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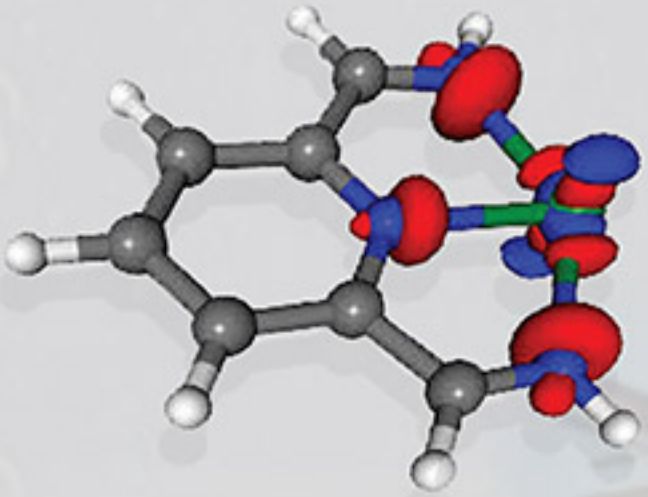

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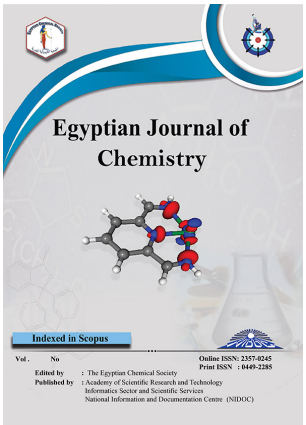

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Characterization of Dry Powder Inhaler Quercetin Solid Lipid Microparticle (SLM) as Lung Delivery System: Effect of Polymer Concentration

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Abstract

The inhalation route for pulmonary treatment can provide many advantages such as high surface area with fast absorption due to high vascularity, avoiding the first pass effect, therapeutic effect can be achieved at much lower doses, as well as targeted drug delivery so as to reduce side effects. Solid lipid microparticle system containing Quercetin which was formed as Dry Powder using glyceryl behenate as lipid and poloxamer 188 as surfactant polymer was prepared. The system was characterised in terms of moisture content, morphology, particle size, flow properties, aerosolization study, mass median aero dynamic (MMAD) and in vitro release. Size and morphology was spherical and smooth with dry and small size met inhalation size (<5 µm). Flow properties showed good; however aerodynamics size based on aerosolization study was not as good as flow characteristics. Recovery dose was between 26 - 43%. In vitro release of Quercetin demonstrated burst release for lower poloxamer concentration, whereas higher surfactant concentration affected the release profile become prolong and controlled release. This study has shown that SLM Quercetin may potential for controlled release respiratory therapy. Further study of stability and activity were highly recommended for lung treatment.

Keywords: SLM, Quercetin, Inhalation, Lung Disease, Poloxamer 188

1. Introduction

Solid Lipid Microparticle (SLM) is a drug delivery system that is used for controlled drug delivery, to improve bioavailability, distribution of drugs in the body and target drugs at specific and predetermined targets [1]. Solid Lipid Microparticle (SLM) is a solid lipid carrier system measuring 1-1000 µm consisting of a naturally occurring solid lipid core stabilized by surfactants [2,3] with various advantages and previously this system could be used as a delivery system that could be targeted at the lungs [4].

Quercetin is a polyphenolic compound found in various plants consumed by humans and is available as dietary supplements. Its physiological effects include antioxidant, anti-inflammatory, immunomodulatory and anti-pathogenic properties, and among several antioxidant compounds, flavonoids are considered to be able to inhibit viral infections. Antioxidants are compounds that can absorb or neutralize free radicals so as to prevent degenerative diseases [5]. Research by Pool et al [6] stated that antioxidants from flavonoids including catechin, luteolin, apigenin, quercetin, and quercetin 7-rhamnoside can inhibit ROS accumulation and apoptosis of virus-infected cells. [7]. Some researchers investigated phytochemical for inhalation [8].

Some of the advantages of drug delivery to the lungs are the large alveolar surface, barrier thin epithelium, wide vascularity and relatively low activity of metabolic enzymes and reduction of side effects [4]. The system of drug delivery through the pulmonary route when compared to conventional drugs is to protect drugs from enzymatic degradation, deliver drugs directly to targeted sites in the lungs, provide controlled drug release, reduce dose frequency and minimize side effects [9]. Inhaled drug delivery system DPI (dry powder inhaler) has advantages that are superior to

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Nebulizers and MDI, which are more environmentally friendly, DPI formulations are more stable, the dose of choice is wider and can improve patient compliance [1].

For inhalation therapy to be effective, the optimal particle size should range from 1 to 5 μm to reach the lower airways. Phagocytic uptake of inhaled particles is one of the main mechanisms of removing insoluble particles from the lung surface. Phagocytosis has been shown to be particle size dependent; particulate drug delivery systems that display optimal particle sizes for inhalation are also more likely to be phagocytized [10].

A recent study conducted using quercetin using lipid-based microparticles received attention because it was considered to be well tolerated due to its biocompatible material, and its high capacity to incorporate lipophilic drugs [11, 12].

Research on drug delivery systems Solid Lipid Microparticle targeted at the lungs, with low water soluble active agents, such as research conducted by Silva et al [10] demonstrated the potential use of solid lipid microparticles as quercetin carriers for testing against pulmonary administration. There are still many formulation studies carried out to produce suitable characteristics, release and stability, one of which is by considering the concentration of lipids in SLM as a carrier and Glyceryl behenate lipids will get much better value of Mass Median Aerodynamic Diameter (MMAD). The Aerosol Consensus Statement stated that an aerosol with a Mass Median Aerodynamic Diameter (MMAD) value of 2-5 μm is the optimal size for delivering drug substances to the respiratory tract and produces better bronchodilation [13].

Concentration of poloxamer 188 used in forming Solid Lipid Microparticles need to be considered, because high concentrations will affect the size of the SLM powder that enters the lungs for it has a smaller surface tension which causes small particle size [14,15].

In addition to the concentration/amount of lipid used, the technique of producing SLM is also an important parameter. In research conducted by Scalia et al., 2013 Quercetin Solid Lipid Microparticle formed using the method melt o/w emulsification [16]. The results show that quercetin SLM can be formulated as a dry powder suitable for the inhalation delivery system with a size (20.5 ± 3.3% Fine Particle Fraction with an aerodynamic size of 64.46 μm).

The purpose of this study was to determine the physical and release characteristics of inhaled SLM with the active agent quercetin at different concentrations of polymeric surfactant, and to determine the effectiveness of aerosols so that they can be well received by the lungs.

2. Experimental

Materials

Quercetin pharmaceutical grade was purchased from Tcichemicals (Japan), Glyceryl behenate (Compritol 888 ATO) was purchased from Gattefose (France), Poloxamer 188 was purchased from Sigma-Aldrich (USA), Na_2HPO_4 pro analysis (Merck); KH_2PO_4 pro analysis (Merck); Aquademineralisata (PT. Bratachem). Methanol, and other chemicals (pharmaceutical grade).

2.1. Production of SLM Quercetin

Solid Lipid Microparticles (SLM) was prepared using the o/w emulsification technique (Table 1). Poloxamer 188 was dissolved using 100 ml of distilled water, then poloxamer 188 and Glyceryl Behenate/Compritol heated on a hot plate to a temperature of 70 °C, after reaching the temperature of 70 °C, Poloxamer 188 is poured into the oil phase Glyceryl Behenate which has melted first and has been mixed with quercetin until homogeneous. The resulting emulsion at 5000 rpm using ultra-turrax (Ultra-Turrax T25, IKA, Germany). for 1 minute and continued at 10,000 rpm for 4 minutes. The results of ultra-turrax were then sonicated for 5 minutes, the sample was allowed to reach room temperature, then dried with freeze dry for 27-30 hours.

Table 1: SLM Formula

Components	Function	F1	F2	F3	F4	F5
Quercetin	Active agents	1%	1%	1%	1%	1%
Glyceryl behenate/Compritol	Lipid	5%	5%	5%	5%	5%
Poloxamer 188	Surfactant	0,2%	0,3%	0,4%	1%	2%
Aquadestillata	Solvent	100 mL	100 mL	100 mL	100 mL	100 mL

2.2.Moisture Content

Moisture content on SLM determined by tool moisture analyser (Mettler Toledo HB43-S Greifense, Switzerland). To determine % MC using the following formula ;

$$\%\ MC = \frac{\text{weight before being analysed} - \text{weight after being analysed}}{\text{weight before being analysed}} \text{ x } 100\%$$

2.3.Yield

SLM yield is calculated from the total amount of dry SLM obtained by the following calculation:

$$\%\ Yield = \frac{\text{Total weight of formed SLM (mg)}}{\text{Weight of constituent components (mg)}} \text{ x } 100\%$$

2.4.SLM Particle Size

Observations were made using a Novel® optical microscope with optilab® camera and Raster J image software as much as 300 particles per formula. The mean diameter were determined.

$$\text{Dmean} = \frac{\sum n.d}{\sum n}$$

2.5. SEM Morphology

Scanning Electron Microscopy (SEM) (FEI Inspect S50, USA) was used to show the morphology and particle size of SLM.

2.6.Drug Loading and Encapsulation Efficiency

Approximately 250 mg of the sample was weighed appropriately into a 50 mL beaker in methanol solvent. The samples were stirred under magnetic stirring for two hours and then centrifuged at 2500 rpm for 10 minutes. The supernatant was analysed by UV spectrophotometry at 373.5 nm (close to the literature wavelength of 375 nm). From these results, it was found that the entrapment efficiency (%) was estimated as the ratio of the percent of drug trapped in the SLM to the total drug added to the formulation. Drug Loading Quercetin (w/v %) was expressed as the percentage ratio of drug to weight of SLM.

$$Drug\ loading = \frac{\text{Quercetin level in completely dry SLM}}{\text{weight of completely dry SLM Quercetin}} \text{x } 100\%$$

$$Entrapment\ efficiency = \frac{\text{Quercetin level in completely dry SLM}}{\text{total weight of Quercetin}} \text{x } 100\%$$

2.7.Flow Properties

2.7.1.Bulk Density and Tapped Density

Bulk density is determined by weighing a certain amount of powder and then put into a measuring cup without compaction. The weight of the powder in the measuring cup is weighed, then calculated:

$$Bulk\ density = \frac{powder\ weight\ (gram)}{initial\ volume\ of\ powder\ (ml)} \text{x } 100\%$$

Tapped Density is determined by filling the material to be tested into a measuring cup, after that by using a motorized tapping in which the device was stomped 500 times, and the final volume of powder was observed:

$$Tapped\ density = \frac{powder\ weight\ (gram)}{volume\ after\ tapping\ (ml)} \text{x } 100\%$$ [17]

2.8.Carr's Index and Haustner Ratio

Carr's index and Hausner-Ratio are determined using the formula:

$$Carr's\ Index = \frac{tapped\ density - bulk\ density}{tapped\ density} \times 100\%$$

$$Hausner\ Ratio = \frac{tapped\ density}{bulk\ density}$$ [17]

2.9. In Vitro Release Study

First, a standard curve was prepared by preparing a standard solution of Quercetin in PBS (Phospate Buffer Saline) solution at pH 7.4 ± 0.05 with 5 different concentrations, namely 5 ppm, 10 ppm, 15 ppm, 20 ppm, and 25 ppm. The standard solution was then transferred to a cuvette and the spectra were observed to determine the maximum wavelength with a UV-Vis spectrophotometer instrument. The five standard solutions of Quercetin at various concentrations were then observed for their absorbance at the maximum wavelength. The obtained wavelength is 373.8 nm (close to the literature wavelength of 375 nm). Furthermore, a standard curve was made from the Quercetin solution in PBS with the concentration of Quercetin as the x-axis and the absorbance value as the y-axis. Then the regression equation is determined with the formula y= ax + b.

Quercetin Release Test from SLM by using a thermoshaker method at 37˚C. at a speed of ± 100rpm with three replications. Each formula was weighed as much as ± 200mg. The release medium of PBS solution pH 7.4 ± 0.05 as much as 100 ml was prepared and the temperature was adjusted at 37 ± 0.05˚C. After the media temperature reached 37 ± 0.05˚C, the SLM was inserted into the media and the thermoshaker was turned on at a speed of ± 100 rpm. Samples were taken as much as 5.0 ml at 0, 15, 30, 60, 90, 120, 150, 180, 210, 240, 300, 360, 420, 480, 540, and 600 in PBS pH 7.4 ± 0.05 media. Each sampling was done by replacing the release medium with PBS (Phospate Buffer Saline) pH 7.4 ± 0.05 under the same temperature of 5.0 ml. This sample is then filtered using Millipore filter paper. The absorbance of the sample solution was then observed with a UV-Vis spectrophotometer at a maximum wavelength of 373.8nm in 7.4 ± 0.05 PBS solution. Quercetin concentration dissolved in each sampling at each time interval can be determined by entering the absorbance value of the test sample solution into the regression equation of the Quercetin standard curve that was previously made. To get the actual concentration by taking into account the dilution of 5.0 ml of media in each sampling, the correction factor in the Wurster equation is used.

The sample level after correction (ppm) was then converted into mg units and multiplied by the amount of media (100mL). Determination of the cumulative amount of Quercetin released from the SLM system each time is obtained from:

$$\%\ \text{cumulative amount of Quercetin} = \frac{\text{Quercetin mass in the 100mL media (mg)}}{\text{Quercetin mass (mg)}} \times 100\%$$

2.10. In Vitro Aerosolization Study

The in vitro aerosolisation study of the SLM Quercetin was evaluated using the Cascade Impactor (CI). The design is flow rate of 28.3 l/min, the aerodynamic cutoff diameters of 8 stages consisiting 0, 1, 2, 3, 4, 5, 6, 7, and F. Whereas the DPI device used to deliver microspheres particles is the Spiriva® Handihealer®. Nine filter millar is cut to the size of the impactor plate. Before use, filter stored for 24 hours in a desiccator, in order to avoid the influence of weight gain from air humidity. Then weighed using an analytical digital balance. Eight stage and container plate filter from cascade impactor washed and then soaked with water in a

sonicator for 20 minutes, then dried in an oven for 60 minutes.

Composition cascade impactor consist of stage (levels) namely 0, 1, 2, 3, 4, 5, 6, 7, and F. Filter Each millar that has been weighed is placed at each level (from the highest to the lowest level) with a pore diameter of < 0.4 : 0.4 : 0.7 : 1.1 : 2.1 : 3, 3: 4.7 : 5.8 : and 9 m. Cascade impactor connected to a flowmeter and a suction pump (electric generator), where the flowmeter is arranged in such a way that the flow rate entering the cascade impactor of 28.3 litres per minute. After everything is connected, about 50 mg of SLM is put into the capsule shell (number 2) and filled into the DPI, then aerosolized by drawing air for 10 seconds at a flow rate of 28.3 litres per minute through the impactor. Then let it flow in cascade impactor. After sampling, weigh the whole filter millar which had previously been put into the container filter millar. From the mass difference of filter after and before the measurement, the percentage of the mass of each level to the total mass of all levels is made. The DPI particle data obtained are entered into the following equation to obtain data recovered dose, emitted dose, and fine particle fraction.

$$Recovered\ Dose\ = \frac{Total\ Net\ Weight\ (mg)}{Total\ of\ sprayed\ Microsphere\ (mg)} \times 100\%$$

$$Emitted\ Dose\ = \frac{Total\ of\ Particle\ on\ 2-F\ plate}{Recovered\ Dose} \times 100\%$$

$$Fine\ particle\ fraction\ = \frac{Total\ of\ Particle\ on\ 4-7\ plate}{Total\ Net\ Weight} \times 100\%$$

Recovered Dose (RD) is taken from the cumulative mass of administered microspheres (mass in inhaler + mass of each impactor plate).

Emitted Dose (ED) is the total mass of microsphere particles coming out of the inhaler. The uniformity of the outgoing dose was determined using Apparatus B (Dosage Unit Sampling Apparatus, DUSA) [18].

Fine Particle Fraction (FPF) or fine particle dose is calculated as the number of microsphere particles obtained until the filtration process.

2.11. Data Analysis

Characteristics and discharge tests were analysed statistically using the one-way Analyse of Variant (Anova) method with a 95% confidence level (α=0.05). The results of the data are 3 times ±SD replication.

3.Results and Discussion

3.1.Moisture Content, Yield and Particle Size of SLM Quercetin

Results of Moisture content, yield and particle size of SLM Quercetin can be seen in table 2. All formulas show moisture content good < 5%. Yield has increased from 49 to 92% for Formula F1 to F5. The manufacture of SLM with the active agent quercetin using the melt o/w emulsification technique resulted in a yield of SLM approaching 100% (Table 2). According to the study of Mezzena et al [1], the SLM formula group using melt o/w emulsification, showed SLM recoveries that were close to 100%. In this research, the freeze dry drying technique was continued to remove water. Freeze dry drying was chosen because it does not use high temperatures so that the stability of the medicinal agents is maintained. Yield is a determining factor for the success of a method used, indicating that the method is effective and efficient [19]. Yield is a parameter or factor supporting the success of a method used in the formulation [20].

Particle size observations showed that the SLM of quercetin in all formulas was less than 5 µm. After analysing the data using one-way ANOVA, sig. 0.040 < 0.05, it can be concluded that there is a significant difference between the formulas but still within the range of particle size for inhalation preparations. The particle size obtained is in accordance with the optimal particle size of 1 to 5 m to reach the bottom of the lung [21], the diameter of the SLM depends on the stirring speed during manufacture, the higher the stirring speed the smaller the size obtained, in addition the use of drying techniques can also be impacted on the small particle size obtained [22].

3.2. SEM Morphology

The SEM results of formula 1 to formula 5 with a magnification of 10000x in Figure 1 show that the morphology of SLM Quercetin formed becomes more round and spherical with increasing surfactant concentration. F5 has a shape that is best round or spherical, smooth and regular. However, the morphology of F1 to F3 still looks like needle crystals, Quercetin outside SLM is in a rather large number compared to F4 and F5, so it is recommended that further observations need to be conducted using Powder X-ray diffraction. The morphological description shows the use of glyceryl behenate as a lipid carrier affects the shape of the SLM [23]. The SEM morphology also showed that surfactant in sufficient concentration would be able to dissolve the Quercetin so that needle crystals did not appear.

The resulting F5 morphology shows the smoothest and most spherical morphology so it is possible that all Quercetins have been trapped in the SLM system. It is also supported by the data on the entrapment efficiency of F5 which is also the highest (Figure 2).

3.3. Drug Loading and Encapsulation Efficiency

Result of drug loading and encapsulation efficiency SLM quercetin formula can be seen in Figure 2. Figure 2 shows that the F5 formula has the highest drug loading and % entrapment efficiency. This is because the effect of the higher surfactant concentration will increase the drug loading and encapsulation efficiency.

Table 2: MC, Yield and Particle Size of SLM Quercetin

FORMULA	MC (%)	Yield (%)	Particle Size (µm)
F1	1.02 ± 0.54	49.27 ± 0.77	1.08 ± 0.06
F2	1.16 ± 0.21	53.85 ± 2.59	1.64 ± 0.15
F3	1.13 ± 0.24	53.21 ± 1.61	1.82 ± 0.09
F4	1.04 ± 0.44	91.07 ± 1.35	1.27 ± 0.07
F5	0.84 ± 0.39	92.67 ± 2.28	1.22 ± 0.06

Table 3: Density riil, Density compressed, Hausner Ratio and Carrs Index

Formula	Density Riil (g/ml)	Density compressed (g/ml)	Hausner Ratio	Carrs Index (%)	Conclusion
F1	0.1233 ± 0.04375	0.1576 ± 0.05012	1.2782	21.7547	Passable
F2	0.1233 ± 0.04728	0.1562 ± 0.06039	1.2668	21.0582	Passable
F3	0.1387 ± 0.05238	0.1792 ± 0.08089	1.2919	22.5962	Passable
F4	0.1019 ± 0.01310	0.1205 ± 0.01955	1.1825	15.4357	Good
F5	0.1145 ± 0.04779	0.1381 ± 0.06310	1.2061	17.0891	Good

3.4. Powder Flow Characteristics

Tables 3 and 4 show that the flow properties of the three formulas F1 to F3 are in the poor category, but formulas F4 and F5 show good flow properties. Many studies say that the aerosolization properties of powders can be improved by reducing the bulk density of the powders [4, 24, 25]. However, some of these investigators emphasized that the lower bulk density of SLM powder could be beneficial for lung deposition. Glyeryl behenate lipids exhibiting bulk densities of 0.1 to 0.2 are considered favourable for aerosolizing powders. After analysing the data using one-way ANOVA, sig. 0.013 < 0.05, it can be concluded that there is a significant difference between all formulas. All formulas met the criteria for aerosolized powders which had the advantage of deposition in the lungs. On the basis of CI value, powder flowability is: 5 – 12% (excellent); 12 – 18% (good); 18– 21% (fair); 21 – 25% (poor) [26].

Tests on flow properties are carried out by observing carr's index and hausner ratio . Parameter value carr's index desired for preparation quercetin SLM is <15% and parameter hausner ratio < 1.8. From the test results quercetin SLM F1 to F3, has a value of carr's index high while F4 and F5 have a value of 15-17% and hausner ratio 1.18-1.29 where the results are included in the flow properties scale which has the criteria of "Good". The flow properties of F1 to F3 are classified as poor, so it is feared that it will affect drug deposition into the lungs and it will be difficult to reach the expected target site.

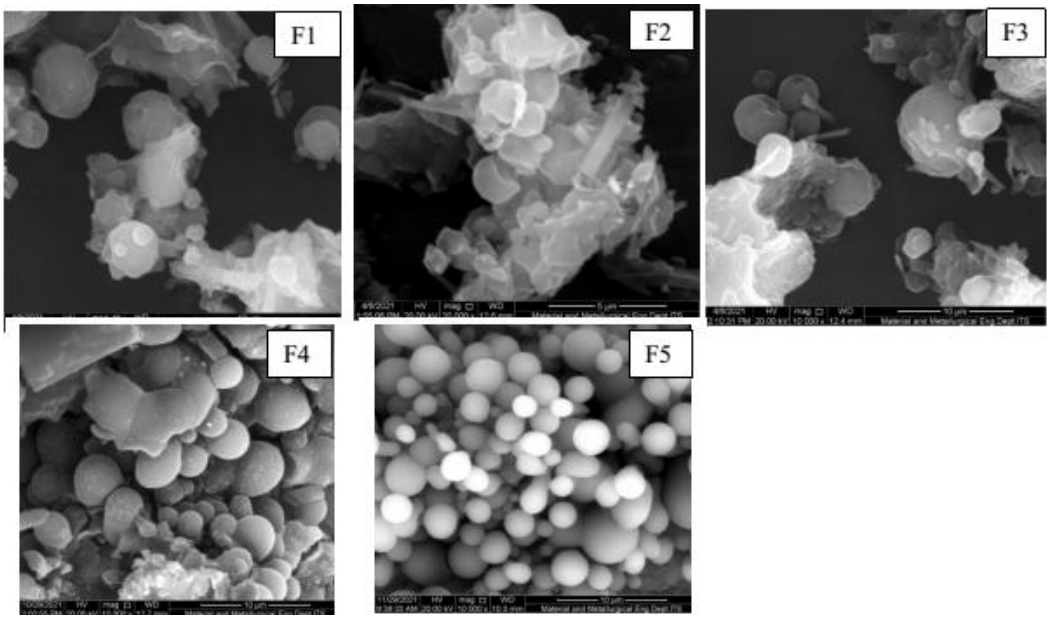

Figure 1. SEM SLM Quercetin at 10000x magnification

Table 4: Flow characteristics

Flow Character	Hausner Ratio	Carrs Index (%)
Excellent/very free flow	1.00 – 1.11	≤ 10
Good/free flow	1.12 – 1.18	11 – 15
Fair	1.19 – 1.25	16 – 20
Passable	1.26 – 1.34	21 – 25
Poor/cohesive	1.35 – 1.45	26 – 31
Very poor/very cohesive	1.46 – 1.59	32 – 37
Approx. non flow	> 1.60	> 38

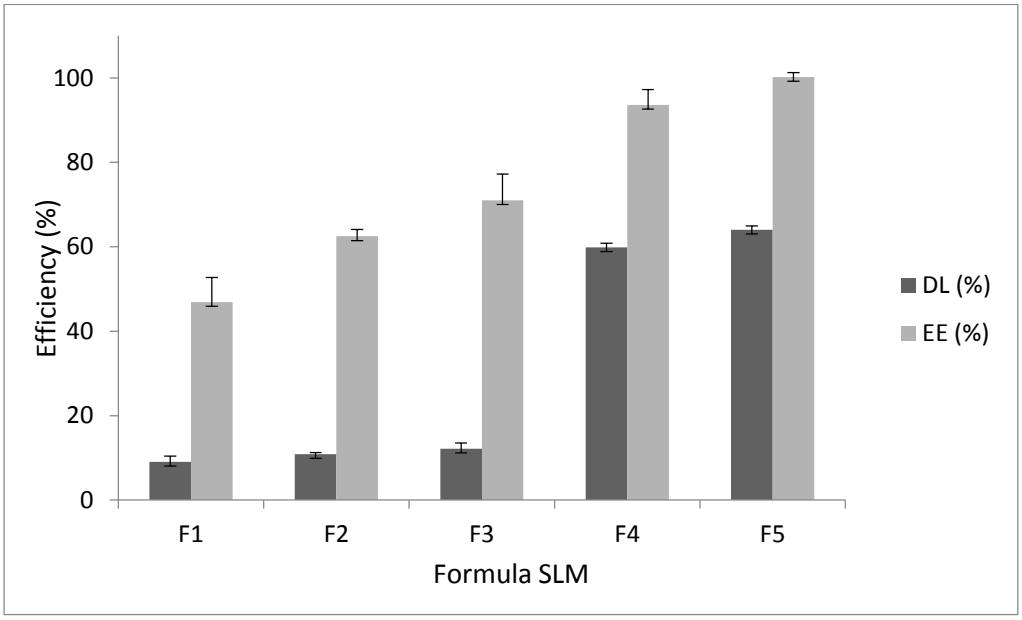

Figure 2. Entrapment Efficiency and Drug Loading of Quercetin SLM Formula

3.5. In Vitro Release Study

The release test was carried out for 10 hours on 5 formulas. Release test using saline phosphate buffer solution with pH 7.4, at 37 ° C with a stirring speed of 100 rpm. Results of the release test can be seen in Figure 3. Release medium with buffered phosphate saline pH 7.4 was used as a simulation of the conditions in the alveolar and based on the pH of the desired target site [27]. From the results of the release test for 10 hours, for each sampling of the media, the volume of the media was replaced as much as the sampling volume.

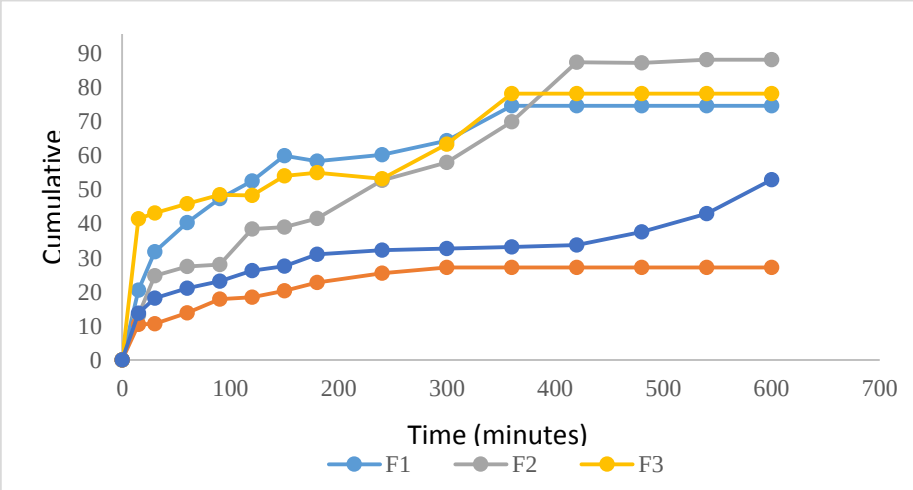

Figure 3. Release test profile on SLM Quercetin preparations.

This aims to keep the media in a sync condition. The SLM Quercetin F3 formula shows burst release in the early minutes followed by a large and rapid release of up to 80% in the 6th hour. For F1 and F2, a release profile phenomenon similar to that of F3 also occurred, namely the release reached 90% in the 6th hour even though the release in the early minutes was still smaller than 40%. F4 showed the slowest release profile among the formulas as well as F5 which was not significantly different also showed prolong release in the first 9 hours, but in F5 there was an increase in Quercetin release which increased up to 50% after 10 hours, this was probably due to the lower surfactant concentration. The highest concentration in the formula causes the solubility of Quercetin to increase up to 50% and it is hoped that in the following hours Quercetin can be completely released 100%.

3.6. In Vitro Aerosolization Study

Results Mass Median Aero Dynamic (MMAD) can be seen in Tables 5 and Figure 4.

It can be seen that percentage drug deposition of Quercetin from each formulation is shown in Figure 4. The ED and FPF were low and need further observation. The total drug recovery from all formulations was within 26 to 43% of the theoretical loaded dose. In general, the F5 formula had the highest aerosol efficiency, however mainly particles still stayed in the throat and on stage 1 (corresponding to particles with an aerodynamic diameter between 3 and 4.7 m). The percentage of the mass of the drug is at the stage of 0 to stage 4, which described the upper respiratory tract area showed all particles were found within the ideal DPI formulation range of less than 5 m. F5 showed the highest RD (43%) which has higher encapsulation efficiency. For RD, it is shown that all formula have low RD. This might be because of aggregation of particles, therefore particles did not reach the desired stage 3-7. The low FPF and low RD were still an issue and need improvement of formulation.

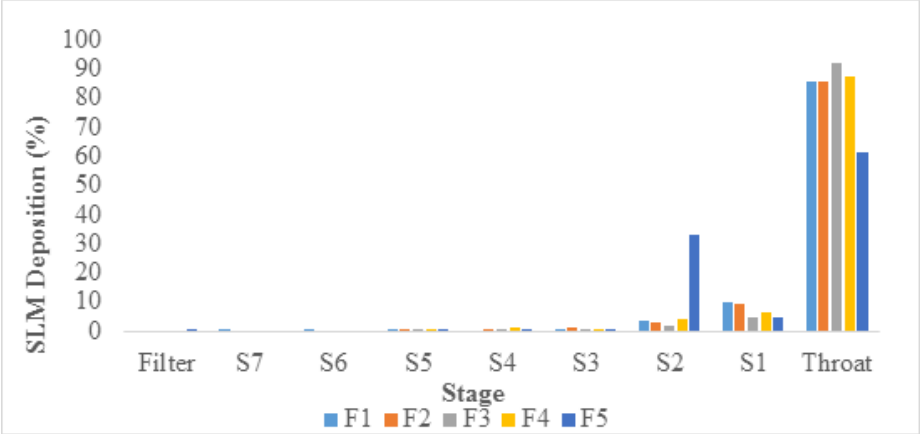

Figure 4. Deposition of SLM Quercetin particle

Table 5: RD and aerodynamic diameter of SLM Quercetin

Formula	Recovered dose (%)	Diameter Aerodynamic (µm)
F1	26.106	11.85
F2	33.766	11.92
F3	32.794	11.63
F4	29.054	12.10
F5	43.446	5.72

4. Conclusion

Increasing the surfactant content of poloxamer 188 formula 1 to formula 5 (0.2% to 2%) resulted in a delivery system solid lipid microparticle (SLM) with physical characteristics (moisture content, yield, good drug loading, and entrapment efficiency) resulted in a smooth and spherical particle morphology as expected and resulted in better flow properties Increasing the concentration of surfactant poloxamer 188 prolongs the release time of quercetin. SLM quercetin produces a size that is in accordance with the requirements of the pulmonary inhalation route, which is < 5µm. To be able to prove that SLM Quercetin is able to reach the alveoli, a test has been carried out aerodynamic mass median (MMAD) to find out how many % of the powder will be deposited on the alveolar. Further testing regarding Quercetin activity and stability quercetin SLM is also highly recommended.

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Conflict of Interest

The authors declare no conflict of interest.

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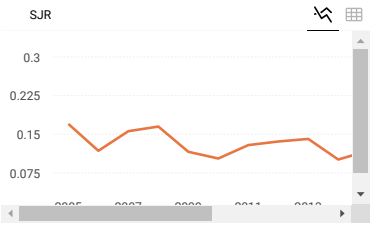

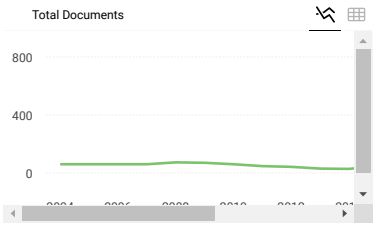

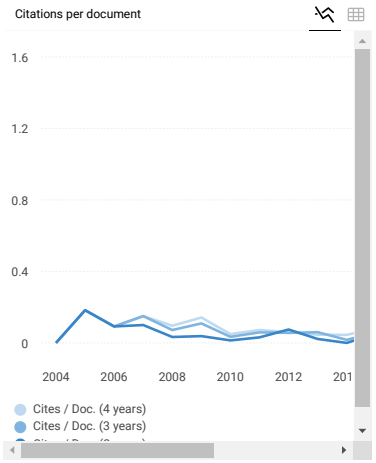

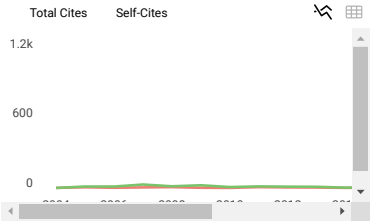

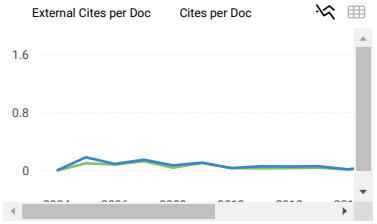

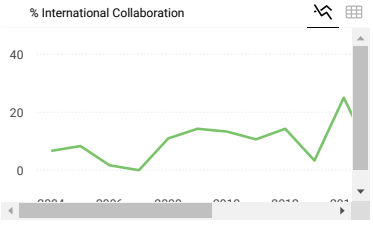

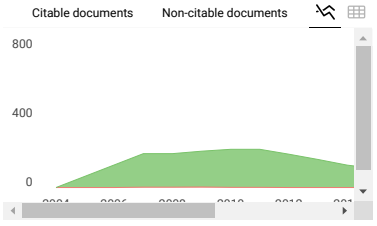

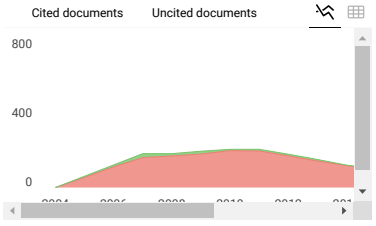

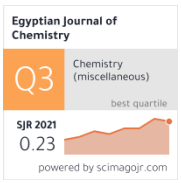

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