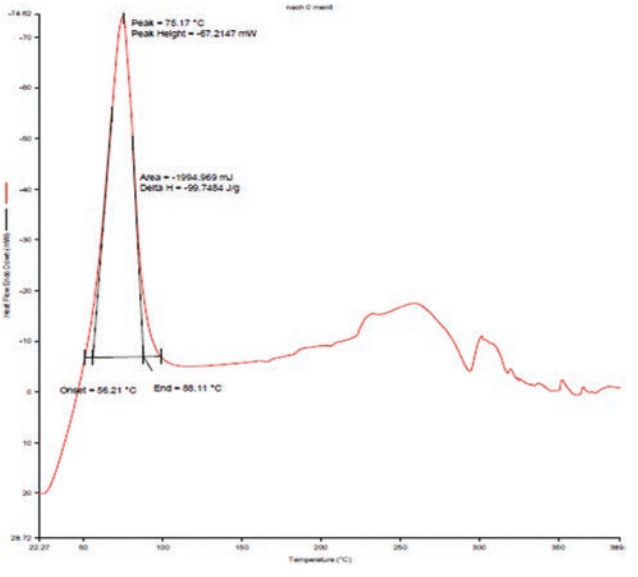

Figure 2. DSC analysis of the MMEO:PEG 4000 (1:1) solid dispersion.

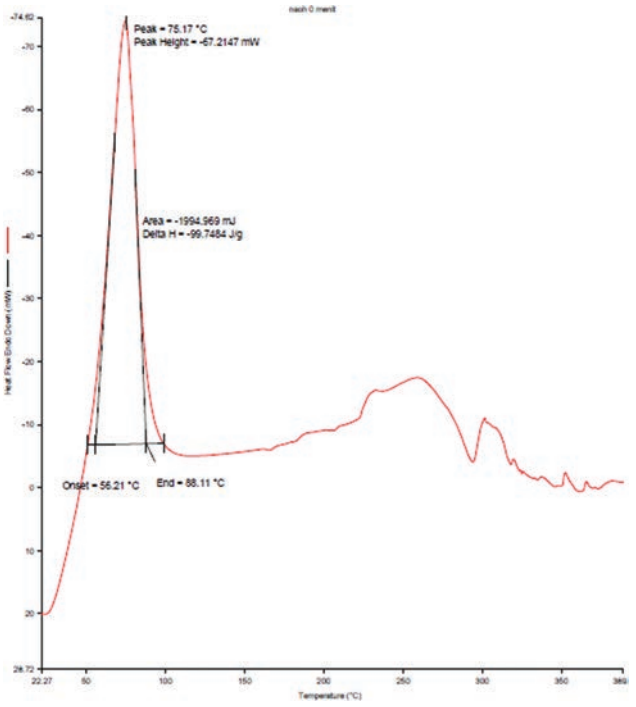

Figure 3. **DSC analysis of the MMEO:PEG 4000 (1:3) solid dispersion.**

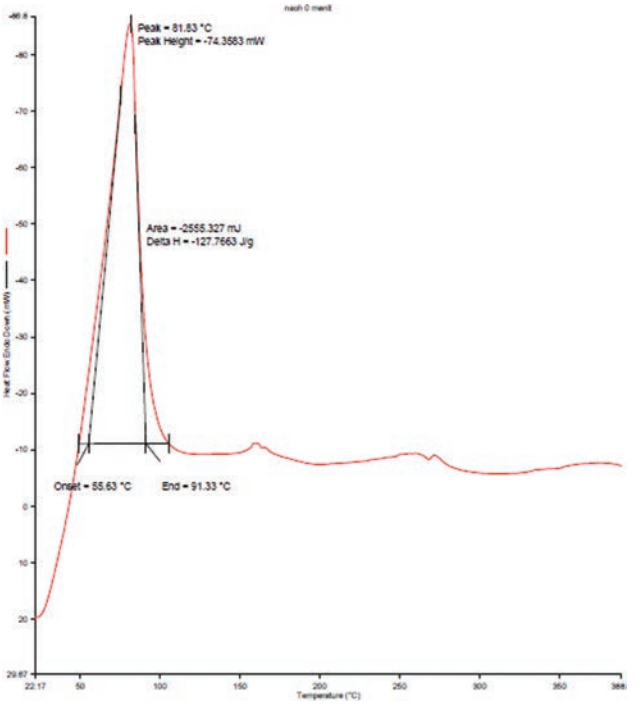

Figure 4. **DSC analysis of the MMEO:PEG 4000 (1:5) solid dispersion.**

the sample holder. Observations were carried out at a voltage of 15 kV and current 20 mA at 100 and 1000 times magnification [7].

Analysis with UV-vis spectrophotometry was performed by dissolving 50 mg MMEO in 10 mL of methanol, and then the maximum wavelength was measured. The solid dispersion results for each ratio were obtained by dissolving 50 mg in 10 mL of methanol, and then the maximum wavelength of each solid dispersion was measured for MMEO-PEG 4000 [10].

To determine the inhibition of MMEO in PANC1 during the Hh inhibition, we knocked down Smo expression in PANC1 cells by siRNA and performed real-time quantitative RT-PCR on its mRNA expression. The Western blotting confirmed that the Smo protein level was totally depleted after a deleting process (Fig. 10, bottom panel). Silencing of Smo siRNA significantly reduced the expression of Ptch mRNA in PANC1-treated MMEO (Fig. 10, upper panel).

RESULTS AND DISCUSSION

The amount of PEG 4000 greatly affected the solubility of MMEO. The solubility of the solid dispersion MMEO-PEG 4000 formula increased. In the solid dispersion of MMEO:PEG 4000 (1:1 w/w) the concentration of dissolved solute was 43.1 ug/mL. In the solid dispersion of MMEO:PEG 4000 (1:3 w/w) the concentration of dissolved solute was as high as 252.7 mg/mL, and in the solid

Table I. **Crystalline size shown by the XRD diffractogram.**

Name	No. peak	2θ	FWHM (°)	Intensity	Crystal size (nm)
MMEO	8	20.1572	1.15870	7898	7.28
	5	16.8855	0.75570	7079	11,16
	9	22.4945	0.78840	6499	10.73
Average			0.90093		9.72
Nanoparticles	10	44.0515	0.16540	2554	54
MMEO	13	64.4132	0.18890	2042	51.94
25 mg/mL	7	39.5379	0.13470	704	68.47
Average			0.163		58.14
Nanoparticles	7	19.9695	1.61330	1954	5.23
MMEO	5	16.8200	1.02640	1786	8.18
50 mg/mL	1	6.1955	0.97840	1343	8.48
Average			1.20603		7.30

Table II. **MMEO nanoparticle size at a concentration of 25 mg/mL.**

Concentration	Count	Total Area	Average Size	% Area	Mean
25 mg/mL	3455	110269827	31916.014	3.602	254 366

Notes: Average particle size = 31916.014 NM2 = 178.65 nm.

Table III. **MMEO nanoparticles size at a concentration of 50 mg/mL.**

Concentration	Count	Total Area	Average Size	% Area	Mean
50 mg/mL	208	151081902.5	726355.3	5236	254 586

Notes: Average particle size = 726355.3 NM2 = 852.26 nm.

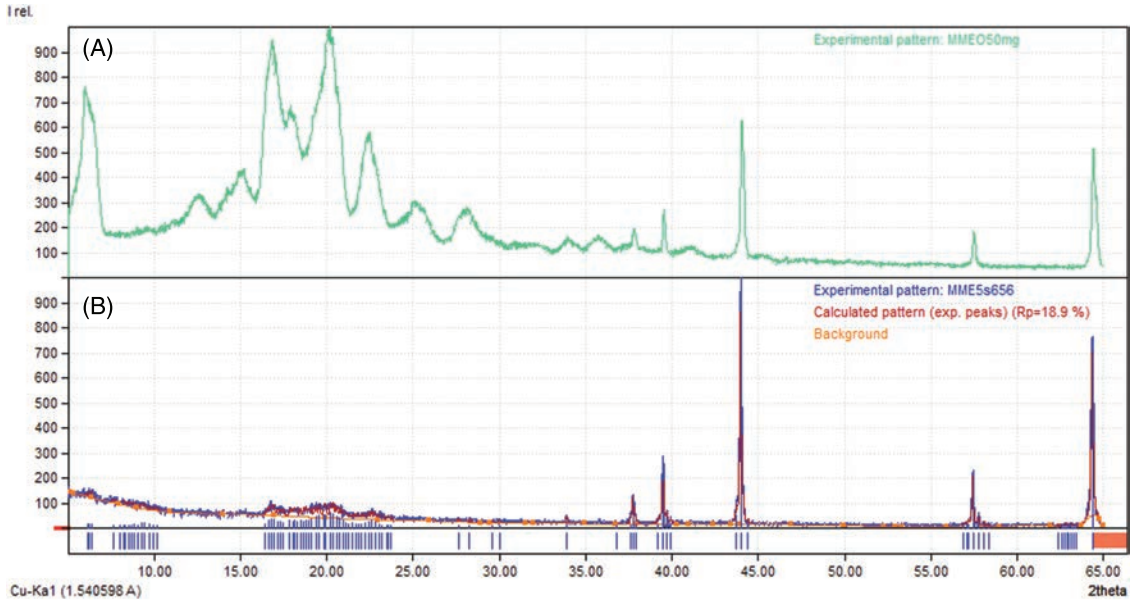

Figure 5. **XRD diffractograms, (A) 50 mg/mL MMEO nanoparticles; (B) 25 mg/mL MMEO nanoparticles.**

MMEO:PEG 4000 (1:5 w/w) dispersion the concentration of dissolved solute was as high as 709.8 mg/mL (Fig. 1).

The thermogram showed that, for MMEO : PEG 4000 (1:1 w/w), there was a single sharp endothermic peak at 75.17 °C with a temperature range of 56.21 °C to 88.11 °C. The results of the comparative thermogram MMEO:PEG 4000 (1:3 w/w) analysis showed a single sharp endothermic peak at 78.53 °C with a temperature range of 52.46 °C to 88.56 °C. The results of the comparative thermogram MMEO:PEG 4000 (1:5 w/w) analysis showed a single sharp endothermic peak at a temperature of 81.83 °C with a temperature range of 55.63 °C to 91.33 °C. In the thermogram results, the solid MMEO:PEG 4000 dispersion (1:1, 1:3 and 1:5) did not crystallize at increased temperature. The melting point of PEG 4000 was 63, 32 °C.

Based on the results of X-ray diffraction, solid dispersions of MMEO:PEG 4000 (1:1, 1:3 and 1:5) each had a high peak intensity more than 200, so the solid dispersions belonged to crystalline phases. The degree of crystallinity in each different comparison signifies the creation of a new crystalline phase for the interaction between MMEO and PEG 4000 (see Figs. 2–4). These results can be seen in Table I, II and III. The degree of crystallinity for solid

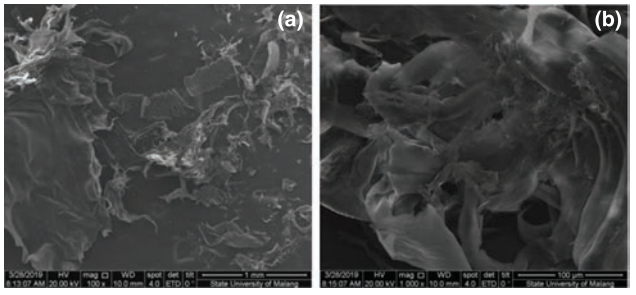

Figure 6. **SEM results for the MMEO nanoparticle concentration of 25 mg/mL, (a) magnification 100×; (b) 1000× magnification.**

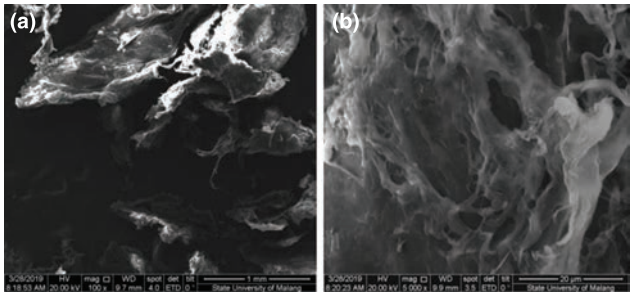

Figure 7. **SEM results for the MMEO nanoparticle concentration of 50 mg/mL, (a) magnification 100×; (b) 1000× magnification.**

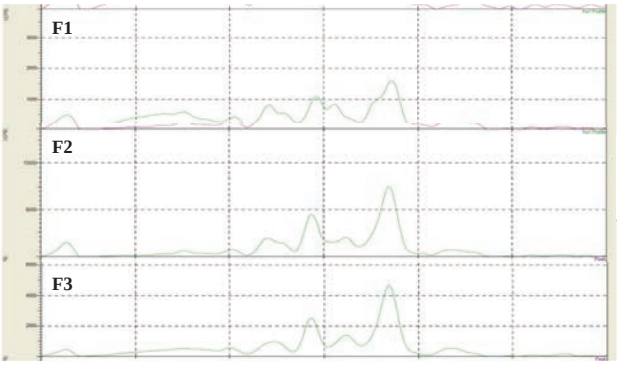

Figure 8. **Results of X-ray diffraction for the MMEO-PEG 4000 solid dispersion:F1 = Solid dispersion by comparison MMEO:PEG 4000 (1:1 w/w); F2 = Solid dispersion by comparison MMEO:PEG 4000 (1:3 w/w); F3 = Solid dispersion by comparison MMEO:PEG 4000 (1:5 w/w).**

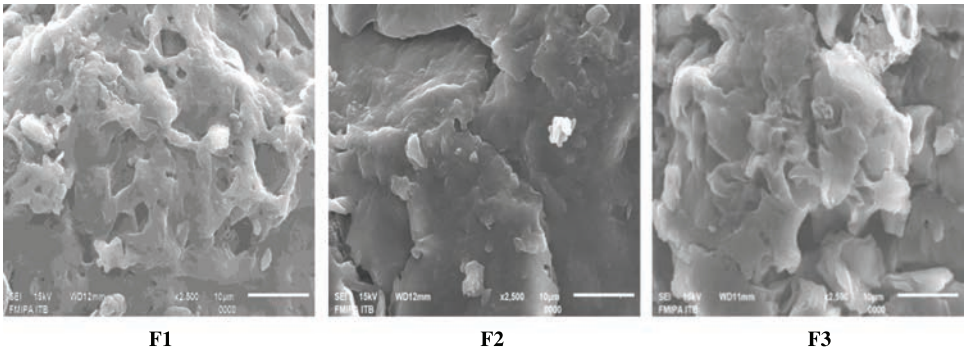

Figure 9. **SEM of (F1) MMEO:PEG 4000 (1:1 w/w) solid dispersion; (F2) MMEO:PEG 400 (1:3 w/w) solid dispersion; (F3) MMEO:PEG 4000 (1:5 w/w) solid dispersion.**

dispersed MMEO:PEG 4000 (1:1, 1:3 and 1:5) diminished which indicates that the crystal size also decreased with the amount of PEG 4000. Meanwhile, comparison of the peak intensities on the XRD diffraction graph showed a decrease in the crystallinity of the MMEO nanoparticles at a concentration of 25 mg/mL compared to 50 mg/mL (Fig. 5).

As shown in Table I, higher concentrations of MMEO resulted in larger crystal sizes. The average crystal size of pure MMEO was 9.7198 nm. MMEO nanoparticles at concentrations of 25 mg/mL and 50 mg/mL had average crystal sizes of 58.14 nm and 7.30 nm, respectively.

Surface morphologies of nanoparticles, produced at concentrations of 25 mg/mL and 50 mg/mL, were different. Figures 6 and 7 show SEM results for MMEO nanoparticles at concentrations of 25 mg/mL and 50 mg/mL, respectively. The morphology of MMEO showed irregular shapes and various sizes (Figs. 6, 7). Image J software, as shown in Tables II and III, showed the average particle sizes of the nanoparticle MMEO samples at concentrations of 25 mg/mL and 50 mg/mL were 852.26 nm and 178.65 nm, respectively.

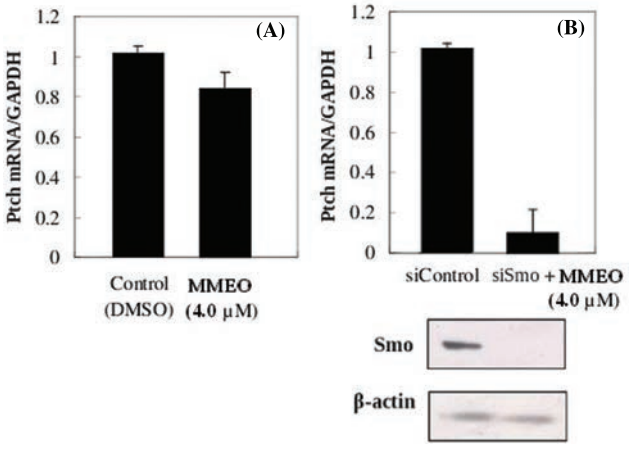

Figure 10. **Expression of Ptch mRNA ((A) upper panel) and Smo protein ((B) bottom panel) in PANC1 treated with MMEO after siRNA-mediated silencing of Smo.**

FTIR analysis of MMEO:PEG 4000 (1:1, 1:3 and 1:5 w/w) showed that the functional groups at each wave number were equal to those on MMEO and PEG 4000. The functional groups on MMEO include ketones, alcohols, ethers, alkanes, and aromatic rings; the functional groups on PEG 4000 include alcohols, alkanes, and ethers. This indicates there was no chemical reaction at the time of melting. In addition, there was no interaction between MMEO and PEG 4000 because there no new wave numbers were recorded.

SEM results of the solid MMEO:PEG 4000 dispersion (1:1, 1:3 and 1:5) can be seen in Figure 8 at a magnification of 2500 times. The results of each SEM showed both irregular and uniform particle morphologies. PEG 4000 greatly influenced the efficiency of the polymer solid dispersion, which also increases the efficiency of the solid dispersion. In Formulas 1, 2, and 3 morphology that resembles that of PEG 4000 could not be found, indicating that PEG MMEO was uniformly dispersed in 4000. The results of the SEM indicate no interaction between MMEO and PEG 4000 to form a new particle morphology, namely morphology solid dispersion for formulas 1, 2 and 3 (Fig. 9).

CONCLUSIONS

MMEO solubility is increased in the solid PEG 4000 polymer dispersion system. The higher the concentration of PEG 4000 greater the solubility of MMEO. The MMEO solid dispersion MMEO:PEG 4000 (1:5 w/w) showed the best results because the concentration of dissolved MMEO was higher than those in the other solid MMEO:PEG 4000 dispersion (1:1 and 1:3 w/w). Image J software showed that at a concentration of 25 mg/mL and 50 mg/mL, the average nanoparticle sizes were 852.26 nm and 178.65 nm respectively. Quantitative RT PCR of MMEO exhibited that the compound inhibited Hh signaling was in an Smo-independent manner.

Ethical Compliance

Research experiments conducted in this article with animals or humans were approved by the Ethical Committee and responsible authorities of our research organization(s) following all guidelines, regulations, legal, and ethical standards as required for humans or animals.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments: We are grateful to Hasanuddin University through internal grant No. 9/E1/KP.PTNBH/2019 and 915/UN4.22/PT.01.03/2021.

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