DEVELOPMENT AND CHARACTERIZATION OF RESVERATROL DRY EMULSIONS USING QUALITY-BY-DESIGN APPROACH

By
MISS Pontip BENJASIRIMONGKOL

A Thesis Submitted in Partial Fulfillment of the Requirements
for Doctor of Philosophy PHARMACEUTICAL ENGINEERING
(INTERNATIONAL PROGRAM)
Graduate School, Silpakorn University
Academic Year 2018

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Title	Development and characterization of resveratrol dry emulsions using Quality-by-Design approach
By	Pontip BENJASIRIMONGKOL
Field of Study	PHARMACEUTICAL ENGINEERING (INTERNATIONAL PROGRAM)
Advisor	Pornsak Sriamornsak

Graduate School Silpakorn University in Partial Fulfillment of the Requirements for the Doctor of Philosophy

..... Dean of graduate school
(Associate Professor Jurairat Nunthanid, Ph.D.)

Approved by

..... Chair person
(Associate Professor Sontaya Limmatvapirat , Ph.D.)
..... Advisor
(Professor Pornsak Sriamornsak , Ph.D.)
..... Co Advisor
(Associate Professor Suchada Piriyaprasarth , Ph.D.)
..... Examiner
(Associate Professor Thawatchai Phaechamud , Ph.D.)
..... External Examiner
(Associate Professor Pienkit Dangprasirt , Ph.D.)

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MISS PONTIP BENJASIRIMONGKOL : DEVELOPMENT AND CHARACTERIZATION OF RESVERATROL DRY EMULSIONS USING QUALITY-BY-DESIGN APPROACH THESIS ADVISOR : PROFESSOR PORNSAK SRIAMORNSAK, Ph.D.

Resveratrol (RVT) possesses various potential advantages to human health. However, RVT has some disadvantages i.e. poor water solubility and photo-sensitivity. The aims of this research were to improve the dissolution property and photostability of RVT. Spray-dried emulsion and emulsion-loaded porous powders were developed to encapsulate RVT. The dissolution and photostability of RVT in the spray-dried emulsion and porous powders were significantly improved, compared to intact RVT. Quality-by-Design (QbD) approach, which emphasizes product and process understanding based on science and risk management, was implemented to provide an extensive understanding of the relationship between product and process factors and responses. In the development of RVT spray-dried emulsion, the possible risks were evaluated in risk assessments, including the Ishikawa diagram and a risk-ranking system. The critical factors were further revealed in a Plackett–Burman design. After the experiment, the risks were re-evaluated based on new understanding. The low-methoxyl pectin (LMP) amount, caprylic/capric glyceride (CCG) amount, homogenization speed, and pump speed were observed to most critically affect the quality of the product and the spray-drying performance. The spray-dried emulsions and spray-drying process were optimized using Box-Behnken design to produce RVT spray-dried emulsion having small redispersed emulsion size, fast dissolution, good flow property, and desirable spraying efficacy. The experimental results have shown that the size of redispersed emulsion was influenced by LMP and CCG amount. The dissolution rate was influenced by CCG. The angle of repose of RVT spray-dried emulsion was influenced by LMP and CCG amount. The spraying efficiency was enhanced due to an increase in pump speed. The optimized formulation containing 2.75% w/w of LMP and 7% w/w of CCG sprayed with pump speed of 10.1 mL/min prepared within design space met satisfy criteria. In the development of RVT-loaded onto porous calcium silicate (PCS) powders, the effects of LMP, ethyl acetate (EA) and RVT to PCS ratio (RVT:PCS) on drug loading capacity, encapsulation efficiency, and drug dissolution were investigated and optimized using Box-Behnken design. EA amount and RVT:PCS had significant effect on the drug loading capacity. The encapsulation efficiency was significantly influenced by EA amount. The RVT:PCS had a significant effect on drug dissolution. The RVT-PCS powders formulation was optimized for achieving high loading capacity, encapsulation efficiency, and rapid drug dissolution. An optimized RVT/PCS powders containing 2.2% w/w LMP, 14% w/w EA and RVT:PCS at 0.15:1 prepared within the design space satisfied all criteria. The RVT products were successfully developed and optimized by implementing the QbD approach.

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LIST OF ABBREVIATION

QbD	Quality-by-Design
DoE	design of experiment
RA	risk assessment
CQAs	critical quality attributes
RVT	resveratrol
LMP	low-methoxyl pectin
CCG	caprylic/capric glyceride
EA	ethyl acetate
PCS	porous calcium silicate
UV	ultraviolet
BCS	biopharmaceutical classification system
O/W	oil-in-water
W/O	water-in-oil
ICH	International council for harmonisation of technical requirements for pharmaceuticals for human use
QTTP	quality target product profile
QRM	quality risk management
RSM	response surface methodology
kDa	kilodalton(s)
Q_5	drug release at 5-min intervals
$T_{50\%}$	time required for 50% drug dissolution
ANOVA	analysis of variance
HPLC	high performance liquid chromatography
% w/w	percent weight by weight
v/v	volume by volume
%	percent
mL	milliliter(s)
μm	micrometer(s)
mm	millimeter(s)

nm	nanometer(s)
kV	kilovolt(s)
min	minute(s)
UV-Vis	ultraviolet-visible
ºC	degree Celsius
%RH	relative humidity in percent
m/s	meter(s) per second
rpm	round(s) per minute
g	gram(s)
RMSE	root mean square error
Cor Total	corrected total sum of squares
>	more than
<	less than
±	plus-minus
º	degree
r^2	coefficient of determination
p-value	probability value
OV	observed value
PV	predicted value
®	registered trademark
%CV	coefficient of variation in percent
Å	angstrom(s)

วิทยานิพนธ์นี้

ได้รับทุนอุดหนุนการทำวิทยานิพนธ์บางส่วน จากเงินงบประมาณแผ่นดินหมวดเงินอุดหนุน

ประจำปีงบประมาณ 2561 (ได้รับการจัดสรรผ่านกองกิจการนักศึกษา)

บัณฑิตวิทยาลัย มหาวิทยาลัยศิลปากร

และ

คณะเภสัชศาสตร์ มหาวิทยาลัยศิลปากร

CHAPTER 1
Introduction

1.1 Statement and significant of the research problem

In recent years, an implementation of high throughput screening increases rate of drug discovery. However, the high throughput screening often results in the discovery of new drug candidates with poor water solubility and high lipophilicity (1). Various strategies have been established to improve drug solubility and enhance bioavailability such as nanoparticles (2), self-emulsifying drug delivery system (3), amorphous solid dispersions (4), drug inclusion in complexes including cyclodextrin (5), particle size reduction (6), crystal engineering technique such as co-crystals, salt formation, and crystalline polymorphs (7–9), and dry emulsion system (10).

The emulsion system is one of the useful strategies for drug delivery system. Emulsions could provide drug solubility enhancement and improve bioavailability (11). Various kinds of drugs with different properties can be encapsulated by using emulsion system. A poorly water-soluble drug can be encapsulated in either internal or interface of the emulsion system depending on desired property. However, emulsion stability such as cracking, creaming, or phase separation is considered as critical influences on patients' safety and efficacy (12). Therefore, a dry emulsion is one of the strategies to solve instability of the emulsion system. The concept of dry emulsions focuses on the transformation of liquid emulsions to dried-solid forms. In the viewpoint of manufacturing products, solid particles enable to be directly filled into capsules, sachets or compressed into tablets. Moreover, solid dosage forms are preferred types of drug delivery systems due to their advantages in dosing and handling.

The dry emulsions can be prepared by using techniques such as spray-drying (13), freeze drying (14), and adsorb onto porous powders (15). Spray-drying is one of the most common techniques used in various industries such as pharmaceutical, chemical, cosmetics, and food (16). Spray-drying process is rapid, continuous, scalable and cost-effective. Another interesting technique is adsorption of emulsions onto porous powders. This method can be prepared using a technique as blending emulsions with the porous powders. Application of spray-drying and porous solid

techniques could be useful strategies to develop dry emulsion formulations. However, pharmaceutical product development requires knowledge gained with an application of scientific-based and risk-based approaches providing a comprehensive understanding of pharmaceutical and manufacturing sciences (17).

Quality-by-Design (QbD) approach also referred to as a science- and risk-based approach is a method introducing a systematic evaluation, understanding, and improving the development of product and process. The QbD approach could be implemented by using tools such as risk assessment (RA) and design of experiment (DoE). RA and DoE are useful in pharmaceutical products development because the pharmaceutical products and their manufacturing processes are complex and typically involved with multiple factors (18,19). Previous studies implemented QbD approach in their researches such as powder blends (20), emulsions (21), nanoparticles (22), and spray-dried powders (23) to understand effects on the product quality attributes. However, factors influence both product qualities and process qualities of the DE prepared by spray-drying and also drug-loaded onto porous powders are still unclear.

In this research, we attempted to develop spray-dried emulsions and emulsion-adsorbed onto porous powders to enhance the dissolution property of poorly water-soluble drug and also light protection of the photosensitive drug model. Resveratrol (RVT) was used as a drug model. QbD approach was implemented in the development and optimization of formulation and process optimization of RVT spray-dried emulsions (Chapter 3) and RVT-loaded onto porous powders (Chapter 4). Influences of formulation and process factors on quality of product and process were discussed based on risk assessment and statistical analysis using DoE. General conclusions of the overall formulation were discussed and some suggestions for the future work were outlined (Chapter 5).

1.2 Objectives

The objectives of this research were:

(1) To develop and characterize RVT spray-dried emulsions and RVT-loaded onto porous powders.

(2) To investigate influences of formulation and process factors on physicochemical characteristics, dissolution properties, process efficiency of RVT products using QbD approach.

(3) To determine the stability of RVT in the spray-dried emulsions and porous powders under ultraviolet (UV) light and on storage.

CHAPTER 2

Literature review

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2.1 Poorly water-soluble drugs

An implementation of a high throughput screening and combinational chemistry in drug discovery introduces new drug candidates with high hydrophobicity and poor water-solubility (1). Poor dissolution property of the drug is a critical aspect and leads to poor bioavailability of the orally administered drug. Orally route administered drug substances have been classified into four classes in the biopharmaceutical classification system (BCS) based on their solubility and permeability by Amidon and co-researchers (24) (Fig. 1).

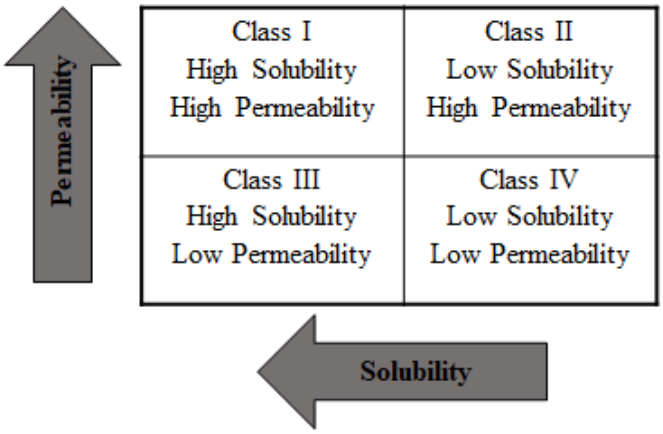

Figure 1. The biopharmaceutical classification system (BCS). Figure was adapted from Figure 1 in reference (25).

The BCS is a guide for formulators to indicate the complication in the development of drug formulation. In case of BCS class I and III drugs, which drugs possess high water solubility. Formulation strategies apply for the drugs in class I and III could not be complicated. On the contrary, the formulation techniques become more challenge for BCS class II and IV drugs. BCS class II drugs possess poor water solubility but high permeability. An improvement of dissolution properties of the BCS class II drugs is often utilized to enhance their bioavailability. Various type of formulations have been developed in drug research and development for improving the dissolution properties of BCS class II drugs such as crystal modification, particle size reduction, amorphization, cyclodextrin complexation, pH modification, and lipid formulations (26).

One of the strategies for achieving high bioavailability of the poorly water-soluble drugs especially highly lipophilic drug is drug dissolving in liquid vehicle or solvent (27). The encapsulated drug in liquid solution is diluted by the surrounding of endogenous fluid once presented in the gastrointestinal tract leading to an increase in drug absorption and bioavailability.

2.2 Emulsions and dry emulsions (DE)

2.2.1 Emulsions

Lipid-based formulation such as emulsion is widely used in various industries such as pharmaceutical, food, cosmetic, nutraceutical industries. In the pharmaceutical industry, emulsion system is used for the improvement of dissolution properties of poorly water-soluble drugs (28). Emulsion system consists of two immiscible liquids, usually oil or solvent and water. One of the liquid phases disperses as small droplets, micron- to nano- size, in a continuous phase. An oil-in-water (O/W) emulsion is the emulsion system, which oil droplets are dispersed in an aqueous phase, while a system consists of dispersed water droplets in an oil phase is a water-in-oil (W/O) emulsion. A presence of poorly water-soluble drugs could be either in the dispersed phase or interface depending on intended purpose of each formulation. In the case of O/W emulsion, the highly lipophilic drug is dissolved by oil or organic solvent, the solubility of the drug can be improved by the oil or organic solvent used in the emulsions.

Oils such as hydrogenated vegetable oils, partial glycerides, ethoxylated glycerides, and esters of various fatty acid and alcohols are widely used as an lipid carrier in drug delivery system. Organic solvents can also be used for preparing emulsions with consideration of health toxicity. Regarding the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q3C (R5) guideline on residual solvents, solvents are classified into 3 classes (29). Class 1 solvents are the solvents which should be avoided due to their risks on human carcinogens properties. Class 2 solvents are limited to be used according to their significant toxicity. Solvents with low toxicity are classified as class 3 solvents. The exposure limit of the class 3 solvents is 50 mg or more per day (29). Therefore,

class 3 solvents, for example ethyl acetate or ethyl ether, are preferred in the pharmaceutical formulation.

Apart from dispersed phase and continuous phase, an emulsifier is the third component required for emulsion system to provide stability of the emulsion. The mechanism on emulsion stabilization depends on types of emulsifier (30). Various kinds of emulsifiers such as polymeric emulsifiers, solid particles, and monomeric surfactants are widely used for emulsion stabilization. In the pharmaceutical industry, polymeric emulsifiers play an important role in the preparation and stabilization of emulsion system. Natural polymers such as proteins, cellulose derivatives, gums, starches, or polysaccharides are commonly used as emulsifiers. Pectin is one of the most useful biopolymers, which possesses emulsifying property (31). Pectin could prevent instability of emulsion by combining steric and electrostatic stabilization mechanism with an increase in the viscosity of continuous phase mechanisms (32). Emulsion stabilization properties of pectin depends on molecular weight, concentration in the formulation, protein content, and acetyl group content (33,34).

Though, emulsion system could increase dissolution properties of poorly water-soluble drugs and protect bioactive compounds. Stability of emulsions is a critical issue to be considered in terms of economic, environmental, and aesthetic factors. Instability of emulsions could occur via one or more several processes such as phase inversion, Ostwald ripening, flocculation, coagulation, creaming, sedimentation, coalescence, and emulsion breakdown (35). Figure 2 illustrates instability processes of emulsion. Emulsion stability could be explained as a thermodynamic basis in Derjaguin, Landau, Verwey, and Overbeek theory (30). Other disadvantages of emulsions are inconveniences in handling and dosing as the same as other liquid formulations. Such instabilities and inconveniences can be solved using an alternative technique that can be referred to as the DE technique.

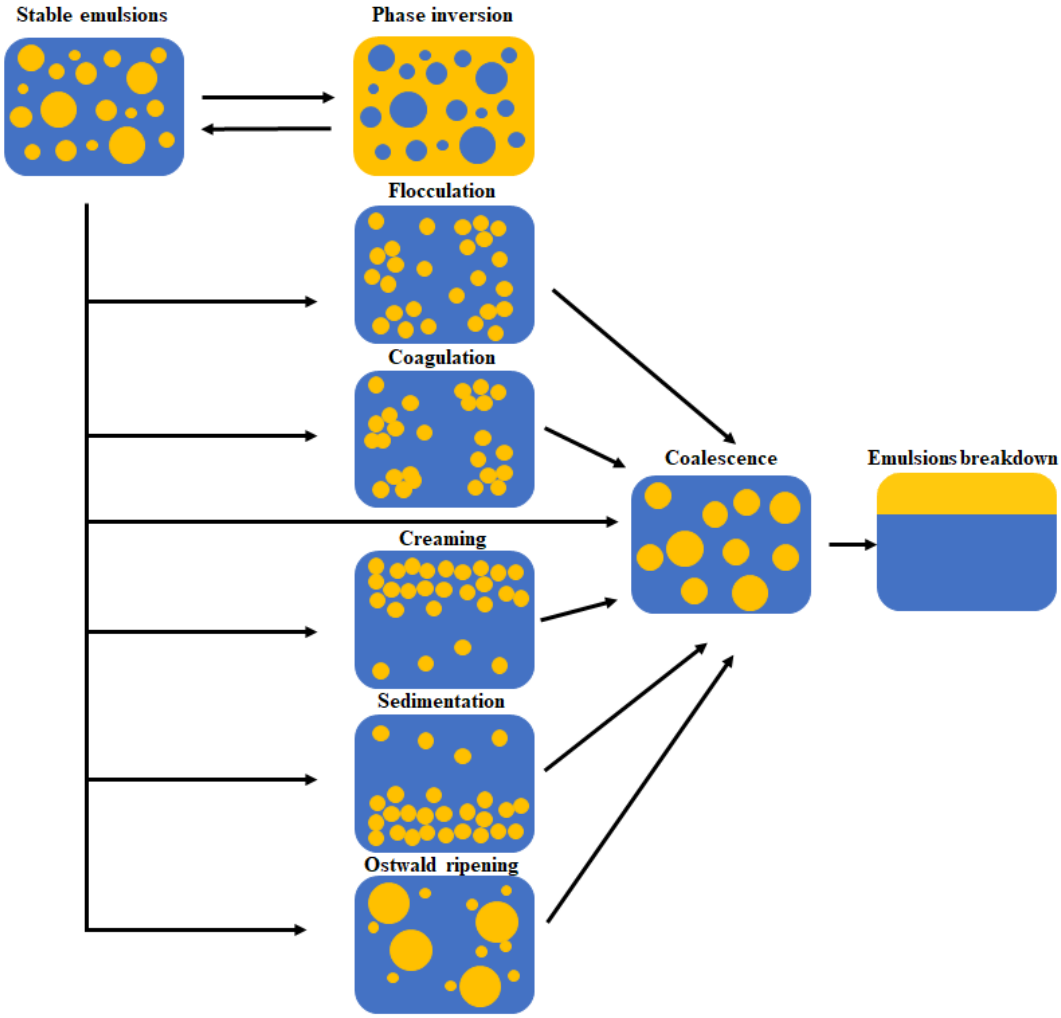

Figure 2. Schematic illustration of instability process of emulsion system. Figure was adapted from Figure 1 in reference (36).

2.2.2 Dry emulsions

The DE technique is one possible way to overcome such disadvantage of conventional emulsions (37,38). An application of DE could encapsulate drug or bioactive compounds inside and provides a number of advantages for example:

1) Providing suitable dissolution characteristic regarding the intended purpose of formulations.

2) Minimizing physical and chemical reactivity of the encapsulated compounds to environmental exposure i.e. moisture, light, and gas.

3) Promoting easier manufacturing, storing, and handling.

4) Masking the taste of encapsulated substances.

Furthermore, solid dosage forms are preferred types of drug delivery system for manufacturers and patients. From the viewpoint of manufacturing, solid powders can be subsequently filled into capsules, sachets or compressed into tablets. Therefore, dry powders provide more production efficiency than liquid formulations. Various types of wall material or encapsulation matrix such as gum acacia, starch, cellulose derivatives, proteins could be used for encapsulation of food and active ingredients (39). An ideal wall material should have the following characteristics:

1) Suitable rheological characteristics to facilitate the manipulation of encapsulation process.

2) Capability to emulsify or disperse the encapsulated substance and stabilize the emulsion.

3) Chemical nonreactive with encapsulated substance during processing and on storage.

4) Maintaining the encapsulated substance during processing and on storage.

5) Providing suitable dissolution regards to intend dissolution purposes.

6) Potential to protect the active ingredient from an exposure to environment i.e. light, heat, and moisture.

7) Ability to be soluble in acceptable solvents i.e. water, ethanol, etc.

8) Safe and economy substance.

2.2.2.1 Wall materials commonly used for encapsulation

2.2.2.1.1 Carbohydrates

Carbohydrates have been used as wall material of DE to encapsulate active substances. Maltodextrins or starches provide good encapsulation due to their low viscosities at a high solid content. An encapsulation using maltodextrins shows good oxidative stability, however, poor emulsion stability and low oil loading capacity are observed due to their poor emulsifying capability (40). Jang *et al.* developed a DE system to encapsulate amlodipine dissolved in Labrafil M 1944 CS using dextrin as a wall material (10). The dissolution and bioavailability of amlodipine was significantly improved by the DE systems. Sugars i.e. sucrose, glucose and starches were reported that they might not suitable for encapsulating sumac flavor prepared by spray-drying according to the caramelization properties, then adhered on the spray dryer surface (41). In addition, starch can cause clogging to the spray dryer nozzles because of its heterogeneous form. In the case of trehalose, which is disaccharide, it can minimize the lipid oxidation of encapsulated substance when it is in glassy state. Trehalose is suggested as a suitable wall material for encapsulation purpose. However, recrystallization of trehalose when stored at high humidity condition could cause rapid oxidation of the encapsulated substance (42). Therefore, the application of trehalose is limited due to its recrystallization properties. In order to modify the kinetics recrystallization of sugars using a combination of other polymers, carbohydrates, and salts may be necessary for improving the stability of encapsulated substances. Carbohydrates with chemical modifications such as modified starches have been used as wall materials. The encapsulation properties of modified starches are more enhanced than that in the common starches according to their improving of surface active properties (43). Pongsamart *et al*. investigated the ability of modified starch as emulsifier and wall material of DE containing fenofibrate. The amorphous fenofibrate encapsulated in the DE showed dissolution rate improvement, however, this system has a limitation for oil loading capacity due to the stability of emulsion (44). Therefore, the DE using modified starch might be suitable for low dose drugs. Pectin is a biopolymer used widely as an emulsifying agent for emulsions. In previous study encapsulated fish oil in sugar beet pectin was prepared using spray-drying technique (45). Low concentration of pectin i.e. 1-2% is

sufficient to prepare a stable emulsion for spray-drying. The DE using sugar beet pectin showed good microencapsulation efficiency and also provided good oxidative stability of the encapsulated oil. High non-encapsulated oil was observed in the formulation containing high oil content. Oil loading capacity may be a limit of DE formulation using sugar beet pectin. However, it was reported that the functional properties of pectin were not affected by the spray-drying process (46).

2.2.2.1.2 Gums

Gums possess film forming and emulsion stabilization properties, which could be applied to encapsulate active agents. Acacia gum (gum Arabic) is one kind of gum that has been used widely in emulsions due to its excellent emulsification properties. The ability to stabilize emulsions of gum arabic is contributed to molecular mass and protein fraction (47). Arabinogalatan-Protein complex in gum arabic is an amphiphilic protein component interfaces with oil droplets. Meanwhile, the carbohydrate fraction in gum arabic is arranged toward the water phase. The emulsion stabilize mechanism is controlled by steric repulsion (48). Previous study reported that gum arabic provided a better encapsulation of cardamom oleoresin than maltodextrins and modified starch (49). In addition, it was found that the microcapsules using gum arabic showed free flowing characteristic. Recently, Bucurescu *et al.* prepared spray dried curcumin DE using gum arabic as a wall material (50). The curcumin DE showed spherical shape with rough surface. The high concentration of gum arabic in the DE could slower the dissolution rate of curcumin DE. Gum arabic could produce stable emulsions with various kind of oils and a wide pH range. Brea gum is an alternative gum, which might be used as a wall material apart from gum arabic. Castel *et al.* investigated the potential of Brea gum to encapsulate corn oil and compared with gum arabic system. The results showed that encapsulation efficiency of DE using Brea gum together with inulin was higher than that used gum arabic as the wall material (51). Another study showed that gum arabic was not efficient to be used as a wall material of monoterpenes i.e. limonene, citral, linalool, β-myrcene, and β-pinene (52). The reduction of monoterpenes in gum arabic was observed under the storage at a controlled temperature. An intrinsic semipermeable property of gum arabic is limited against oxidation. Oxygen can

penetrate through gum arabic capsule and oxidation might occur between oxygen and encapsulated substances. Therefore, a limited in protective capability might be a disadvantage in order to encapsulate drug or bioactive agents. other disadvantages of gum arabic include high cost, limited supply, variations in quality, and impurities have restricted the utilization in pharmaceutical development.

2.2.2.1.3 Proteins

Proteins have various functional properties such as film formation, emulsification, emulsion stabilization, and water solubility. Gelatin is a protein commonly used as a wall material for encapsulation. Shu *et al.* produced lycopene spray-dried microcapsules using gelatin and sucrose as wall materials (53). The result showed that some isomerization of lycopene was observed, which might cause by heat exposure during spray-drying process. However, the storage stability of lycopene was good in the spray-dried microcapsules. In a comparison with maltodextrin, pullulan, glucose, maltose, and mannitol, gelatin showed effective encapsulation capability due to its high emulsifying and emulsion stabilization properties. In addition, gelatin could form a fine dense network during the drying process. Other proteins i.e. soy proteins, whey protein concentrate, skimmed milk powder, and caseinates have been widely studied for their encapsulation capability. The stabilization mechanism of proteins is the change of their structure via unfolding upon emulsification and oil/water interface adsorption. The protein layer is formed around the droplets and also with repulsive forces, which facilitates emulsion stability. Whey proteins have been successfully encapsulated fish oils using a spray-drying technique (54). The results showed that high oil contents of 33% to 90% on dry weight were encapsulated in whey protein capsules using a spray dryer. However, the particles containing high amount of oil showed some leakage. Vega *et al.* studied encapsulation property different between sodium caseinate and micellar casein (skim milk) (55). The result showed that sodium caseinate provided better encapsulation than skim milk because of strong amphiphilic characteristics of sodium caseinate allows better distribution around the surface of encapsulated oil. However, a critical disadvantage of proteins is protein denaturation by heat exposure. The heat could induce proteins aggregation results in turbidity, increased viscosity, emulsion breakdown,

precipitation, and gelation (56). Therefore, DE formulation using spray-drying with high temperature might affect functional properties of proteins and might not be suitable.

2.2.2.2 Preparation methods for DE

DE is usually prepared from O/W emulsions containing a soluble solid carrier in the water phase using various techniques such as spray-drying (10), freeze drying (57), or adsorption onto adsorbent porous powders (58). Each processing technique has the advantages and limitations differently. In order to select a suitable technique, factors such as physicochemical properties of drugs and excipients, manufacturing process, facilities, and environment should be considered. Spray-drying and adsorption onto the porous powders are interesting methods for preparing dry emulsions as they can be applied with emulsions containing oils or solvents in the formulations

2.2.2.2.1 Spray-drying technique

Spray-drying is widely used in pharmaceutical, chemical, cosmetic, and food industries (16). Spray-drying technique is very interesting for both laboratory and industrial scale because it is rapid, continuous, reproducible, and scalable without major modifications. Final drying step is not required as it is single step process. Spray-drying process starts from the transformation of liquid into dry powders by atomization into hot air. The fundamental processing steps of spray-drying include the following (33);

1) Atomization of liquid feed: The liquid feed is pump through an atomizer of a nozzle by a peristaltic pump. The liquid is atomized into small droplets, which usually in micrometer scale.

2) Drying of spray into drying gas: The small droplets are subjected to fast evaporation by the drying gas.

3) Formation of dry particles: Since the evaporation takes place, particles start drying and dried particles can be formed at this stage.

4) Separation and collection of the dry product from the drying gas: The dry particles flow-through pipe by drying gas. Then, they are separated in a

cyclone separator. The spray-dried particles are collected by a collector at the bottom of the cyclone. The drying gas goes out of the cyclone as an exhaust gas. The schematic of spray-drying process is illustrated in Figure 3.

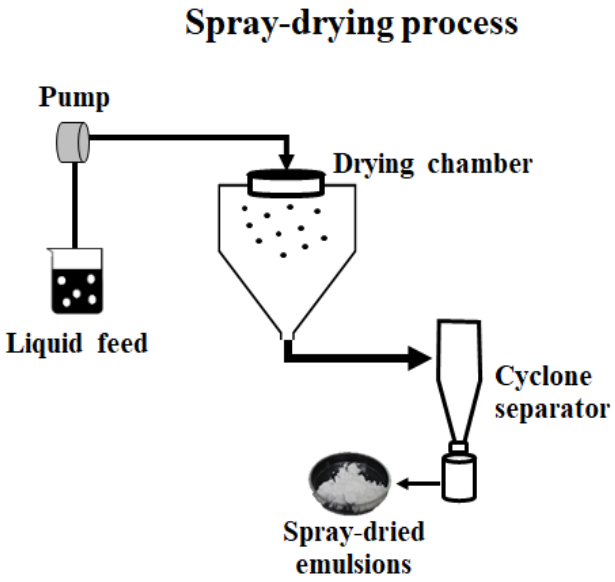

Figure 3. Schematic illustration of spray-drying process.

Two main designs for spray dryer are classified regarding the flow patterns of drying gas and product (16). The first model is co-current flow, which the product and air flow pass through the drying chamber in the same direction. The co-current flow design is widely used and suitable for heat sensitive product as the final particles contact with the coolest air in the drying chamber. Another model is counter-current flow, which the product and air enter at the opposite direction. The counter-current flow model provides effectiveness in drying performance as the final particles are subjected to the hottest air stream. This counter-current flow model is not suitable for heat-sensitive products. The other design is mixed-flow spray dryer, which incorporates both co-current and counter-current design together.

Spray-dried particles can be formed via two steps, which happen extremely fast in the drying chamber. The first period is “constant rate period”, in which the fluid droplet and moisture can migrate easily from inside of the droplet to its surface. The droplet keeps saturated conditions in constant rate makes liquid droplet shrinks into smaller size as water is evaporated. In this stage, the droplet temperature drops to wet bulb temperature of the drying air. Then, the moisture content of the droplet becomes too low to keep saturation conditions. The droplet surface starts to form skin or thin solid crust. After this point, the particle enters the second period calls “falling rate period”. The crust becomes thicker and makes water evaporation decrease and particle temperature increase (59). Figure 4 illustrates schematic simulation of single spray-dried emulsion particle during spray-drying process.

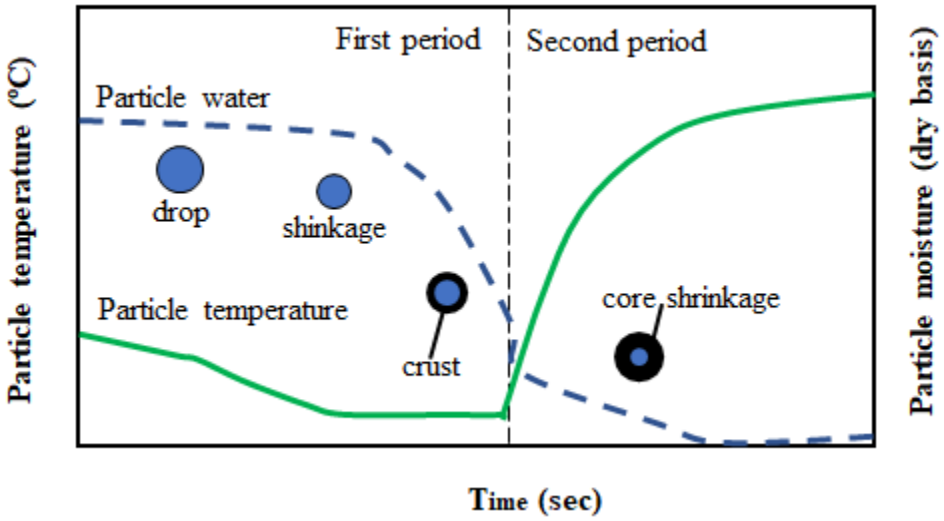

Figure 4. Schematic simulation of spray-drying mechanism of a single spray-dried emulsion particle. Figure was adapted from Figure 8 in reference (60).

The spray-dried emulsions could encapsulate various kind of drugs including bioactive compounds. The encapsulation of lipophilic compounds i.e. fish oil, soya oil applied DE technique has been studied (61,62). Aghbashlo *et al.* demonstrated that the encapsulation efficiency of spray-dried fish oil depended on type of wall material (61). The quality of fish oil was still remained on storage when encapsulated with milk-originated wall materials. Furthermore, the encapsulation could protect sensitive compounds such as antioxidants form environmental exposure including light, oxygen and moisture (63). Oliveira *et al.* developed spray-dried pequi oil and investigated on the degradation of its bioactive compounds (64). The results showed that the DE could provide a protection of the antioxidant properties of bioactive substances i.e. β-carotene, δ-carotene, and lycopene. Though the spray-dried emulsions could be applied in various compounds, there are possible factors such as formulation composition, emulsions droplet size, feed viscosity, feed rate, drying temperature, or else influence the quality of the final product. In addition, the physical properties of the spray-dried emulsions may varied depending on formulation compositions and some process factors (65).

Effect of spray-drying conditions on final spray-dried particles have been studied. The most common spray-drying factors i.e. inlet air temperature, drying air flow rate, and feed rate. Inlet temperature represents the temperature of the drying air, which is the temperature of the air at the first contact moment with the liquid feed. Inlet temperature could directly affect the drying rate of spray-dried particles and final water content. The low evaporation rate occurs at low inlet temperature might cause high moisture residue and poor flow properties of spray-dried particles (66,67). Meanwhile, high inlet temperature might cause too high evaporation and results membrane ruptures, which may lead to a loss of encapsulated substances (68). Kim e*t al.* demonstrated effect of inlet temperature on spray-dried milk powder characteristics (59). The results showed that an increase in the inlet temperature could minimize the surface lipid according to the rapid formation of membrane at high drying temperature. At the low inlet temperature, spray-dried particles have shriveled shape because the membrane remains moist and soft, then the hollow particles can deflate when they cool.

Air flow rate is another spray-drying factor that could have influenced on final spray-dried particles' quality. The air flow rate can represent the volume of drying air supplied to the spray dryer per unit time. It also describes the spray-dried particles drying level and separation level in the cyclone separator unit. At low air flow rate, the spray-dried particles move slowly in the spray-drying system results in the longer drying of the particles. However, the particles' separation efficiency and high yield might be achieved at high air flow rate (69). In addition, Wang *et al* found effect of air flow rate on spray-dried soy sauce characteristics such as particle size and powder cohesiveness (70). At high air flow rate, small feed droplets were formed resulted in small size of spray-dried particles produced. The small size of particles also showed high powder cohesiveness, which indicated poor flow properties of the spray-dried particles.

Feed rate also can have an influence on final product quality because it relates to the drying level of the spray-dried particles. Toneli *et al.* developed a spray-dried inulin and found that a reduction of feed rate and increasing an inlet temperature could increase the mass production rate (71). The increase in feed flow rate can cause the clogging of the feed at atomization nozzle, which resulted in the reduction of product yields. The similar tendency of low yields was also observed in the work of Tonon *et al.* at higher feed rate (72). At high feed rate, part of the feed was not atomized and drop passed straight to the drying chamber, which caused a lower yield. In addition, the moisture content of spray-dried particles could increase when produced at a high feed rate condition (73). The feed contact time could be reduced when the feed rate is increased, results in less efficiency of heat transfer and low water evaporation. In addition, the greater moisture content in the spray-dried particles can cause stiffness and resulted in a formation of inter-particle bridges led particle collapse. The collapse might cause particle's leakage and leading chemical degradation of the encapsulated substances (74). However, the slower heat transfer at the high feed rate provides lower energy supply to the spray-dried particles resulted in lower degradation of heat sensitive compound such as β-carotene (75).

In order to obtain spray-dried emulsions with desirable characteristics, optimization of formulation and spray-drying process factors is essential. Exploring criticality of spray-dried emulsions formulation and process are important to understand how formulation and process factors affect the product qualities and spray-drying process efficiency.

2.2.2.2.2 Adsorption onto porous powders technique

An alternative method of producing dry emulsions is an adsorption of emulsions onto an inert adsorbent porous powder. By using this technique, dry emulsions can be prepared by a simple method of blending porous powders into the liquid emulsion. Calcium silicate, magnesium aluminometasilicate, silicon dioxide, hydrophilic silica, and hydrophobic silica are widely used as the adsorbent carrier for loading various drugs (76–78).

Porous powders are classified according to pore diameter into three classes including macroporous (pore diameter > 50 nm), mesoporous (pore diameter 2 - 50 nm), and microporous (pore diameter < 2 nm) (79). Different preparation conditions leading in different pore diameter of porous powders (80,81). Common preparation method of porous powders is the use of templating agents within particles followed by removing of those templates to crate pores of the particles. The templating agents can be volatile agents e.g. hydrogen peroxide (82), ammonium carbonate (83) and sodium bicarbonate (84) or inorganic compounds e.g. sugars (85) and salts (86). Evaporation is a method for removal of volatile templating agents, while washing with solvent is commonly used to remove inorganic templates. Another preparation method is synthesis of the porous powders. This method is mainly used for silicon-based porous powders. The starting agents for preparation of silicon-based porous powders could be an alkyoxysilane or sodium silicate derivatives. The synthesis involves hydrolysis of the starting agent to form silanol groups (Si-OH) followed by alcohol condensation to form a siloxane bridge (Si-O-Si). The formation of siloxane bridge leading the aggregation of the silicon-based porous powders (79).

The porous powders can be applied in drug delivery systems for various purposes including drug dissolution property improvement, designing drug release pattern, floating in the gastrointestinal tract, and protecting the bioactive compound from physical degradation (87). The dissolution improvement mechanism of poorly water-soluble drugs depends on the formulation of adsorbent such as lipid-based formulation or solvent-based formulation as reported in previous studies (3,88,89). The porous powders used in adsorption of spontaneous emulsifying formulation was found to have significant and positive effects in dissolution and oral bioavailability (3). Furthermore, the reduction in crystal size of drug by adsorption onto porous powders allows enhancement of drug solubility (88,89). Apart from those advantages of porous powders, they also provide excellent flow property, which is appropriate for filling directly into capsules or mixed with other excipients for tablet production (3).

Various porous materials, e.g. porous silicon, porous silica and porous calcium silicate (PCS), have been studied as adsorbent carriers for drug delivery systems. These materials can be applied for oral administration because of their biocompatibility, stability, and nontoxicity. They are generally recognized as safe as the food ingredient in accordance with good manufacturing practices under 21 CFR 182.2227 (90). PCS is one option for enabling improvements in the dissolution properties of drugs possessing poor water solubility (91,92). The high number of pores in PCS provides a large surface area and pore volume, leading to a high adsorption capacity. Preparation method of drug-loaded onto porous powders may not complicate. However, some factors such as specific surface area, porous powders size, porosity, emulsion formulation, adsorption techniques may influence on the quality of the final product (15). Therefore, a deeper understanding of how the formulation factors could influence on the product qualities is important for the formulation using porous powders.

2.3 Resveratrol (RVT)

2.3.1 Physicochemical and pharmacological properties of RVT

Resveratrol (RVT), *trans*-RVT; *trans*-3,5,4'-trihydroxystilbene, is a polyphenolic compound naturally found in grapes, peanuts, and other foods that exhibited applications against multiple disorders, including cancer, diabetes, cardiovascular disease, and aging (93). Figure 5 shows chemical structure of RVT. RVT could increase nitric oxide production and reduce rhythm disturbances, cardiac infarction size, lactate dehydrogenase, and creatine kinase level in plasma (94). Cancer prevention property of RVT was reported by Mikula-Pietrasik *et al* that RVT could inhibit adhesion of all ovarian cancer cell line in their study (95). Antioxidant effect of RVT was studied on the prevention of motor impairment in neuronal injury in rats (96). RVT exists in two isoforms *cis*- and *trans*- configurations. The *trans*-RVT is more biologically active than the *cis*-isomer. Rius *et al* reported that *trans*-RVT could inhibit angiotensin II-induced arteriolar leukocyte adhesion, which causes vascular inflammatory, while *cis*- isoform could not effectively reduce this effect (97). RVT is a weak acid with three acidic dissociation constants ($pK_{a1,2,3}$ = 8.8, 9.8, and 11.4) and a log *P* of 3.17 (98). RVT has poor water solubility (approximately 23 μg/mL) (99). However, RVT possesses high permeability and it is classified as class-II compound in the BCS (24). An improving the solubility of RVT using a suitable approach was important for developing solid drugs that can be orally administered.

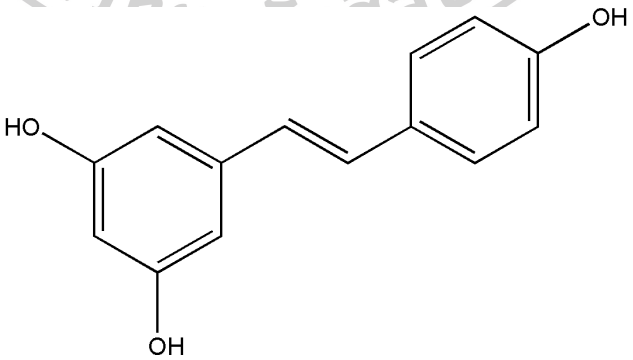

Figure 5. Chemical structure of trans-resveratrol.

2.3.2 Techniques for improvement dissolution properties and chemical stability of RVT

Over a decade, various techniques have been used to overcome the disadvantages of RVT. The main objectives of previous researches were encapsulation of RVT molecules which help improving dissolution, preventing isomerization, and maintaining RVT activities. RVT was encapsulated using various carriers, including cyclodextrin (100), yeast cells (101), and incorporation in nanoparticles (102). These encapsulation techniques could improve the dissolution of RVT without diminishing its activity. However, some limitations such as scale up and reproducibility could be the drawbacks of these techniques in the case of industrial application. The emulsion system is another applicable method to enhance the dissolution properties of encapsulated RVT (99,103). Table 1 summarized reports of different drug delivery systems utilized for RVT.

Table 1. Summary of the different drug delivery systems available for resveratrol (RVT).

Encapsulation technology	**Encapsulation details**	**Evaluations on the encapsulated resveratrol**	**Reference**
Emulsion-based	O/W emulsions.	Chemical stability, drug release, and antioxidant activity.	(103)
	Water-in-oil-in-water emulsions	Drug release and encapsulation efficiency.	(104)
	O/W emulsions using solvent evaporation and freeze drying	Drug solubility, encapsulation efficiency, antioxidant and anti-inflammatory activity.	(102)
Inclusion complexes	Inclusion in yeast cells	Chemical stability and drug release	(101)
Biopolymer particles	Cationic chitosan-and anionic alginate–coated poly(d,l-lactide-co-glycolide) nanoparticles	Controlled drug release and light protection	(105)
	Encapsulate by gliadin-zein using liquid antisolvent precipitation	Chemical stability	(106)
	Solid dispersions	Chemical stability and drug release	(107)
	Anti-solvent solution and spray-drying using pectin cross-linked with zinc		

2.4 Quality-by-Design (QbD) approach

European Medicines Agency (EMA) and the United States Food and Drug Administration (USFDA) currently initiate an implementation of science-based and risk-based regulatory processes or QbD approach (108). The ICH describes a QbD approach for developing pharmaceutical products in guideline Q8 (R2). As defined by ICH Q8, "QbD is a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding, based on sound science and quality risk management" (17). The process of the QbD approach for product development is explained in Figure 6.

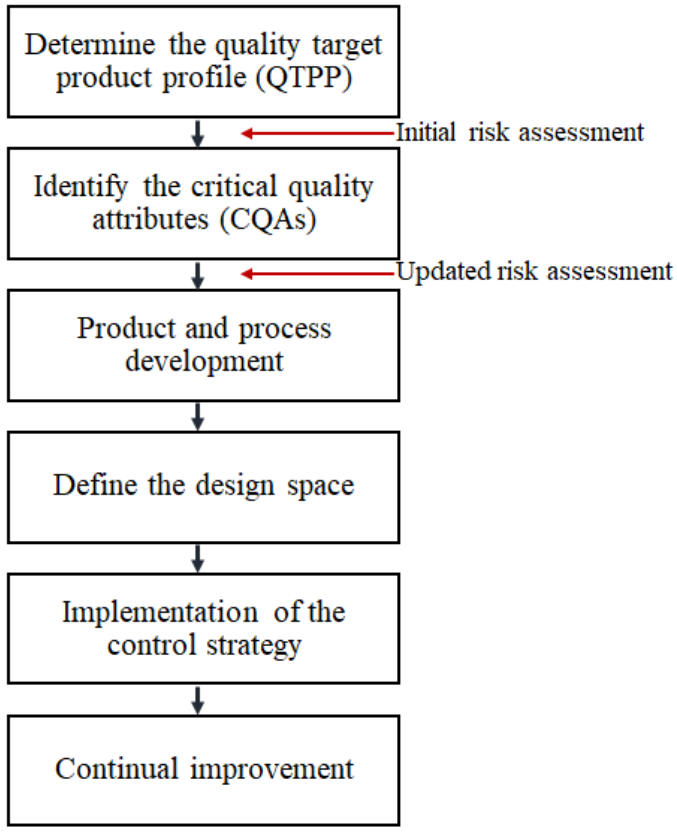

Figure 6. Principle steps of product development using the Quality-by-Design (QbD) approach (17).

Determining quality target product profile (QTTP) is the first step of the QbD approach. QTTP could summarize the quality characteristics of the developed product. The desired quality usually involved with safety and efficacy of the product. The next step is critical quality attributes (CQAs) identification. CQAs are the property of product characteristics that should be within an appropriate range, limit, or distribution to ensure the desired product quality regarding ICH Q8 (17). Therefore, critical factors affected CQAs should be identified to investigate the greatest influence on CQAs. The influences of critical factors are possibly explained by the fundamental knowledge of pharmaceutical operations or experiments. Typically, fundamental knowledge or first principle models are quite limited for applying in pharmaceutical development due to the variety of formulation and process factors. Tools such as risk assessment or DoE are useful for identifying the critical product or process factors affecting CQAs of the product and understanding relationship of variables to product quality attributes. DoE helps creating design space for product and process operating ranges. The relationship between the independent factors and responses could be described using design space (17). The satisfy quality attributes of the product could be met when producing within the design space region. The design space provides sufficient assurance of product on quality, safety, and efficacy. After design space establishment, the control strategy can be defined based on manufacturing data within the defined design space to ensure that the criteria are always achieved. The control strategy should include controls of material, production process, design space monitoring, and ensuring consistency of final product quality. The last step is a continual improvement, which is a GMP requirement. The continual improvement must be applied to all pharmaceutical products. The continual improvement step is an on-going program to gather and conduct data analysis to observe the process consistency and validity.

2.4.1 Risk assessment

Regarding ICH Q8, quality risk management (QRM) should be implemented in a systematic process to facilitate science-based decision making. Risk assessment is one of the process steps in the QRM. ICH Q9 stated that risk assessment is an identification of hazards including analysis and evaluation of risk to those hazards (109). Basically, three fundamental questions help assessing the risk include;

1) What might go wrong?
2) What is the probability it will go wrong?
3) What are the consequences?

All relevant factors influenced product qualities should be identified before conducting the risk assessment. This step should include literature review, data from preliminary experiments or past experiences (110). Risk assessment was observed to be useful while developing pharmaceutical products because the products and their manufacturing processes were complex and generally involved multiple relevant factors (19). Further, risk identification and evaluation were based on current knowledge related to the formulation and manufacturing processes and were assisted by risk assessment tools such as the Ishikawa diagram and risk-ranking systems. Prior to conducting experimental work, the Ishikawa diagram helped to clarify the overall relevant factors that affected the predetermined quality of the targeted pharmaceutical product (111). Risk assessment methods, such as failure mode and effect analysis and risk-ranking systems, can further prioritize the relevant risks and evaluate their criticality. (112).

2.4.2 Design of experiment (DOE)

Although initial risk assessment identified the relevant factors that affected the product quality, it may not adequately elucidate the influences of these factors on the product quality. A DoE provides a quantitative understanding of the product quality attributes and process performance that typically preceded the extensive experiments (113). DoE is useful as it could be used for various purposes such as screening, developing design space, optimization, and maintain a controlled process or evaluate process capability (114). For product development, two objectives for implementation of DoE are screening and optimization purposes. Screening experiments are used to indicate the most important factors influence on responses. Practically, two-level fractional factorial design or Plackett-Burman design is used in the screening experimental design. The number of runs is relatively small due to the design structure. Examples of screening designs are summarized in Table 2. Another purpose of DoE in the drug development is optimization study. The most critical factors obtained from screening experiment are determined in more detail using response surface methodology (RSM). This design helps quantify the relationships between one or more responses and the input factors by creating a response surface map. The outputs of the optimization study include combination of factors to predict the optimum response, region of possible factor combinations to predict acceptable results, or to predict process performance in the experimental region (114). Several designs often used for RSM study such as central composite, Box-Behnken, 3-level factorial, Doehlert matrix, or D-Optimal design (Table 3). DoE is used in pharmaceutical area researches in order to fulfill principles of QbD approach and comprehensive knowledge. Examples of studies used DoE were shown in Table 4.

Table 2. Examples of common design used for screening experiments.

DoE*	Levels	Number of experiments	Estimated effects
Two-level full factorial	2 levels for each factor	2^{k**}	Main effects, interactions (2-way, 3-way and k factors).
Two-level fractional factorial	2 levels for each factor	2^{k-p***}	R****=III (main effects confounded with 2-factors interactions), R=IV (main effects confounded with 3-factors interactions and 2-factors interactions confounded with 2-factors interactions), R=V (main effects confounded with 4-factors interactions and 2-factors interactions confounded with 3-factors interactions).
Plackett-Burman	2 levels for each factor	N (multiple of 4)	Main effects confounded with (fractions) of the 2 and higher order interactions.
Asymmetrical or mixed level factorial	Factors have different numbers of levels	Various number	Main effects and 2-factors interactions.
D-optimum	Factors have different numbers of levels	Any number equal to or greater than the number of independent coefficients in the model	Main effects and all interactions depend on the chosen model.

*Design of Experiments; **number of factors; ***number of independent generators;

****resolution.

Table 3. Examples of common design used for optimization experiments.

DoE	Levels	Number of experiments
Central composite	3 or 5	$2^k+2k+Cp$*
Box-Behnken	3	$2k(k-1)+Cp$
Full factorial design at 3 levels	3	3^k
Doehlert Matrix	Difference for each factor	k^2+k+Cp
D-Optimum	Difference for each model, Irregular experimental domains	Selected subset of all possible combinations

*number of center points.

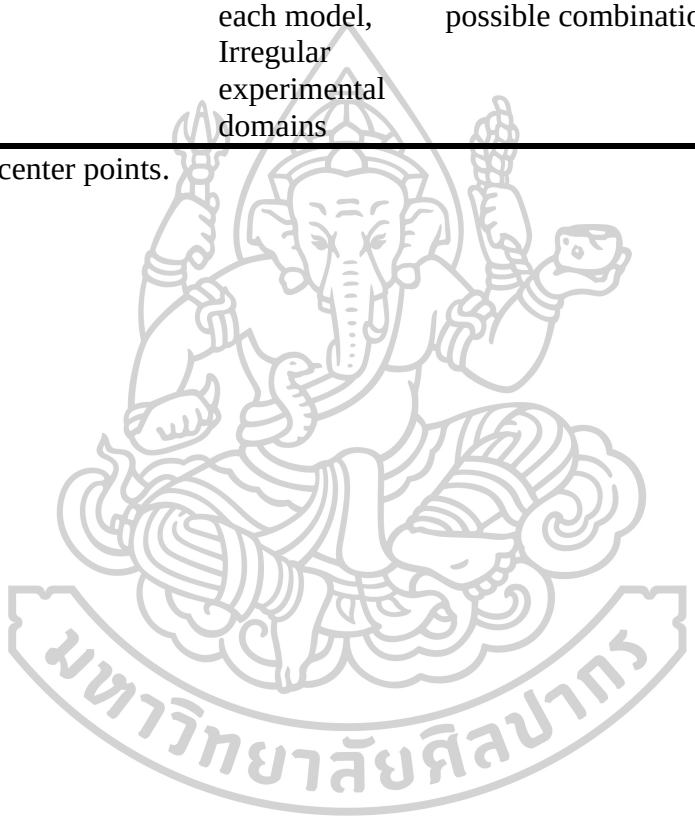

Table 4. Research studies implemented design of experiment (DoE).

Purpose	DoE	Unit operation	Studied factors	Results	Reference
Screening main factors	Plackett-Burman design	Roller-compaction	Disintegrant level, tablet compression force, speed of granulator, roll pressure, active pharmaceutical ingredient, particle size, source of lubricant, binder grade, ratio of roll speed to feed screw speed, blending time, and glidant level	Main factors: -Roll pressure and lubricant source (granule particle size) -Gliadant addition (Carr's index) -Compression force and roll pressure (tablet breaking force) -Binder grade (disintegration time and dissolution)	(115)
Screening main factors	Fractional factorial design	nanosuspension	Drug content, stabilizer type, stabilizer concentration, processing temperature, milling time, and homogenization pressure	All factors except drug content were critical factors to nanoparticle formation and stability. Interaction between homogenization pressure, temperature, and milling time affected particle size	(116)
Optimiza-tion	Cubic central design	Spray-drying	Inlet temperature, aspiration, and flow rate	Higher yields were obtained at higher aspiration and lower flow rates. High Inlet temperature caused aggregation and increased particle size.	(117)
Optimiza-tion	Central composite design	Spray-drying	Inlet temperature, air flow rate, polymer concentration	Higher yields were obtained from higher polymer concentration and inlet temperature. Higher polymer concentration decreased spray rate and drying efficiency	(118)

CHAPTER 3

Development of RVT spray-dried emulsions

3.1 Introduction

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3.3 Results and discussion

3.3.1 Risk assessment and screening experiment of RVT spray-dried emulsions

3.3.1.1 Initial risk assessment of product factors and process factors

3.3.1.2 Identification of the critical factors using Plackett–Burman design

3.3.1.2.1 Particle sizes of RVT spray-dried emulsions

3.3.1.2.2 Loading capacity of RVT spray-dried emulsions

3.3.1.2.3 Moisture content of RVT spray-dried emulsions

3.1 Introduction

RVT exhibited various therapeutic applications, however, the bioavailability of RVT may be limited because of its poor water solubility. Therefore, improving the solubility of RVT using a suitable approach was important for developing solid drugs that can be orally administered. Previous studies implemented various methods in order to improve the solubility of RVT by encapsulation techniques such as solutions, emulsions, liposomes, inclusion into cyclodextrin, and biopolymer-particles (119). Another disadvantage of RVT was chemical degradation; for instance, RVT was isomerized by exposure to ultraviolet light and a pH of greater than 8 (120). Previous studies have shown that RVT encapsulated in nanoemulsions could improve photostability of RVT (103,121).

Encapsulation increased the solubility of RVT and protected it along the gastrointestinal tract. Among the aforementioned encapsulation techniques, the emulsion system was observed to be popular because it efficiently encapsulated and maintained the chemical stability of RVT (122). However, the quality of the encapsulated substances was easily degraded by the instabilities that were associated with the emulsion such as cracking, creaming, or phase separation (35). Such instabilities can be prevented using an alternative technique that can be referred to as the spray-dried emulsion technique.

Although spray-drying improved the manipulability and scalability of industrial processing, multiple factors that were associated with the development of the RVT spray-dried emulsions were observed to affect the quality attributes of the product. Identifying the product and process factors that considerably influenced the product quality, such as the process efficiency, drug dissolution, and drug loading, was considered to be essential. Guideline Q8 (R2) of the ICH described a QbD approach for developing pharmaceutical products. Risk assessment was observed to be useful while developing pharmaceutical products because the products and their manufacturing processes were complex and generally involved multiple relevant factors (19). Further, risk identification and evaluation were based on current knowledge related to the formulation and manufacturing processes and were assisted by risk assessment tools such as the Ishikawa diagram and risk-ranking systems.

Although initial risk assessment identified the relevant factors that affected the quality of a product, it may not adequately elucidate the influences of these factors on the product quality. A DoE provided a quantitative understanding of the product quality attributes and process performance that typically preceded the extensive experiments (113). A common screening DoE was the Plackett–Burman design, which screened multiple factors using a small number of trials (123). A Plackett–Burman design of the spray-dried particles identified the polymer concentration and the spray-drying process as critical factors (124,125). However, it was unclear whether these factors critically affected the final product quality and the spray-drying efficiency of the spray-dried emulsions. Further, the homogenization process factors should be considered in the screening DoE to determine the effect on the quality of the spray-dried emulsions before optimization study.

Furthermore, the physical properties of a spray-dried emulsion may vary depending on the formulation composition and some process factors (65). Redispersibility is one of the most critical properties of the spray-dried emulsions with regard to emulsion size following reconstitution. Small-sized emulsion droplets can enhance intestinal adsorption due to the large interfacial surface area (126). In addition, the dissolution rate of a drug encapsulated in spray-dried emulsions can depend on the emulsion droplet size (127). The ready release of emulsion droplets at appropriate and rapid rates following reconstitution may guarantee bioavailability. A previous study reported that the droplet size of a redispersible spray-dried emulsion was influenced by formulation composition and homogenizing conditions (128). Another key attribute of a spray-dried emulsion is flowability, which is critical with regard to the ease of handling, processing, and final application. The flowability of a spray-dried emulsion could be influenced by particle size and shape, surface composition, and moisture content, as previously reported (129,130). Preparing a redispersible spray-dried emulsion with an optimum formulation and spray-drying conditions could result in particles with small redispersed emulsion sizes, fast dissolve, and suitable flow properties. However, predetermined particle characteristics, i.e., redispersed emulsion sizes, dissolution, and flow properties, are not the only concerning issues in manufacturing. The performance of the spray-drying process is a factor that should be optimized to ensure cost-effectiveness of the

process. From an industrial perspective, optimization considering both product and process characteristics could be efficient, ensuring quality in the entire process.

In this study, the screening DoE investigated the effects of the RVT spray-dried emulsion formulation, homogenization process, and spray-drying process on the quality of the RVT spray-dried emulsion. The factors that were evaluated included the particle size, drug loading capacity, moisture content, drug dissolution, and spraying efficiency of the spray dryer. Further, a RVT spray-dried emulsion was developed using the QbD approach. The most critical factors that affected the quality of the RVT spray-dried emulsion and the spray-drying process were identified using risk-assessment tools (such as the Ishikawa diagram and the relative risk-ranking system). Plackett–Burman design were applied for identifying the most critical factors affecting the quality of the RVT spray-dried emulsions and spray-drying process.

Box-Behnken design was used to optimize and investigate effect of critical factors obtained from the screening DoE study on the quality of the RVT spray-dried emulsions i.e. the redispersibility and flowability of RVT spray-dried emulsions and spray-drying efficiency. RVT spray-dried emulsions and the spray-drying process were optimized to produce a spray-dried emulsion with high quality and spraying efficiency. A design space was developed based on small redispersed emulsion sizes, fast dissolve, and suitable flowability achieved using high a spraying efficiency. The optimized formulation was characterized with high redispersibility, drug dissolution, flowability, and chemical stability.

3.2 Materials and methods

3.2.1 Materials

RVT was purchased from Sami Lab Ltd. (Tumkur, India). Low-methoxyl pectin (LMP; molecular weight = 70 kDa, degree of esterification = 38%) was obtained from Herbstreith & Fox Corporate Group (Neuenbürg, Germany). Caprylic/capric glyceride (CCG; Imwitor® 742) was purchased from Sasol Germany GmbH (Hamburg, Germany). Distilled water was the aqueous phase that was used in all the preparations. All the other chemicals were of pharmaceutical grade and were used as received without performing any further purification.

3.2.2 Risk assessment of product factors and process factors of RVT spray-dried emulsions

The initial risk assessment was conducted using an Ishikawa diagram and a relative risk-ranking system. The Ishikawa diagram contained the factors that affected the quality of the RVT spray-dried emulsions and the spray-drying process. The candidate factors were further determined using a relative risk-ranking system, which assessed the quality attribute of each product to be high, medium, or low risk. The risk levels were assessed by the effect of the risky factor on the CQAs of the RVT spray-dried emulsions. The high-risk factors potentially exhibited extreme consequences, and their background knowledge was considered to range from weak to moderate. The medium-risk factors potentially exerted moderate effects, and their background knowledge was observed to be weak. However, the low-risk factors were unlikely to result in a serious outcome (131). Prior to reassessing using the same risk-ranking system, the criticality of each high-risk factor was determined in a Plackett–Burman design.

3.2.3 Identification of the critical factors of RVT spray-dried emulsions using Plackett–Burman design

Data collection

The high-risk factors that were obtained during the initial risk assessment were assigned as independent factors in the Plackett–Burman design. This design was used to perform $k = N - 1$ analyses, where k denoted the number of factors and N denoted the number of experimental runs. N was a multiple of 4 (e.g., 12, 20, 24). When $k < 11$, the statistical procedures required dummy factors that were not assigned any values (123). The factors were assigned as standard orders (S) according to the Plackett–Burman design pattern (Table 5) and were further randomly arranged as experimental orders by Design-Experts® version 8.0.7.1 software (Stat-Ease Inc., Minneapolis, USA). The CQAs of the RVT spray-dried emulsion product and process were determined as responses (namely, the particle size, drug loading capacity, moisture content, drug dissolution at 5-min intervals (Q_5), process yield, and spraying efficiency).

Data analysis

The relations between independent factors and responses were analyzed using analysis of variance (ANOVA). The results were considered to be significant at the $p < 0.05$ level. The effect of each factor on the responses was computed as a *t*-value and was ranked using a Pareto chart. When the factor effect exceeded the standard *t*-limit in the Pareto chart, the factor exerted a significant effect on the response. Further, factors with effects of lower than the standard *t*-limit were not observed to significantly influence the response. Additionally, the significances of the factors were determined by the Bonferroni limit in the Pareto chart (132). The standard *t*-limit and Bonferroni limit were depicted as a black line and a red line in the Pareto chart, respectively. The positive and negative effects of each factor on each response were depicted using the yellow and blue bars in the Pareto chart, respectively.

Table 5. Standard and experimental order sequence in the Plackett–Burman design.

Standard order (S)	Experimental order	A	B	C	D	E	F	G	H	I	J	K
1	5	1	1	−1	1	1	1	−1	−1	−1	1	−1
2	1	−1	1	1	−1	1	1	1	−1	−1	−1	1
3	12	1	−1	1	1	−1	1	1	1	−1	−1	−1
4	8	−1	1	−1	1	1	−1	1	1	1	−1	−1
5	11	−1	−1	1	−1	1	1	−1	1	1	1	−1
6	4	−1	−1	−1	1	−1	1	1	−1	1	1	1
7	9	1	−1	−1	−1	1	−1	1	1	−1	1	1
8	2	1	1	−1	−1	−1	1	−1	1	1	−1	1
9	6	1	1	1	−1	−1	−1	1	−1	1	1	−1
10	3	−1	1	1	1	−1	−1	−1	1	−1	1	1
11	7	1	−1	1	1	1	−1	−1	−1	1	−1	1
12	10	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1

3.2.4 Optimization of RVT spray-dried emulsions formulation and process using Box-Behnken design

In the optimization study, a Box–Behnken design was used to optimize RVT spray-dried emulsions formulation and process and investigate the effect of selected independent factors on the responses. The levels of each independent factor were selected based on results obtained from the screening experiment. Design-Experts® version 8.0.7.1 (Stat-Ease Inc., Minneapolis, USA) was utilized to design the experiments, which created 15 experiments and two center points. All the experiments from standard order (S1–17) were randomized and performed as per the run order to avoid any bias. Partial model sum of squares and lack-of-fit tests were performed for linear, two-factor interaction and quadratic models for each response. A significant *p*-value from the partial model sum of squares analysis and a non-significant lack-of-fit *p*-value were used as criteria to select the model. The obtained model was simplified using backward elimination to remove unimportant predictors in the equation and improve model adequacy. The model of each response was verified from the results of additional experiments and the deviation from the predicted value was determined based on the root mean square error (RMSE) as follows:

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} (\hat{y}_i - y_i)^2}{n}} \qquad (1)$$

where, $(\hat{y}_i - y_i)$ is the residual or difference between the predicted value $(\hat{y}_i)$ and observed value (y_i) for i = 1 to n, where n is number of the experiment (133).

The design space was developed according to the criteria of each response and the desirability function. An optimized batch was produced from the optimal value within the obtained design space. The residual and percent error of results obtained from the optimized and predicted values were calculated as follows:

$$Residual = \hat{y}_i - y_i, \qquad (2)$$

$$Error\ (\%) = \left|\frac{\hat{y}_i - y_i}{y_i}\right| \times 100 \qquad (3)$$

3.2.5 Preparation of RVT spray-dried emulsions

In the screening study, The RVT O/W emulsions were prepared using a homogenization process with varying LMP amounts (2–3% w/w), CCG amounts (5–10% w/w), homogenization time (5–10 min), and homogenization speed (12,000–18,000 rpm). The oil phase containing RVT (9.5 mg/mL) was CCG. The aqueous phase was prepared by dissolving LMP in distilled water. Both the phases were homogenized in a homogenizer (Polytron, PT 10-35 GT, Kinematica AG, Luzern, Switzerland) immersed in an ice bath to avoid overheating. The RVT emulsions were further spray-dried using a laboratory co-current flow spray dryer (SD-06 model, Labplant UK Ltd., North Yorkshire, UK). The prepared emulsions were pumped into the drying chamber through a peristaltic pump with a 0.5-mm spray nozzle. The emulsions were spray-dried at different inlet temperatures (120–200ºC), pump speeds (4.7–15 mL/min), drying airspeeds (2.7–4.3 m/s), and de-blocking speeds (slow–fast). The range of formulation concentrations and process ranges were obtained from preliminary experiments by considering emulsion stability and viscosity, droplet size, and spray-dried emulsions' physical characteristics, including minimum and maximum operating value of spray dryer factors. The RVT spray-dried emulsions samples were eliminated from the cyclone-separating chamber using a stainless-steel laboratory spoon. To calculate the spraying efficiency and yield, the emulsion and

spray-dried emulsions were weighed before and after spray-drying, respectively, and the time that was spent in the spray-drying process was also recorded.

RVT spray-dried emulsions in the optimization experiments were prepared using the same method as the screening study. The homogenization speed and time were 18,000 rpm and 10 min, respectively. The emulsion was sprayed at an inlet temperature of 160ºC, drying air speed of 4.3 m/s and medium speed of de-blocking with varying a pump speed (4.7 – 11.6 mL/min).

3.2.6 Characterization of RVT spray-dried emulsions

3.2.6.1 Particle morphology and size measurement

The morphologies of the RVT spray-dried emulsions were investigated using a scanning electron microscope (SEM; model LEO 1450 VP model, LEO Electron Microscopy Ltd., Cambridge, UK). The samples were fixed on the SEM stub and coated with gold before conducting the investigation. Images were obtained at an accelerating voltage of 10.0 kV and at a working distance of 11 mm. The SEM images were analyzed using JMicro Vision software (version 1.2.7). The average size of the spray-dried emulsions particles was considered to be Martin's diameter and was presented as mean ± standard deviation (n = 100).

3.2.6.2 Loading capacity of RVT spray-dried emulsions

The RVT content was determined using a high-performance liquid chromatography (HPLC; model Jasco PU-2089 plus a quaternary gradient inert pump, and a Jasco UV-2070 plus multi wavelength UV–vis detector, Jasco, Tokyo, Japan) system. First, the RVT spray-dried emulsions was dissolved in a mixed mobile phase of water, methanol, and glacial acetic acid (440:550:10 v/v/v). The sample solutions were filtered through a 0.45-μm nylon syringe filter and were further applied to a reversed-phase C18 column (Phenomenex; 250 × 4.60 mm; particle size = 5 μm; Phenomenex Inc., California, USA). The injection volume and flow rate were 20 μL and 1 mL/min, respectively. The RVT concentration was determined from the UV absorption that was measured at 306 nm. Further, the RVT retention time was approximately 5 min. Linear calibration curve was performed with RVT standard at

known concentrations (0.01–50 μg/mL). Further, the drug loading capacity in the RVT spray-dried emulsions was calculated as follows:

$$\text{Drug loading capacity (\%)} = \frac{\text{Total amount of RVT (mg)} \times 100}{\text{Amount of RVT spray-dried emulsions (mg)}} \quad (4)$$

3.2.6.3 Moisture content of RVT spray-dried emulsions determination

The moisture content of the RVT spray-dried emulsions was determined by weighing the sample before and after drying in a thermo-controlled infrared dryer (model YTC01L, Sartorius AG, Göttingen, Germany). Therefore, the sample was spread on an aluminum weighing dish that was placed on the balance. The sample was heated and dried until a constant weight was reached. The moisture loss was recorded as a percentage value, and the results were reported as the means of triplicate analyses.

3.2.6.4 In vitro dissolution study of RVT spray-dried emulsions

In the screening study, the drug dissolution from the RVT spray-dried emulsions was measured *in vitro* using a flow-through cell apparatus (USP apparatus 4; Sotax CE7 smart with a CY 7 piston pump, Sotax AG, Aesch, Switzerland). Further, the flow-through cells (diameter = 12 mm) were prepared by placing a 5-mm-diameter ruby bead in a cone and by packing the cone with 1-mm-diameter glass beads. The operation was performed in a closed temperature-controlled (37°C ± 0.1°C) system. In each experiment, two glass microfiber filters with different diameters (2.7 and 0.7 μm) were placed in the cell before the filter head. The sink condition in the dissolution medium was provided by the RVT spray-dried emulsions (equivalent to 0.2 mg of RVT) placed in the flow-through cell. A 100-mL volume of acetate buffer (pH 4.5) was circulated at a flow rate of 8 mL/min. At 5, 10, 15, 30, 60, 90, and 120 min, 3 mL of the samples were replaced with a fresh medium. After filtering, the sample was analyzed using HPLC. The drug dissolutions were reported as the means of triplicate analyses.

In the optimization study, *in vitro* drug dissolution was carried out similarly to the screening study. The sample cell was fitted without the glass microfilters. The dissolution medium was pH 4.5 acetate buffer with 0.05% of tween 80. Samples of 4

mL were withdrawn at 5, 10, 15, 30, and 60 min intervals and were replaced with equivalent fresh media. These samples were filtered using a 0.45-μm nylon filter before analyzing using a T60 UV-Visible spectrophotometer (PG instrument Ltd., Leicestershire, UK) at 307 nm. The time required for 50% drug dissolution ($T_{50\%}$) was determined. RVT concertation in each batch of RVT spray-dried emulsion was assessed using UV spectrophotometry prior to dissolution testing, which was performed in triplicates.

3.2.6.5 Redispersed emulsion size of RVT spray-dried emulsions measurement

RVT spray-dried emulsion was dispersed in acetate buffer pH 4.5 using the same volume of distilled water used in the emulsion before spray-drying. The sample was stirred using an environmental shaker (Model ES-20, Biosan, Riga, Latvia) at 100 rpm at 25ºC ± 5ºC. After 10 min, a sample was withdrawn for droplet size measurements using static light scattering. These samples were diluted in deionized water with gentle stirring. Mean particle size was measured using a laser-scattering particle size distribution analyzer (Partica LA-950, Horiba Instruments Inc., California, USA) under continuous stirring. Measurements were performed in triplicate, and mean diameters of emulsion droplets were reported.

3.2.6.6 Measurement of RVT spray-dried emulsions flowability

Flowability of RVT spray-dried emulsion was determined by measuring the angle of repose using a hollow cylinder of 50-mm diameter and 100-mm height. The cylinder with RVT spray-dried emulsion was placed on a laboratory table and was gradually raised until RVT spray-dried emulsion formed a symmetric conical shape on the table. The angle of repose was calculated as follows:

$$\tan \alpha = \frac{\text{height (cm)}}{\text{0.5 base (cm)}} \qquad (5)$$

3.2.7 Efficiency of the spray-drying process

The efficiency of the spray-drying process was determined from the spraying efficiency and yield, which were calculated as follows:

$$\text{Spraying efficiency (g/min)} = \frac{\text{Total amount of emulsions sprayed (g)}}{\text{Spray-drying time (min)}}, \qquad (6)$$

$$\text{Yield (\%)} = \frac{\text{Total amount of collected RVTspray-dried emulsions (g)}}{\text{Total amount of sprayed solid (g)}} \times 100 \qquad (7)$$

3.2.8 Stability studies of RVT in spray-dried emulsions

3.2.8.1 Photostability of RVT in spray-dried emulsions

RVT spray-dried emulsions sample was spread onto an area of 20 cm^2 of glass plates (approximately 20 mg/cm^2) before exposure to UVA (365 nm) emitted by Spectroline UV lamp model ENF-260/FE (Spectronics Corporation, New York, USA). After irradiated for 30 min and 60 min intervals, samples were withdrawn for RVT content analysis using HPLC as described in section 3.2.6.2. Enhancement of RVT photostability was verified by comparing the percentage of remained *trans*-RVT in RVT spray-dried emulsions with that of intact RVT in CCG.

3.2.8.2 Chemical stability of RVT in spray-dried emulsions on storage

RVT spray-dried emulsions sample was stored in an open vial protected from light at 25ºC and 40ºC/75% relative humidity (RH). RVT concentrations were evaluated by HPLC after 3 months of storage.

3.3 Results and discussion

3.3.1 Risk assessment and screening experiment of RVT spray-dried emulsions

3.3.1.1 Initial risk assessment of product factors and process factors

Risk assessment is the initial step of the product development process. The factors that affect the quality of the RVT spray-dried emulsions, including the raw materials, manufacturing environment, and manufacturing process, were obtained from literature reviews (35,119) and preliminary experiments (data not shown). The raw materials, manufacturing environment, homogenization, and spray-drying process factors were related to the quality of the RVT spray-dried emulsions that were observed in an Ishikawa (fishbone) diagram (Figure 7). These factors were assessed for their criticality with respect to the quality of the RVT spray-dried emulsions and the spray-drying process. In this experiment, the factors (type of raw materials, type of equipment, and production scale) were fixed, and the manufacturing environment was controlled. Therefore, these factors were omitted from further risk assessment. Further, the risk effects were evaluated and prioritized by a risk-ranking system.

Table 6 classifies the initial risks as production and processing factors that influence the RVT spray-dried emulsions, which comprise LMP and CCG. The main manufacturing steps of the emulsions were considered to be homogenization and the spray-drying process. The risk level of each factor was qualitatively evaluated by the potential effect on the CQAs (product and process qualities) of the RVT spray-dried emulsions. The CQAs of the spray-dried powder, such as the particle size, drug loading capacity, moisture content, and drug dissolution, can significantly affect the quality and efficacy of the final spray-dried powder (134). In the preliminary experiments, the physical properties of the spray-dried powder and the spray-drying performance were observed to be influenced by the LMP and CCG amounts, the time and speed of homogenization, the inlet temperature, pump speed, drying air speed, and de-blocking speed (data not shown). The effect of each factor was observed to be dependent on its CQA. For performing risk evaluation, the results of the preliminary experiments were combined with those of the previous studies. The formulation composition affected the oil encapsulation and spraying efficiency (51,75,118). A high solid content enhanced the oil encapsulation and reduced the moisture content of

the spray-dried particles (51,75); however, the increased feed viscosity can reduce the spraying efficiency (118). Further, the homogenization time and speed were observed to affect the emulsion characteristics. In our preliminary experiments, increasing the time and speed of the homogenization reduced the droplet size (data not shown). The effects of the emulsion characteristics (such as droplet size) on the quality of the spray-dried particles, especially on the drug loading efficacy, were reported in previous study (135). In emulsions containing small droplets, the unencapsulated oil on the spray-dried particle surface was observed to be relatively low (135). The spray-drying factors (inlet temperature, pump speed, and drying airspeed) significantly influenced the spray-dried particle characteristics and the spray-drying performance (72,118). The characteristics of the spray-dried emulsions were also indirectly affected by the speed of the elimination of blockage from the nozzle (the de-blocking speed). In particular, blocking the spray dryer nozzle could considerably affect the yield and spraying efficiency. According to the initial risk evaluation, all the eight factors exhibited a high-risk level because of their effects on the quality of the RVT spray-dried emulsions and the spray-drying performance. However, the criticality of the high-risk factors cannot be determined by a qualitative risk assessment and required a quantitative analysis based on the Plackett–Burman design.

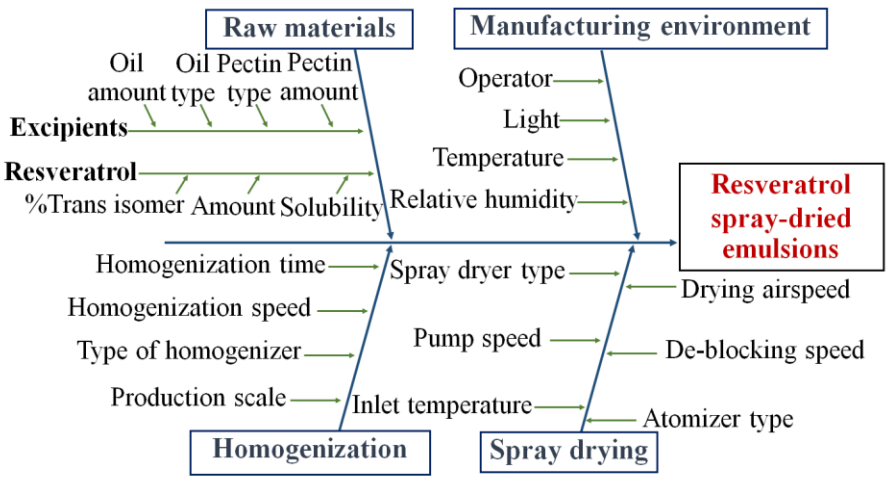

Figure 7. Ishikawa diagram of the product and process factors of the resveratrol (RVT) spray-dried emulsions.

Table 6. Initial risk assessment for the product and process factors of RVT spray-dried emulsion.

Unit operation	Factors	Critical quality attributes					
		Particle size	Drug loading capacity	Moisture content	Drug dissolution	Yield	Spraying efficiency
Formulation compositions	Level of raw materials	High	High	High	High	High	High
Homogenization	Homogenization time	High	High	Low	Medium	Low	Low
	Homogenization speed	High	High	Low	Medium	Low	Low
Spray-drying	Inlet temperature	High	High	High	High	High	High
	Pump speed	High	High	High	High	High	High
	Drying airspeed	High	High	High	High	High	High
	De-blocking speed	Medium	Medium	Medium	Medium	High	High

3.3.1.2 Identification of the critical factors using Plackett–Burman design

The risk-ranking results identified the eight high-risk factors that were to be investigated. The independent factors (*A*–*H*) were assigned to two treatment levels, as summarized in Table 7. Three dummy factors (*J*–*L*) were not assigned any values. The factor levels were determined in preliminary studies that specified the formulation and process-operating ranges. Further, 12 design experiments are summarized in Table 5, whereas the responses are summarized in Table 8. The *p*-values of the response models were shown in Table A1 (Appendix).

Table 7. Factors studied in the Plackett–Burman design and their assigned treatment levels.

Unit operation	Code	Independent factors	Level	
			Low (-1)	High (+1)
Formulation compositions	A	LMP amount (% w/w)	2	3
	B	CCG amount (% w/w)	5	10
Homogenization	C	Homogenization time (min)	5	10
	D	Homogenization speed (rpm)	12,000	18,000
Spray-drying	E	Inlet temperature (°C)	120	200
	F	Pump speed (ml/min)	4.7	15
	G	Drying airspeed (m/s)	2.7	4.3
	H	De-blocking speed	Slow	Fast
Dummy factors	J	Dummy	(−1)	(+1)
	K	Dummy	(−1)	(+1)
	L	Dummy	(−1)	(+1)

Table 8. Summary of responses from the Plackett–Burman design of RVT spray-dried emulsions.

Standard order (S)	Critical quality attributes					
	Particle size	Drug loading capacity	Moisture content	*Q_5	Yield	Spraying efficiency
	(μm)	(%)	(%)	(%)	(%)	(g/min)
1	8.30 ± 0.76	0.69 ± 0.06	9.85 ± 1.07	62.17 ± 6.40	30.29	14.32
2	6.80 ± 0.44	0.78 ± 0.01	10.87 ± 1.30	52.00 ± 3.40	34.51	16.04
3	6.67 ± 0.31	0.53 ± 0.03	11.87 ± 1.71	51.05 ± 4.25	30.28	14.59
4	6.37 ± 0.44	1.11 ± 0.01	10.25 ± 2.61	55.03 ± 3.33	26.29	5.10
5	6.32 ± 0.32	0.61 ± 0.01	9.53 ± 1.15	48.87 ± 4.69	36.16	15.52
6	6.42 ± 0.30	0.61 ± 0.01	9.33 ± 0.80	51.58 ± 6.69	37.56	14.90
7	8.44 ± 0.41	0.72 ± 0.01	10.72 ± 2.29	36.75 ± 2.01	28.38	5.22
8	8.64 ± 0.74	0.67 ± 0.01	9.39 ± 1.17	47.36 ± 11.40	23.24	13.83
9	7.17 ± 0.46	0.68 ± 0.01	7.96 ± 2.17	41.07 ± 2.60	42.56	3.96
10	6.24 ± 0.36	0.73 ± 0.01	9.14 ± 1.25	57.35 ± 5.17	27.78	5.05
11	7.91 ± 0.62	0.63 ± 0.03	12.77 ± 1.24	36.77 ± 0.89	27.45	3.93
12	6.22 ± 0.34	0.63 ± 0.01	11.08 ± 2.52	43.31 ± 9.23	36.54	5.23

**drug dissolution at 5-min intervals.*

3.3.1.2.1 Particle sizes of RVT spray-dried emulsions

The mean diameter of the RVT spray-dried emulsions ranged from 6.22 to 8.64 μm (Table 8). In the Pareto chart (Figure 8a), the *t*-value of the LMP amount exceeded the standard *t*-limit for a particle size, indicating that the size of the RVT spray-dried emulsions most critically responded to the LMP amount. Furthermore, the Pareto bar was yellow, indicating that the LMP amount positively influenced the particle size. Panels (a) and (b) of Figure 9 denote the SEM images of the RVT spray-dried emulsions containing 2% w/w and 3% w/w LMP, respectively. Thus, the particles are smaller in the former as compared to that in the latter formulation. The increased particle size at a high LMP concentration may be explained by the high amount of solid content, which increased the viscosity of the emulsions. As depicted in Figure A1 (Appendix), the RVT spray-dried emulsions containing 3% w/w LMP were significantly more viscous as compared to those containing 2% w/w LMP. In a previous study, a high emulsion viscosity resulted in large-sized droplets in the spraying steps of the spray-drying process (16). Further, the size of the spray-dried particles reportedly depended on the concentration of the solid content in the liquid feed (72,136).

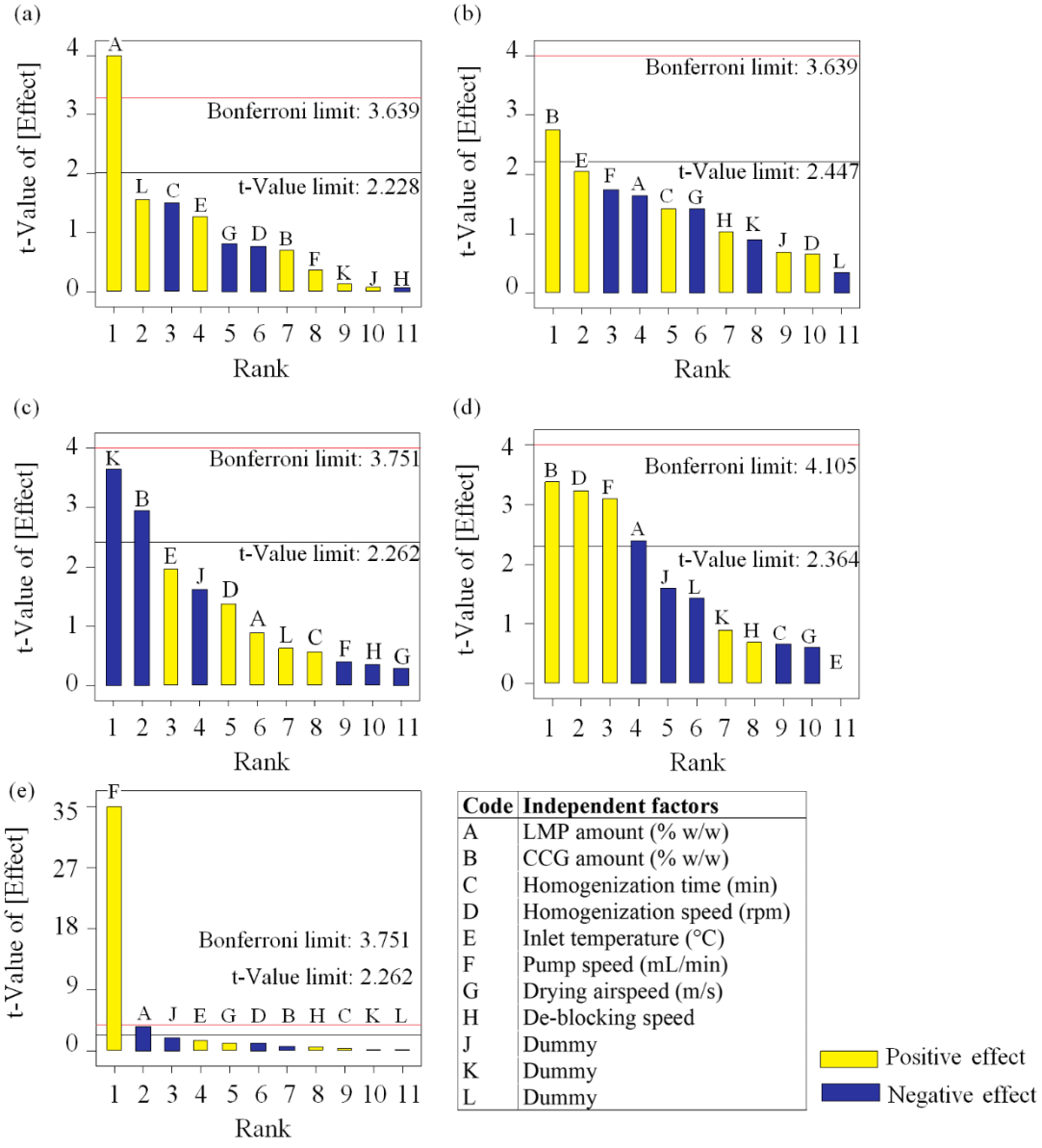

Figure 8. Pareto charts of the response values of the (a) particle size, (b) drug loading capacity, (c) moisture content, (d) drug dissolution at 5-min intervals, and (e) spraying efficiency.

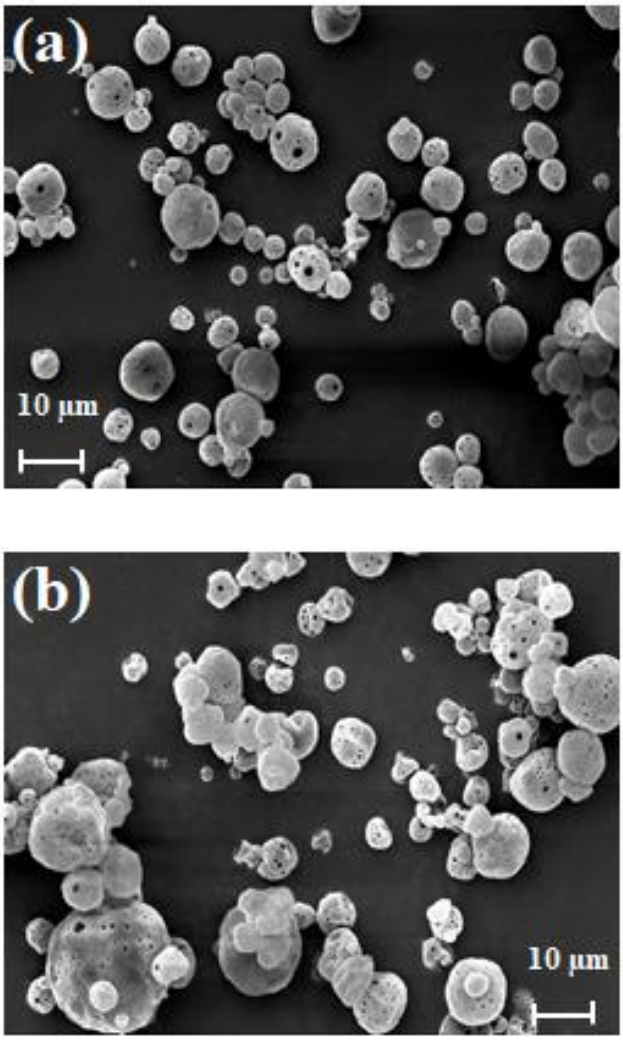

Figure 9. Scanning electron microscope (SEM) images of the RVT spray-dried emulsions containing (a) 2% w/w of low-methoxyl pectin (LMP) and (b) 3% w/w of LMP.

3.3.1.2.2 Loading capacity of RVT spray-dried emulsions

The drug loading capacity of the RVT spray-dried emulsions varied from 0.53% to 1.1% (see Table 8). In the Pareto chart (Figure 8b), the *t*-value of the CCG amount exceeded the standard *t*-limit for the drug loading capacity, indicating that the CCG amount most critically affected the drug loading capacity of the RVT-spray-dried emulsions. The positive association between the CCG amount and the drug loading capacity was observed to be statistically significant. Because RVT was dissolved in CCG (the oil phase of the emulsions), its content depended on the amount of CCG in the formulation. Increasing the CCG amount in the initial emulsion increased the amount of RVT-containing oil in the spray-dried emulsions. Previous studies reported similar tendencies (137,138), with a positive association between the amount of oil in the spray-dried particles and the amount of oil in the emulsion before spray-drying (137).

3.3.1.2.3 Moisture content of RVT spray-dried emulsions

The moisture content of the spray-dried emulsions ranged from 7.96% to 12.77% (Table 8). In the Pareto chart, the CCG amount was more than the standard *t*-limit for moisture content (Figure 8c), indicating that this factor significantly affected the residual moisture in the product. Furthermore, the Pareto line was blue, indicating a negative association between the CCG amount and moisture content of the spray-dried emulsions. The emulsion formulation with a high amount of CCG contained a low amount of water (aqueous phase) in the formulation, which was more effectively evaporated as compared to the large aqueous phase. Therefore, the oil concentration can affect the moisture content of the spray-dried powders (139,140). In the present study, the moisture content was also significantly influenced by the dummy factor, possibly via a two-factor interaction that occurred among the considered factors. Further, the two-factor interactions were confounded with the main factor in the Plackett–Burman design structure (141). Therefore, the significant factors that influenced the moisture content required further investigation.

3.3.1.2.4 *In vitro* dissolution study of RVT spray-dried emulsions

Figure 10 depicts the dissolution profiles of the RVT spray-dried emulsions S1–S12 relative to intact RVT. The dissolution of intact RVT was ~20% after 120 min, indicating the intrinsically poor solubility of RVT in water. The drug dissolutions of all the RVT spray-dried emulsions were higher than that of intact RVT, suggesting that the spray-dried emulsions significantly improved the dissolution property of RVT. The Q_5 values of the 12 experimental samples ranged from 36.75% to 62.17% (Table 8), and the drug dissolution of each sample remained steady between 30 and 120 min. At 30 min, the average drug dissolution was 68% ± 2.55%. The spray-dried emulsions showed rapid dissolve, therefore an information of in the initial stage of dissolution might be necessary (142). Subsequently, the factors affecting Q_5 were statistically determined in the Pareto chart of Figure 8d. The *t*-values of the CCG amount, homogenization speed, pump speed, and LMP amount were more than the standard *t*-limit, confirming their significant influences on Q_5. Further, Q_5 was enhanced by increasing the CCG amount, homogenization speed, and pump speed; however, Q_5 was retarded by increasing the LMP amount. The drug dissolution of dried emulsions was already known to depend on the particle characteristics (2,143). For instance, the drug dissolution was increased by increasing the amount of encapsulated drug in the spray-dried emulsions (143); however, the drug dissolution was retarded by increasing the amount of pectin (an obstructive polymer) (2). The homogenization speed directly impacted the emulsion characteristics even though high-speed homogenization (18,000 rpm) achieved unimodal dispersion and small-sized emulsion droplets (data not shown). Therefore, the homogenization speed directly impacted the emulsion characteristics. Further, the drug dissolution from a dry emulsion was more rapid in emulsions having a small droplet size as compared to that in emulsions with larger droplets (127). Increasing the pump speed can reduce the contact time between the emulsions and the drying air, leading to incomplete drying of the particles (144). Therefore, increasing the pump speed accelerates the drug dissolution. According to a previous study, increasing the pump speed enhances the dissolution by reducing the wettability time of the spray-dried particles (145).

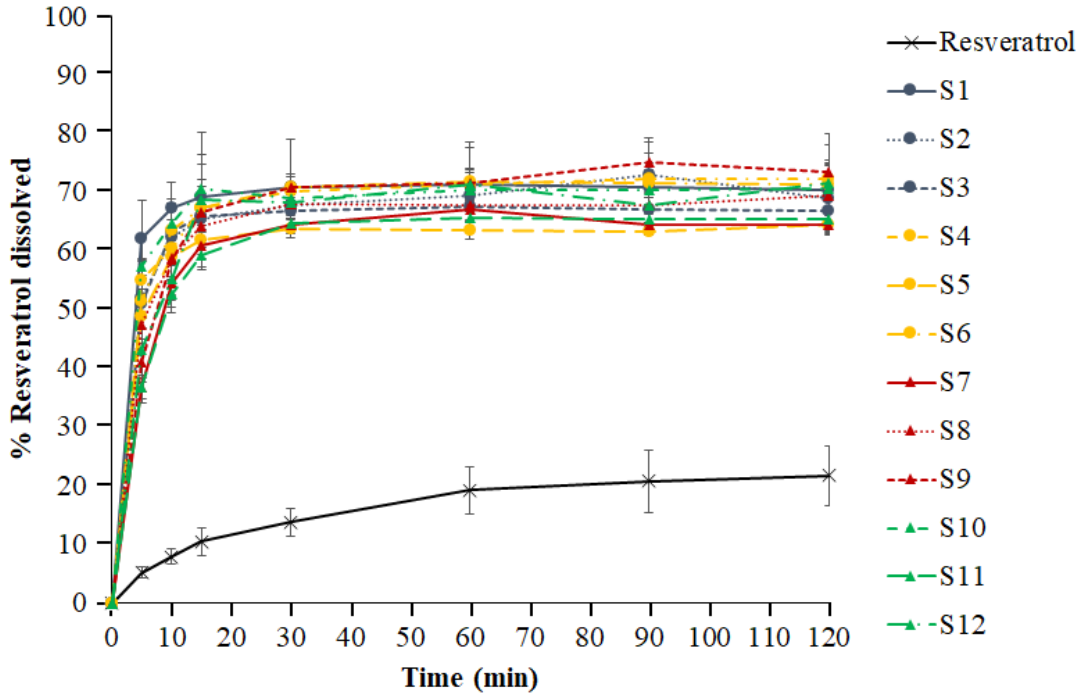

Figure 10. Dissolution profiles of RVT spray-dried emulsions standard order (S) 1–12 and intact.

3.3.1.2.5 Efficiency of the spray-drying process

The performance of the spray-drying process was evaluated in terms of the spraying efficiency and yield. As summarized in Table 8, the spraying efficiency varied from 3.93 to 16.04 g/min. In the Pareto chart (Figure 8e), the *t*-values of the pump speed and LMP amount exceeded the standard *t*-limit for spraying efficiency, indicating that both the factors significantly affected this response variable. Increasing the pump speed also increased the spraying efficiency in the conventional spray-drying process and efficiently reduced the spray-drying process time (118,144). However, increasing the LMP amount reduced the spraying efficiency. This effect may be caused because of the increased polymer amount, which increased the feed viscosity and the time that was required for the spray-drying process (118). The emulsions containing 3% w/w LMP were more viscous as compared to those containing 2% w/w LMP (Figure A1 in Appendix).

The yields of the spray-drying process ranged from 23.24% to 42.56% (average ± standard deviation = 31.75% ± 5.66%; see Table 8). These low yields could be attributed to the small batch size produced by the laboratory spray dryer. Typically, low yields were caused by the adherence of the spray-dried particles to the drying chamber walls, amount of loss being relatively constant (33). Laboratory spray dryers were renowned to produce low yields, especially when the sample volume was small (118,134).

3.3.1.3 Updated risk assessment of product factors and process factors

Based on the experimental results using the Plackett–Burman design, the critical factors influencing each response were identified. The criticalities of the product and process factors were further re-evaluated using the same risk-ranking tool. The updated risks of all the factors are summarized in Table 9. This step refined the risk level based on the product improvement and enhanced understanding of the process. After risk refinement, the highest risk factors were identified as the LMP and CCG amounts. These risk factors affected most of the product-quality factors such as the particle size, drug loading capacity, moisture content, and Q_5. The composition of the spray-dried formulation considerably influenced the final product quality (23). The homogenization speed was a high-risk factor for Q_5. Further, the pump speed

posed the highest risk to Q_5 and the spraying efficiency. Controlling these high-risk factors would improve the robustness of the product and the fabrication process (109).

Table 9. Updated risk assessment of the product and process factors of RVT spray-dried emulsions.

Unit operation	Factors	Critical quality attributes					
		Particle size	Drug loading capacity	Moisture content	Drug dissolution	Yield	Spraying efficiency
Formulation compositions	Level of raw materials	High	High	High	High	Low	High
Homogenization	Homogenization time	Low	Low	Low	Low	Low	Low
	Homogenization speed	Low	Low	Low	High	Low	Low
Spray-drying	Inlet temperature	Low	Low	Low	Low	Low	Low
	Pump speed	Low	Low	Low	High	Low	High
	Drying airspeed	Low	Low	Low	Low	Low	Low
	De-blocking speed	Low	Low	Low	Low	Low	Low

3.3.2 Optimization of RVT spray-dried emulsions formulation and process

According to our screening study, four independent factors including LMP concentration, CCG concentration, homogenization speed, and pump speed showed significant influences on RVT spray-dried emulsions characteristics and spray-drying process (146). However, a monodispersed pattern emulsion droplet size distribution was obtained when using homogenization speed of 18,000 rpm. Therefore, this factor was set as constant in this optimization experiment. The LMP concentration, CCG concentration, and pump speed were selected in this study. The independent factors with the design levels and responses are shown in Table 10.

Seventeen formulations of RVT spray-dried emulsions were prepared using the Box–Behnken design with the following formulation factors: LMP concentration (X_1), CCG concentration (X_2), and pump speed (X_3). The responses of RVT spray-dried emulsions that were analyzed were redispersed emulsion size (Y_1), time required for 50% drug dissolution ($T_{50\%}$; Y_2), angle of repose (Y_3), and spraying efficiency (Y_4). The results of the RVT spray-dried emulsion formulations and responses are shown in Table 11.

Table 10. Independent factors in Box-Behnken design and responses of RVT spray-dried emulsions.

Independent factors	Level		
	Low (-1)	Medium (0)	High (+1)
X_1: Low-methoxyl pectin (LMP) concentration (% w/w)	2	2.5	3
X_2: Caprylic/capric glyceride (CCG) concentration (% w/w)	5	7.5	10
X_3: Pump speed (mL/min)	4.7	8.15	11.6
Responses			
Y_1: Redispersed emulsion size (μm)			
Y_2: Time required for 50% drug dissolution ($T_{50\%}$; min)			
Y_3: Angle of repose (°)			
Y_4: Spraying efficiency (g/min)			

Table 11. Box-Behnken experimental design layout and experimental results of RVT spray-dried emulsions.

Standard order (S)	Run order	Independent factors			Responses			
		LMP amount (% w/w)	CCG amount (% w/w)	Pump speed (mL/min)	Redispersed emulsion size (μm)	*$T_{50\%}$ (min)	Angle of repose (º)	Spraying efficiency (g/min)
1	9	2	5	8.15	5.88 ± 0.40	3.9 ± 0.6	27.4 ± 3.4	8.29
2	6	3	5	8.15	4.51 ± 0.06	6.1 ± 2.3	46.2 ± 4.0	8.40
3	1	2	10	8.15	7.37 ± 0.61	4.6 ± 1.9	19.7 ± 1.8	7.99
4	3	3	10	8.15	6.02 ± 0.56	3.2 ± 0.3	21.1 ± 4.2	8.24
5	15	2	7.5	4.7	6.34 ± 0.26	3.7 ± 0.7	29.6 ± 1.3	3.49
6	10	3	7.5	4.7	5.29 ± 0.35	3.7 ± 0.9	26.3 ± 2.4	4.11
7	8	2	7.5	11.6	6.55 ± 0.16	4.9 ± 1.4	21.6 ±5.8	12.31
8	11	3	7.5	11.6	5.82 ± 0.03	2.8 ± 0.3	36.9 ± 5.6	12.27
9	5	2.5	5	4.7	4.73 ± 0.52	6.6 ± 2.3	35.8 ± 5.4	4.10
10	2	2.5	10	4.7	5.47 ± 0.44	4.1 ± 0.5	23.6 ± 3.4	3.22
11	7	2.5	5	11.6	5.11 ± 0.19	7.3 ± 3.0	25.0 ± 0.8	12.31
12	14	2.5	10	11.6	6.35 ± 0.23	2.8 ± 0.1	32.4 ± 4.3	12.31
13	16	2.5	7.5	8.15	5.91 ± 0.36	4.1 ± 0.4	32.8 ± 3.7	8.42
14	12	2.5	7.5	8.15	6.42 ± 0.15	3.7 ± 0.8	25.5 ±2.3	8.42
15	13	2.5	7.5	8.15	5.76 ± 0.07	3.7 ± 0.1	31.0 ± 0.4	8.45
16	17	2.5	7.5	8.15	6.49 ± 0.17	3.6 ± 1.0	30.0 ± 3.9	8.28
17	4	2.5	7.5	8.15	5.72 ± 0.52	3.9 ± 0.3	31.9 ± 4.4	8.46

* Time required for 50% drug dissolution

3.3.2.1 Redispersed emulsion size of RVT spray-dried emulsions

Redispersed emulsion size is one of the most critical characteristics of RVT spray-dried emulsions in terms of the effectiveness of the emulsion. Small droplet size provides larger interfacial surface area, which in turn enhances intestinal absorption (126). The droplet sizes of emulsions in this study were determined after the reconstitution of RVT spray-dried emulsions and ranged from 4.51 to 7.37 μm (Table 11). ANOVA was performed to determine the significance and effect of each independent factor. The mathematical model describing the relationship between factors and redispersed emulsion size of RVT spray-dried emulsions in terms of coded factors is presented below.

Redispersed emulsion size (μm): $Y_1 = + 6.03 - 0.56X_1 + 0.62X_2 + 0.25X_3 - 0.35X_2^2$ (8)

The mathematical model for predicting redispersed emulsion sizes of RVT spray-dried emulsions was significant at p = 0.0002, whereas the lack of fit was non-significant (Table 12). The model predicting redispersed emulsion size had a high r^2 of 0.8187, representing the reliability of the model. ANOVA results show that the effects of LMP (X_1) and CCG concentrations (X_2) were significant.

Figure 11 illustrates the influence of LMP and CCG concentrations on the redispersed emulsion size. LMP amount negatively influenced the redispersed emulsion size. Conversely, CCG concertation positively influenced the redispersed emulsion size. Emulsion size was influenced by the concentration of each formulation composition as previously reported (147). Pectin (e.g., LMP) is a polymeric surfactant that facilitates emulsion stability by covering the surfaces of oil droplets. Under low LMP concentrations in the redispersed emulsion, LMP concertation was inadequate to stabilize the newly formed droplets from the reconstituted RVT spray-dried emulsions; as a result, coalescing could cause an increase in the redispersed emulsion size. An increase in LMP concentration may stabilize the newly formed droplets from the reconstitution of RVT spray-dried emulsions. Similarly, the effect of CCG concentration on the redispersed emulsion size can be explained by the stability of the reconstituted emulsion. The high amount of oil droplets required adequate emulsifier concentrations to cover the surfaces of the oil droplets to decrease coalescence. Therefore, an increase and decrease in LMP and CCG concentrations, respectively, may result in small redispersed emulsion size.

Table 12. ANOVA results for model to predict redispersed emulsion size (Y_1), time required for 50% drug dissolution ($T_{50\%}$; Y_2), angle of repose (Y_3), and spraying efficiency (Y_4) of RVT spray-dried emulsions.

Source	Sum of Squares	Degree of freedom	Mean square	F value	*p*-value, Prob>F
For Y_1 (μm)					
Model	6.66	4	1.67	13.55	0.0002
X_1	2.53	1	2.53	20.59	0.0007
X_2	3.1	1	3.1	25.22	0.0003
X_3	0.5	1	0.5	4.07	0.0666
X_2^2	0.53	1	0.53	4.3	0.0602
Residual	1.47	12	0.12		
Lack of Fit	0.93	8	0.12	0.86	0.6054
Pure Error	0.54	4	0.14		
*Cor Total	8.13	16			
Other statistics: r^2 = 0.8187, adjusted r^2 = 0.7583, RMSE = 0.35					
For Y_2 (min)					
Model	17.85	4	4.46	6.02	0.0099
X_1	0.21	1	0.21	0.28	0.6051
X_2	10.58	1	10.58	14.27	0.0036
$X_1 X_2$	3.24	1	3.24	4.37	0.0631
X_2^2	3.81	1	3.81	5.15	0.0467
Residual	7.41	10	0.74		
Lack of Fit	7.29	8	0.91	14.38	0.0666
Pure Error	0.13	2	0.063		
Cor Total	25.26	14			
Other statistics: r^2 = 0.7065, adjusted r^2 = 0.5891, RMSE = 0.86					
For Y_3 (º)					
Model	563.72	6	93.95	6.24	0.0079
X_1	129.28	1	129.28	8.59	0.0168
X_2	177	1	177	11.75	0.0075
X_3	0.038	1	0.038	0.0025	0.9611
X_1X_2	75.17	1	75.17	4.99	0.0523
X_1X_3	86.68	1	86.68	5.76	0.04
X_2X_3	95.55	1	95.55	6.35	0.0328
Residual	135.53	9	15.06		
Lack of Fit	110.97	6	18.49	2.26	0.2688
Pure Error	24.56	3	8.19		
Cor Total	699.24	15			
Other statistics: r^2 = 0.8062, adjusted r^2 = 0.6770, RMSE = 3.88					
For Y_4 (g/min)					
Model	147.11	2	73.56	1050.41	< 0.0001
X_2	0.22	1	0.22	3.21	0.0986
X_3	146.89	1	146.89	2097.62	< 0.0001
Residual	0.84	12	0.07		
Lack of Fit	0.82	10	0.082	8.01	0.116
Pure Error	0.02	2	0.01		
*Cor Total	147.95	14			
Other statistics: r^2 = 0.9943, adjusted r^2 = 0.9934, RMSE = 0.26					

Note: X_1=LMP amount; X_2=CCG amount; X_3=pump speed; $p < 0.05$ is considered as significant; *corrected total sum of square

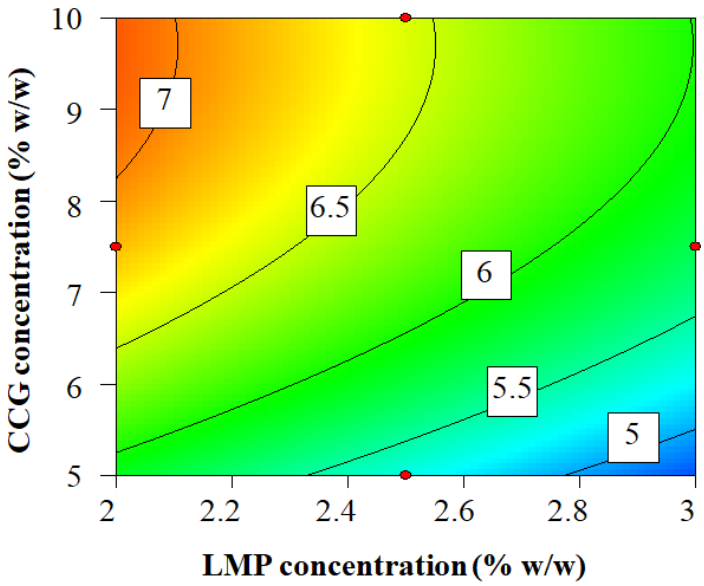

Figure 11. Contour plot showing effect of LMP amount and caprylic/capric glyceride (CCG) amount on the redispersed emulsion size (μm) of RVT spray-dried emulsions.

3.2.2.2 In vitro drug dissolution study of RVT spray-dried emulsions

Drug dissolution is a critical step in drug absorption of poorly water-soluble drugs into the medium and is based on the dosage forms. Enhancing dissolution results in better adsorption and higher bioavailability. Spray-dried emulsion is a reliable system for improving drug dissolution an bioavailability (143). From the Plackett-Burman design experiment, the results showed that the solubility of RVT was significantly improved by using spray-dried emulsion. Furthermore, the RVT spray-dried emulsions showed fast dissolve as seen in the Plackett-Burman design result. Therefore, the dissolution rate, especially in the initial stage, of RVT spray-dried emulsion was assessed in this part. The time required for 50% drug dissolution ($T_{50\%}$) was determined to show the dissolution rate of RVT spray-dried emulsions. $T_{50\%}$ of the RVT spray-dried emulsions ranged from 2.8 to 7.3 min (Table 11). The mathematical model describing the relationship between factors and $T_{50\%}$ in terms of coded factors is presented below.

$$T_{50\%} \text{ (min)}: Y_2 = + 3.81 - 0.16X_1 - 1.15X_2 - 0.9\ X_1X_2 + 1.01X_2^2 \quad (9)$$

The mathematical model for predicting $T_{50\%}$ was significant at p = 0.0099, whereas the lack of fit was non-significant (Table 12). The model predicting $T_{50\%}$ had a high r^2 of 0.7065, representing the reliability of the model. ANOVA results show that the effects of CCG concentrations (X_2) and its quadratic term (X_2^2) were significant.

Figure 12 illustrates the influence of CCG concentrations on the $T_{50\%}$. CCG amount negatively influenced the $T_{50\%}$. At the high CCG concentration, the $T_{50\%}$ of RVT spray-dried emulsions was shorter than that in RVT spray-dried emulsions contained low CCG concentration. The result was in accordance with the Plackett-Burman experiment (section 3.3.1.2.4) that CCG was the most significant factor affected the drug dissolution. An increase in CCG amount resulted in an enhancement of Q_5 of the RVT spray-dried emulsions. Fast drug dissolution of the spray-dried emulsion could be due to the release of oil deposited at the surface of the spray-dried emulsion. A presence of oil at the surface of the spray-dried emulsion was caused by the large size of the initial emulsion droplet in the formulation contained high amount of oil (148). Therefore, an increase CCG concentration may result in faster $T_{50\%}$.

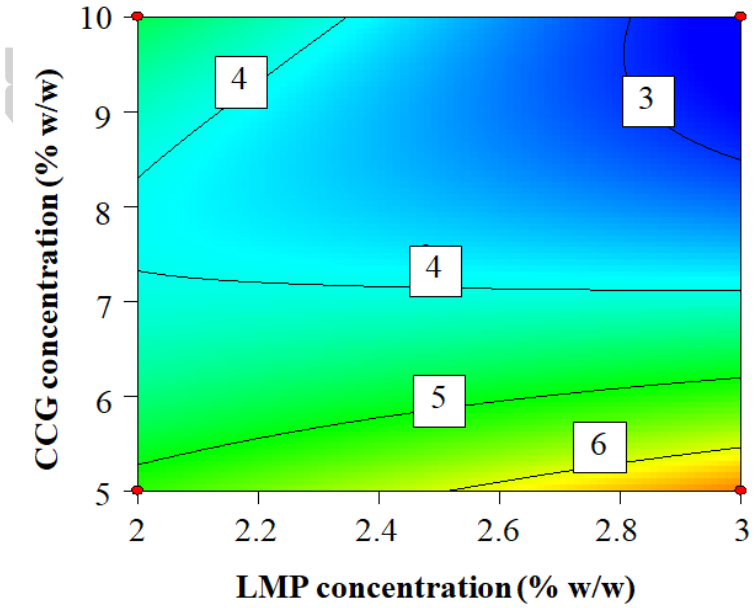

Figure 12. Contour plot showing effect of LMP amount and CCG amount on the time required for 50% drug dissolution ($T_{50\%}$; min) of RVT spray-dried emulsions.

3.3.2.3 Flowability of RVT spray-dried emulsions

The angle of repose was measured to determine flowability of RVT spray-dried emulsion. The flowability of powders is one of the critical criteria for manufacturing satisfactory drugs. The angle of repose of the RVT spray-dried emulsion formulations varied from 19.7º to 46.2º as summarized in Table 11. According to the criteria set by the United States Pharmacopeia angles of repose < 25º, 25º–35º, 36º–40º, 41º–45º, and 46º–55º represent excellent, good, fair, passable, and poor flow properties, respectively (149). Based on our results, the flow property of RVT spray-dried emulsion formulations varied from excellent to poor based on value of the angle of repose. The mathematical model, in terms of coded factors, describing the relationship between factors and angle of repose of RVT spray-dried emulsion is shown below.

$$\text{Angle of repose (º): } Y_3 = +28.99 + 4.02X_1 - 4.7X_2 + 0.069X_3 - 4.34X_1X_2 + 4.65X_1X_3 + 4.86X_2X_3 \quad (10)$$

The mathematical model for predicting the angle of repose in RVT spray-dried emulsion formulations was significant with a p value of 0.0079, whereas the lack of fit was non-significant (Table 12). The mathematical model predicting the angle of repose showed a high r^2 of 0.8062, representing the reliability of the model. ANOVA analysis results showed that the effects of LMP concentration (X_1), CCG concentration (X_2), an interaction between LMP concentration and pump speed (X_1X_3), and interaction between CCG concentration and pump speed (X_2X_3) were significant.

A contour plot (Figure 13a) indicated that the effect of LMP concentration was observed at high pump speed. At lower LMP amounts, RVT spray-dried emulsion had an angle of repose of <25º, indicating excellent flow based on the criteria set by USP. The flowability of RVT spray-dried emulsion reduced with an increase in LMP concentration. At higher LMP concentrations, RVT spray-dried emulsion exhibited an angle of repose of >35º, indicating fair flow properties. The high LMP amount in the formulation could retard the flow properties of RVT spray-dried emulsion. SEM images of RVT spray-dried emulsion produced using the same

CCG amount at high pump speed containing LMP amount of 2% w/w (Figure 14a) and 3% w/w (Figure 14b) exhibited varied particle morphology. More spherical shapes of RVT spray-dried emulsions were obtained from formulations containing less LMP concentrations. Conversely, shriveled shapes were observed in formulations with high LMP concentrations (Figure 14b). A previous study reported that shriveled spray-dried particles can be obtained from more viscous liquids due to slow film formation around droplets (150). From our screening experiments, emulsions containing 3% w/w of LMP showed relatively higher viscosity than those containing 2% w/w of LMP (Figure A1 in Appendix). Similarly, film formation in RVT spray-dried emulsion with higher LMP concentrations can be slower than that in RVT spray-dried emulsion with relatively lower LMP concentrations, and result in the formation of shriveled particles. Particle morphology could influence the flow properties of spray-dried particles. Particles with spherical morphology often exhibit good flow properties than particles with the deviation from the spherical shape. Consequently, RVT spray-dried emulsion containing lower LMP concentration exhibited good flow properties than that containing higher LMP concentration.

The effects of interactions between pump speed and CCG concentration can be observed from the contour plot (Figure 13b). Excellent flow in RVT spray-dried emulsion can be observed when with CCG concentration and low pump speed. Conversely, RVT spray-dried emulsion with lower CCG concentration produced at the low pump speeds exhibited fair flow properties. The flow properties of the powders could be influenced by moisture content in the spray-dried emulsions. From our screening of DoE, we observed that CCG concentration influences moisture content in RVT spray-dried emulsion. In the present study, moisture content in RVT spray-dried emulsion with 5% w/w of CCG and 10% w/w of CCG produced using 2.5% w/w of LMP at a pump speed of 4.7 mL/min was 7.98% ± 0.17% and 6.18% ± 1.02%, respectively. An increase in CCG concentration can reduce moisture content in RVT spray-dried emulsion. Effective evaporation can be obtained in the feed formulation with relatively low water content. The moisture content of spray-dried powders can be influenced by oil concentrations in emulsions (140). Excellent flow properties were observed in formulations containing higher CCG concentrations, which implied low moisture contents. The flowability of the spray-dried powders

could be enhanced by decreasing the moisture content, leading to a decrease in liquid bridges and capillary forces between the particle surfaces (129).

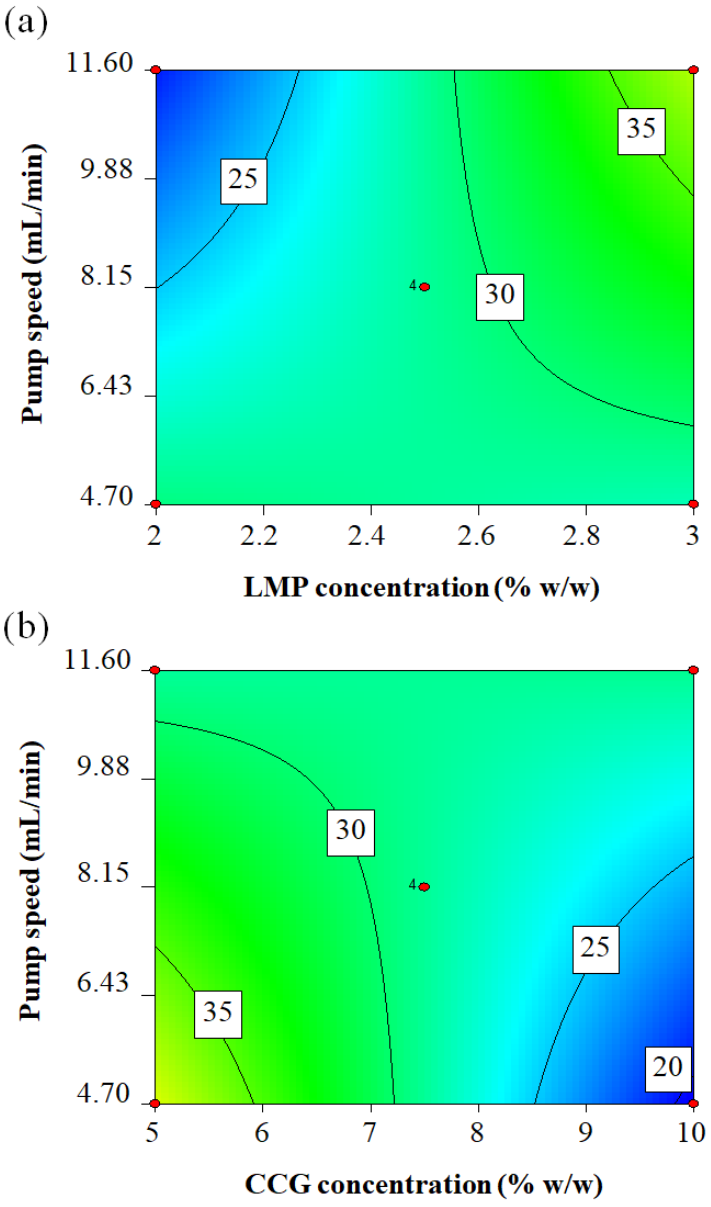

Figure 13. Contour plot showing effect of (a) LMP amount and pump speed and (b) CCG amount and pump speed on the angle of repose (°) of RVT spray-dried emulsions.

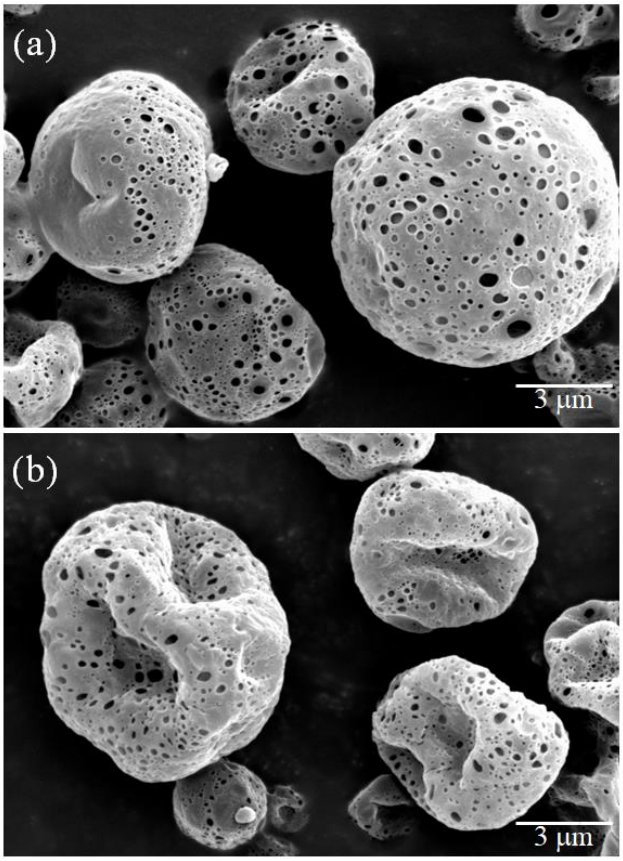

Figure 14. SEM images of the RVT spray-dried emulsions comprising LMP amount of (a) 2 % w/w and (b) 3 % w/w with CCG amount of 7.5 % w/w produced at pump speed of 11.6 mL/min.

3.3.2.4 Efficiency of the spray-drying process

Spraying efficiency is a critical process characteristic from manufacturing and economic perspectives. An increase in spaying efficiency may reduce the time spent in the spraying operation per production batch. The spraying efficiency revealed the performance of spray-drying process. As summarized in Table 11, the spraying efficiency varied from 3.49 to 12.31 g/min. The mathematical model in terms of coded factors describing the relationship between factors and spraying efficiency is presented below.

Spraying efficiency (g/min): $Y_4 = + 8.15 - 0.17X_2 + 4.29X_3$ (10)

The mathematical model for predicting spraying efficiency was significant at $p < 0.0001$, and the lack of fit was non-significant (Table 12). The model predicting

spraying efficiency had a high r^2 of 0.9943, representing the reliability of the model. ANOVA showed that effect of pump speed (X_3) was significant.

The contour plot (Figure 15) indicated that the spraying efficiency considerably depended on the pump speed of the spray dryer. An increase in the pump speed resulted in higher amounts of liquid feed to be sprayed with a shorter time. Therefore, production rates of RVT spray-dried emulsion can be enhanced by increasing pump speeds of the spray dryer. From a manufacturing point of view, a reduction in process time could be efficient and cost effective.

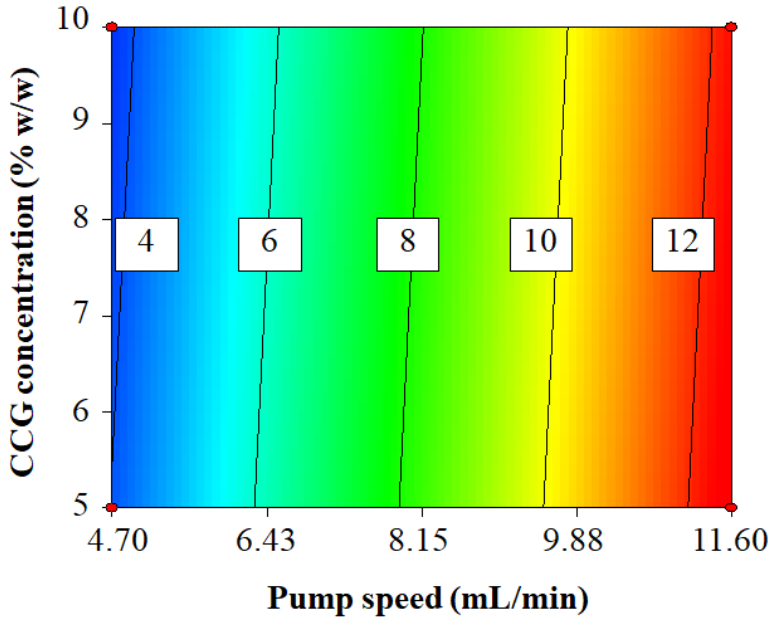

Figure 15. Contour plot showing effect of pump speed and CCG amount on the spraying efficiency (g/min) of RVT spray-dried emulsions.

3.3.2.5 Verification of mathematical model from RVT spray-dried emulsions responses

In order to verify the adequacy of the obtained mathematical models, three additional batches of RVT spray-dried emulsions were produced and their product and process characteristics determined. Table 13 presents the levels of each factor, observed values, and predicted values (PV) of the verification (V) batches. The results obtained from all the verification batches were within 95% of the prediction intervals of the PV (data not shown). Errors in the model prediction were determined using RMSE. All models had low RMSE values in the range of 0.25-2.70, which indicated the suitable predictability of the model. Therefore, the models could be used for prediction purposes.

Table 13. Verification experimental results for model obtained from Box-Behnken design of RVT spray-dried emulsions.

		Verification run (V)				
		V1	V2	V3	V4	
Independent factors	LMP amount (% w/w)	2.8	2.3	2.6	2.2	
	CCG amount (% w/w)	8	7	9	6	
	Pump speed (mL/min)	9.5	8.8	6.8	5.4	
Responses						RMSE
Redispersed size (μm)	*OV	6.33±0.16	6.58±0.13	6.21±0.36	5.87±0.10	
	**PV	5.90	6.17	6.07	5.67	0.32
$T_{50\%}$ (min)	OV	3.6±0.9	4.4±1.3	3.5±0.3	4.0±0.6	
	PV	3.4	4.1	3.3	4.6	0.36
Angle of repose (º)	OV	30.1±2.0	31.6±3.3	22.0±1.0	31.1±2.8	
	PV	31.5	27.5	24.9	32.4	2.70
Spraying efficiency (g/min)	OV	10.08	9.24	6.68	4.94	
	PV	9.79	8.99	6.37	4.83	0.25

*Observed value; **Predicted value.

3.3.2.6 Optimization of RVT spray-dried emulsions formulation and process

The optimal operation ranges of RVT spray-dried emulsion formulation and spray-drying process were determined from valid mathematical models. RVT spray-dried emulsion produced within the optimal ranges exhibited product and process qualities with desired characteristics. The criteria for optimization of RVT spray-dried emulsion characteristics and spray-drying process included minimizing droplet sizes of redispersed emulsions (not more than 6 μm), $T_{50\%}$ (not more than 5 min), and angles of repose (excellent to good flow) and maximize the spraying efficiency (not less than 8 g/min). In the present study, we aimed to produce RVT spray-dried emulsion with good to excellent flowability. The droplet sizes of redispersed emulsions should be small to enhance intestinal absorption and increase RVT bioavailability. In addition, fast spray-drying process is also preferred for its cost-effectiveness. The design space was established according to the criteria meeting all the desired requirements (Figure 16). The optimized formulation can be produced using a high pump speed of 10.1 mL/min. LMP and CCG concentrations could be used in the range presented in the yellow area of the design space. An optimized formulation was produced using 2.75% w/w of LMP and 7% w/w of CCG. Optimized RVT spray-dried emulsion results are shown in Table 14. The optimized formulation exhibited a small redispersed emulsion droplet size (5.49 ± 0.23 μm) and rapid drug dissolution (Figure 17), which $T_{50\%}$ was 3.7 ± 0.2 min. The angle of repose of the formulation was 31.0° ± 2.5°, showing good flow properties of the optimized RVT spray-dried emulsion. The spraying efficiency of the optimized RVT spray-dried emulsion was high (10.63 g/min), indicating that a relatively high amount of the emulsions was sprayed within a relatively short process time. The percentage error from predicted value each response of the optimized formulation was less than 10%, confirming the predictability of the models.

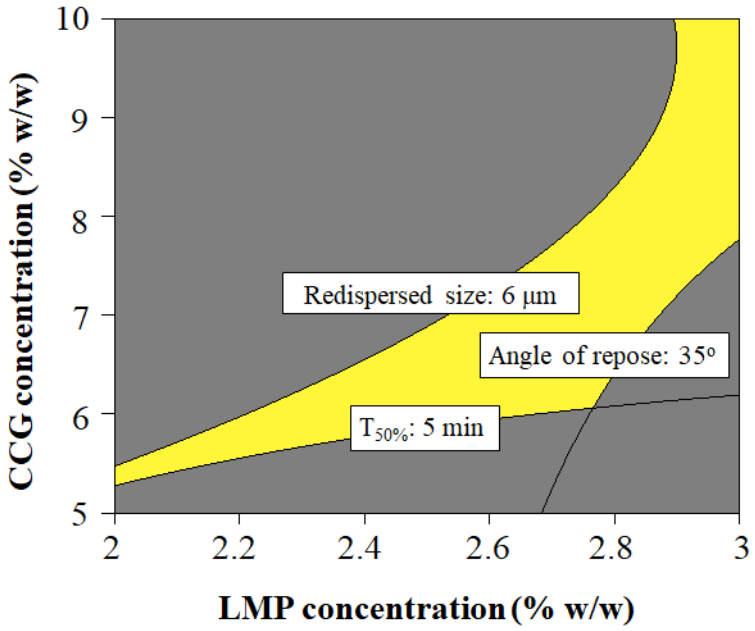

Figure 16. Overall design space for satisfy criteria of all responses of RVT spray-dried emulsions. The design space was represented in the yellow area.

Table 14. Experimental results of the optimized RVT spray-dried emulsions.

Responses	Observed value	Predicted value	Residual	Error (%)
Redispersed emulsion size (µm)	5.49 ± 0.23	5.75	0.26	4.60
$T_{50\%}$ (min)	3.7 ± 0.2	4.09	0.64	9.54
Angle of repose (º)	31.0 ± 2.5	33.18	2.2	6.63
Spraying efficiency (g/min)	10.63	10.60	-0.03	0.28

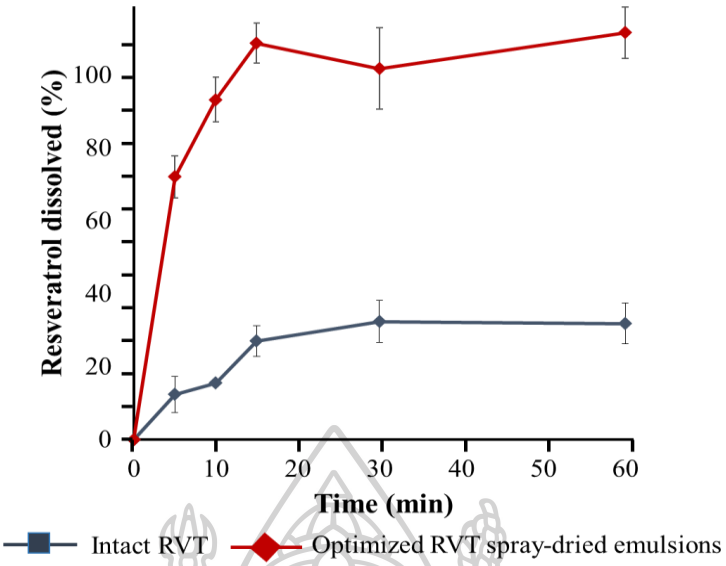

Figure 17. Dissolution profiles of optimized RVT spray-dried emulsions and intact.

3.3.2.7 Chemical stability of RVT in the optimized RVT spray-dried emulsions

Photostability and short-term stability of the optimized RVT spray-dried emulsion were assessed to evaluate the effectiveness of spray-dried emulsion formulation in protecting RVT from environmental exposure. RVT is more photolabile in oil solution than in solid form; this is because RVT molecules are exposed to light in transparent oil. While RVT was dissolved in CCG in the spray-dried emulsions, RVT was evaluated in oil solution to compare it with RVT in RVT spray-dried emulsion. In the present study, the percentage of intact RVT that retained *trans*-RVT configuration of 37.27% ± 5.33% and 10.5% ± 0.22% after UV irradiation for 30 and 60 min, respectively (Figure 18). Conversely, *trans*-RVT in the optimized RVT spray-dried emulsion exhibited higher photostability than intact RVT. After UV irradiation for 30 and 60 min, residual *trans*-RVT in RVT spray-dried emulsion was 92.56% ± 0.45% and 86.25% ± 4.04%, respectively. In a recent study, for RVT encapsulated in Eudragit® microcapsules, only 60% of RVT remained after 60 s of UV irradiation (151). Therefore, RVT was effectively protected from UV irradiation by encapsulation using LMP in spray-dried emulsion in the present study. In addition, residual RVT concentrations in RVT spray-dried emulsion after storage at 25ºC and 40ºC/75%RH for 3 months were 95.24% ± 1.01% and 96.60% ± 0.73% of the freshly

prepared sample, respectively. RVT in RVT spray-dried emulsion was stable following storage under both conditions.

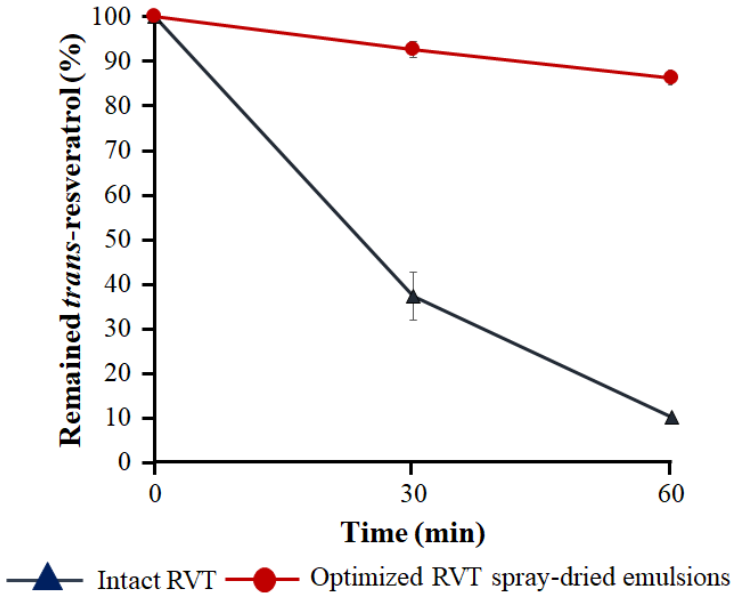

Figure 18. Remaining trans-RVT after UV exposure of intact and optimized RVT spray-dried emulsions.

3.4 Conclusion

The systematic product and process development of the RVT spray-dried emulsion were facilitated by the QbD approach. RVT is a model example of a poorly water-soluble drug. Prior to the experimental design, the possible risks were evaluated in a formal risk assessment, including the Ishikawa diagram and a risk-ranking system. The critical factors were further revealed in a Plackett–Burman design experiment. After the experiment, the risks were re-evaluated based on our new understanding of the process. The LMP amount, CCG amount, homogenization speed, and pump speed were observed to most critically affect the quality of the product and the spray-drying performance. To mitigate their risk level, the high-risk factors must be related to the product quality and the spray-drying process. Although this relation required further investigation, the application of both risk assessment and the Plackett–Burman design in the early stage of product development can effectively identify the most critical factors that influence the product quality and the process quality attributes.

In the optimization study, the influence of formulation compositions and process factors such as pump speed on RVT spray-dried emulsion properties and spray-drying performance were investigated using the Box–Behnken design. Regression models were established to predict RVT spray-dried emulsion properties and process efficiency. The effects of factors on responses were analyzed using statistical approaches. Redispersed emulsion sizes decreased with an increase in the LMP concentration and with a decrease in the CCG concentration. Time required for 50% drug dissolution was depended on CCG concentration. High CCG concentration may cause oil deposition at the surface of RVT spray-dried emulsion and resulted in faster drug dissolution. The flowability of RVT spray-dried emulsion depended on formulation composition and its interactions with pump speed. Particles with a spherical shape and low moisture content demonstrated good flow properties. Pump speed had a significant effect on the spraying efficiency. The formulations and spray-drying processes were optimized to produce RVT spray-dried emulsion with small redispersed emulsion sizes, fast dissolution, good flow properties, and a spray-drying process with desirable efficiency based on the developed mathematical models. Validation experiments showed good predictability of the mathematical models. The design space was established to specify optimum ranges of formulations and processes. RVT spray-dried emulsion produced within the design space achieved the desired requirements. Optimization elucidated effects of formulation composition and pump speed of spray dryer on RVT spray-dried emulsion properties and process efficiency. In addition, the chemical stability of RVT improved by encapsulation using LMP in RVT spray-dried emulsion.

CHAPTER 4

Development of RVT-loaded onto porous powders

4.1 Introduction

According to our results from RVT spray-dried emulsions part (Chapter 3), the dissolution properties and chemical stability of RVT were effectively improved using spray-dried emulsions formulation. However, the RVT loading in the formulation was quite low and some degradation form UV irradiation was still observed. Therefore, some improvement is required to enhance the drug loading capacity and photostability of RVT.

PCS is one option for enabling improvements the drug loading capacity and photostability of RVT. The high number of pores in PCS provides a large surface area and pore volume, leading to a high adsorption capacity. Nevertheless, PCS has certain disadvantages for drug delivery systems. The intrinsically low density of PCS retards pharmaceutical development from the viewpoint of the manufacturing process (152). Density differences between the components during formulation may cause non-homogeneous mixing and segregation during processing. A previous study used a wet granulation technique to increase the bulk density of PCS powders during formulation (153); however, adsorption of the drug onto PCS requires many processing steps. Therefore, the implementation of faster techniques is required to increase the bulk density of PCS.

To obtain RVT/PCS powders with the desired quality, e.g. having a high drug loading capacity, encapsulation efficiency or drug dissolution, the relationship between formulation factors and product quality must be clearly understood using DoE. Previous studies have applied the DoE approach to experiments on porous materials (154,155).

In the present work, we developed RVT-loaded PCS powder formulations using an emulsion system and solvent evaporation. PCS powders were used to improve the dissolution properties of RVT. The effects of formulation factors on the characteristics of RVT-loaded PCS powders, including drug loading capacity, encapsulation efficiency and drug dissolution, were determined using the Box–Behnken design. The RVT-loaded PCS powder formulations were optimized to be produced with specific properties within a developed design space. Based on the optimization study, the optimal range for each formulation factor was revealed.

4.2 Materials and methods

4.2.1 Materials

PCS (Florite® RE) was a gift from Eisai R&D Management Co., Ltd. (Kobe, Japan). Ethyl acetate (EA) was purchased from RCI labscan Ltd. (Bangkok, Thailand). All other materials were described in section 3.2.1.

4.2.2 Optimization RVT loaded onto porous calcium silicate (PCS) powders formulation using Box-Behnken design

In the present study, a Box-Behnken design was used to optimize RVT/PCS powders formulation and investigated the effect of selected independent factors on the responses. The method was the same as described in section 3.2.4. The independent factors with the design levels and responses were shown in Table 15. The levels of each independent factor were selected from preliminary experimental results.

Table 15. Independent factors in Box–Behnken design and responses of RVT-loaded porous calcium silicate (PCS) powders.

Independent factors	Level		
	Low (-1)	Medium (0)	High (+1)
X_1: LMP amount (% w/w)	2	2.5	3
X_2: EA amount (% w/w)	10	15	20
X_3: ratio of RVT to PCS (RVT:PCS)	0.1:1	0.15:1	0.2:1
Responses			
Y_1: Drug loading capacity (%)			
Y_2: Encapsulation efficiency (%)			
Y_3: Time required for 50% drug dissolution ($T_{50\%}$; min)			

4.2.3 Preparation of RVT/PCS powders

O/W emulsions containing RVT were prepared by a homogenization process. The LMP amounts (2–3% w/w) and EA amounts (5–10% w/w) were varied in the RVT emulsions. The dispersed phase was EA, containing RVT at a concentration of 17 mg/mL. LMP dissolved in distilled water was used as the aqueous phase. Homogenization was performed using the same method as described in optimization part of section 3.2.5. The RVT emulsion was adsorbed onto PCS powders by varying the ratio of RVT to PCS (RVT:PCS; 0.1:1–0.2:1) using a mortar and pestle. The RVT emulsion-loaded PCS powders were dried overnight at 40°C in a vacuum oven

(model Vacucell 55, MMM Medcenter Einrichtungen GmbH, Munich, Germany) to yield RVT/PCS powders. The EA containing RVT was also loaded onto PCS powders, as a control formulation, under the same conditions.

4.2.4 Evaluation of drug loading capacity and encapsulation efficiency of RVT/PCS powders

The RVT content was measured and the drug loading capacity was calculated using the same method as described in section 3.2.6.2. The encapsulation efficiency of the RVT/PCS powders were calculated as follows (156):

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Total RVT amount (mg)} \times 100}{\text{Initial amount of RVT (mg)}} \qquad (11)$$

4.2.5 Determination of RVT/PCS powders morphology

The morphology of RVT/PCS powders were determined using the same method as described in section 3.2.6.1.

4.2.6 Porosimetry of RVT/PCS powders measurement

Surface area, pore volume and pore size of intact PCS and RVT/PCS powders were evaluated using a surface area and pore size analyser (model Nova 2000e, Quantachrome, USA). Samples were degassed for 2 hours at 100°C using a vacuum to remove residual water. Adsorption and desorption isotherms were collected at 77 K. Surface area, pore volume and pore size were calculated using the Berret–Joyner–Halenda (BJH) method.

4.2.7 Viscosity measurement

Selected samples were evaluated for viscosity by a dynamic shear rheometer (model Kinexus, Malvern Panalytical Ltd., Malvern, UK) endowed with a cone–plate geometry with a diameter of 50 mm. The shear rate profile was 0.1 to 100 S^{-1}. Measurement of each sample was performed in triplicate at 25°C.

4.2.8 *In vitro* dissolution study of RVT/PCS powders

In vitro dissolution study of RVT/PCS powders and intact RVT was carried out using the same method as described in optimization part of section 3.2.4.6. The dissolution medium was pH 4.5 acetate buffer. 4 mL of sample was withdrawn at 5, 10, 15, 30, 60, and 120 min intervals and replaced with fresh medium. The sample was centrifuged at a speed of 15,000 rpm for 30 min, and the supernatant was analysed using T60 UV-Visible spectrophotometer (PG instrument Ltd., Leicestershire, UK) at 307 nm. The time required for 50% drug dissolution ($T_{50\%}$) was determined. The RVT content was measured by the UV method prior to the dissolution test. The drug dissolution was carried out in triplicate.

4.2.9 Bulk density RVT/PCS powders determination

The bulk density of intact PCS, optimized RVT/PCS powders and PCS containing the same amount of RVT as the optimized formulation were measured using a graduated cylinder. A 2-g sample was poured into a 50-mL graduated cylinder and the volume observed was used in the calculation of bulk density, as follows:

$$\text{Bulk density } (g/cm^3) = \frac{\text{Sample weight (g)}}{\text{Sample volume } (cm^3)} \quad (12)$$

4.2.10 Stability studies of RVT/PCS powders

4.2.10.1 Photostability of RVT in PCS powders

Photostability study of RVT in RVT/PCS powders was carried out using the same method as described in section 3.2.8.1.

4.2.10.2 Chemical stability of RVT in PCS powders on storage

RVT/PCS powders sample was stored in an open vial protected from light at 25ºC. The RVT content was evaluated by HPLC after 1-year storage.

4.3 Results and discussion

Seventeen RVT/PCS powder formulations were prepared using the Box–Behnken design. The formulation factors were LMP amount (X_1), EA amount (X_2) and RVT:PCS (X_3). The responses of the RVT/PCS powders were drug loading capacity (Y_1), encapsulation efficiency (Y_2) and Time required for 50% drug dissolution ($T_{50\%}$; Y_3). The formulations of the RVT/PCS powders and the response results are shown in Table 16.

Table 16. Box–Behnken experimental design layout and response results for RVT/PCS powders.

Standard order (S)	Run order	Independent factors			Responses		
		LMP amount (% w/w)	EA amount (% w/w)	RVT:PCS	Drug loading capacity (%)	Encapsulation efficiency (%)	$T_{50\%}$ (min)
1	6	2	10	0.15:1	0.51 ± 0.07	60.34 ± 0.41	4.9 ± 0.7
2	5	3	10	0.15:1	0.50 ± 0.05	66.49 ± 6.31	4.8 ± 0.4
3	12	2	20	0.15:1	1.39 ± 0.01	89.65 ± 0.95	6.4 ± 1.6
4	8	3	20	0.15:1	1.38 ± 0.15	98.80 ± 0.81	6.5 ± 1.3
5	3	2	15	0.1:1	1.55 ± 0.07	90.99 ± 0.31	6.4 ± 1.5
6	9	3	15	0.1:1	1.43 ± 0.03	88.64 ± 1.89	4.3 ± 0.2
7	11	2	15	0.2:1	0.78 ± 0.05	91.22 ± 5.36	24 ± 7.3
8	14	3	15	0.2:1	0.72 ± 0.05	84.71 ± 4.30	9.5 ± 2.3
9	7	2.5	10	0.1:1	0.86 ± 0.01	75.82 ± 0.63	4.6 ± 0.3
10	13	2.5	20	0.1:1	2.16 ± 0.04	97.61 ± 1.73	3.9 ± 0.4
11	2	2.5	10	0.2:1	0.32 ± 0.05	62.20 ± 1.58	11.9 ± 3.1
12	16	2.5	20	0.2:1	1.03 ± 0.07	88.76 ± 1.93	13.5 ± 2.5
13	15	2.5	15	0.15	0.83 ± 0.01	72.82 ± 0.96	4.6 ± 0.6
14	1	2.5	15	0.15	0.89 ± 0.06	81.50 ± 2.43	5.9 ± 1.4
15	4	2.5	15	0.15	0.99 ± 0.07	86.72 ± 5.73	4.5 ± 0.2
16	17	2.5	15	0.15	0.98 ± 0.08	81.89 ± 3.12	13.5 ± 2.5
17	10	2.5	15	0.15	0.87 ± 0.09	71.54 ± 2.20	5.9 ± 1.2

4.3.1 Drug loading capacity of RVT/PCS powders

A high amount of drug loaded into a powder is a desirable formulation property. The effect of each independent factor on the drug loading capacity of the RVT/PCS powders was investigated using statistical analysis. The drug loading capacity of RVT/PCS powders varied from 0.32% ± 0.05% to 2.16% ± 0.04%, as summarised in Table 16. The significance and the effect of each independent factor were determined

using ANOVA. A mathematical model used to describe the relationship between the various factors and drug loading capacity of the RVT/PCS powders is shown below.

Drug loading capacity (%): $Y_1 = + 0.93 + 0.47X_2 + 0.39X_3 + 0.15X_2X_3 + 0.18\ X_3^2$ (13)

From the ANOVA results (Table 17), the mathematical model for predicting drug loading capacity of RVT/PCS powders was significant (p-value < 0.0001) and lack of fit was not significant. The model predicting drug loading capacity showed a high r^2 value of 0.9863, which demonstrates the reliability of the model. ANOVA showed that effect of EA amount (X_2), RVT:PCS (X_3), the interaction between EA amount and RVT:PCS (X_2X_3), and quadratic term of RVT:PCS (X_3^2) were significant. All of the significant factors showed positive association with drug loading capacity of the RVT/PCS powders. The drug loading capacity increased with increasing EA amount and also the ratio of RVT:PCS. The effect of each significant factor was illustrated using a contour plot. From the contour plot (Figure 19), an increase in EA amount and a ratio of RVT:PCS evidently enhanced the drug loading capacity of the RVT/PCS powders. The content of RVT depended on the amount of the dispersed phase (EA containing RVT) in the emulsion. An increase in the dispersed phase amount in the formulation led to an increase in the RVT amount adsorbed on the RVT/PCS powders. The formulation containing a high amount of RVT had a high amount of emulsion in the formulation, which subsequently led to a higher amount of RVT in the RVT/PCS powders. A previous study reported that varying the volume of the solvent in the dried adsorbed powder formulation induces differences in drug adsorption onto porous powders (89). In a similar manner, the drug loading capacity of RVT/PCS powders was dependent on the EA amount and RVT:PCS in the formulation.

Figure 20 shows the surface morphology of intact PCS and RVT/PCS powders (batch S10). Intact PCS exhibited petal-like flakes on its surface, with a large number of pores. In comparison, the surface of the RVT/PCS powder, which contained the highest amount of RVT among all the RVT/PCS powders, showed the some formation of LMP stacks on the PCS surface (Figure 20-b2). The porosimetry results showed that intact PCS has a surface area of 83.82 m^2/g, pore radius of 16.93 Å and

pore volume of 0.46 cc/g, whereas the surface area, pore radius and pore volume of the RVT/PCS powders were 63.29 m^2/g, 16.23 Å and 0.34 cc/g, respectively. The decrease in pore volume in the RVT/PCS powder, as compared with the intact PCS, was due to the adsorption of RVT and LMP onto the PCS powder. The decrease in surface area was due to a blocking of pores in the presence of the drug (157). In the case of the RVT/PCS powder, the reduction of both pore volume and surface area could be due to the adsorption of both RVT and LMP on the PCS surface. The pore size of intact PCS and the RVT/PCS powders was similar, indicating that the emulsion did not affect the pore size of PCS.

Table 17. ANOVA results for model predicting drug loading capacity (Y_1), encapsulation efficiency (Y_2) and $T_{50\%}$ (Y_3).

Source	Sum of Squares	Degree of freedom	Mean square	F value	*p*-value, Prob>F
For Y_1 (%)					
Model	3.24	4	0.81	215.86	< 0.0001
X_2	1.78	1	1.78	473.37	< 0.0001
X_3	1.24	1	1.24	330.47	< 0.0001
X_2X_3	0.087	1	0.087	23.19	0.0004
X_3^2	0.14	1	0.140	36.39	< 0.0001
Residual	0.045	12	0.004		
Lack of fit	0.025	8	0.003	0.64	0.7239
Pure error	0.02	4	0.005		
*Cor Total	3.29	16			
Other statistics: r^2 = 0.9863, adjusted r^2 = 0.9817, RMSE = 0.061					
For Y_2 (%)					
Model	1756.57	3	585.52	17.07	< 0.0001
X_2	1511.68	1	1511.68	44.07	< 0.0001
X_3	85.61	1	85.61	2.5	0.1382
X_3^2	159.29	1	159.29	4.64	0.0505
Residual	445.91	13	34.3		
Lack of fit	277.92	9	30.88	0.74	0.6789
Pure error	167.99	4	42		
Cor Total	2202.48	16			
Other statistics: r^2 = 0.7975, adjusted r^2 = 0.7508, RMSE = 5.86					
For Y_3 (min)					
Model	324.5	4	81.13	8.27	0.0019
X_1	35.28	1	35.28	3.59	0.0823
X_3	199	1	199	20.28	0.0007
X_1X_3	39.69	1	39.69	4.04	0.0673
X_3^2	50.53	1	50.53	5.15	0.0425
Residual	117.78	12	9.81		
Lack of fit	61.17	8	7.65	0.54	0.7874
Pure error	56.61	4	14.15		
Cor Total	442.28	16			
Other statistics: r^2 = 0.7337, adjusted r^2 = 0.6449, RMSE = 3.13					

Note: X_1=LMP amount; X_2=EA amount; X_3=RVT:PCS; $p < 0.05$ is considered as significant; *corrected total sum of square

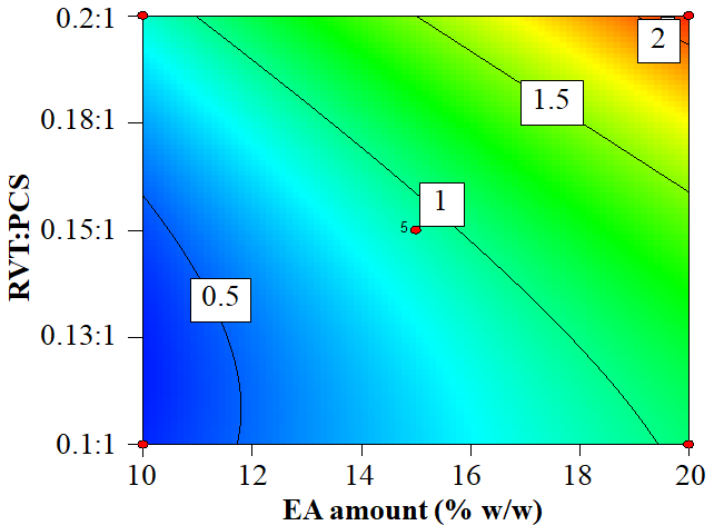

Figure 19. Contour plot showing effect of porous calcium silicate (PCS) amount and ethyl acetate (EA) amount on drug loading capacity of resveratrol (RVT)/PCS powders.

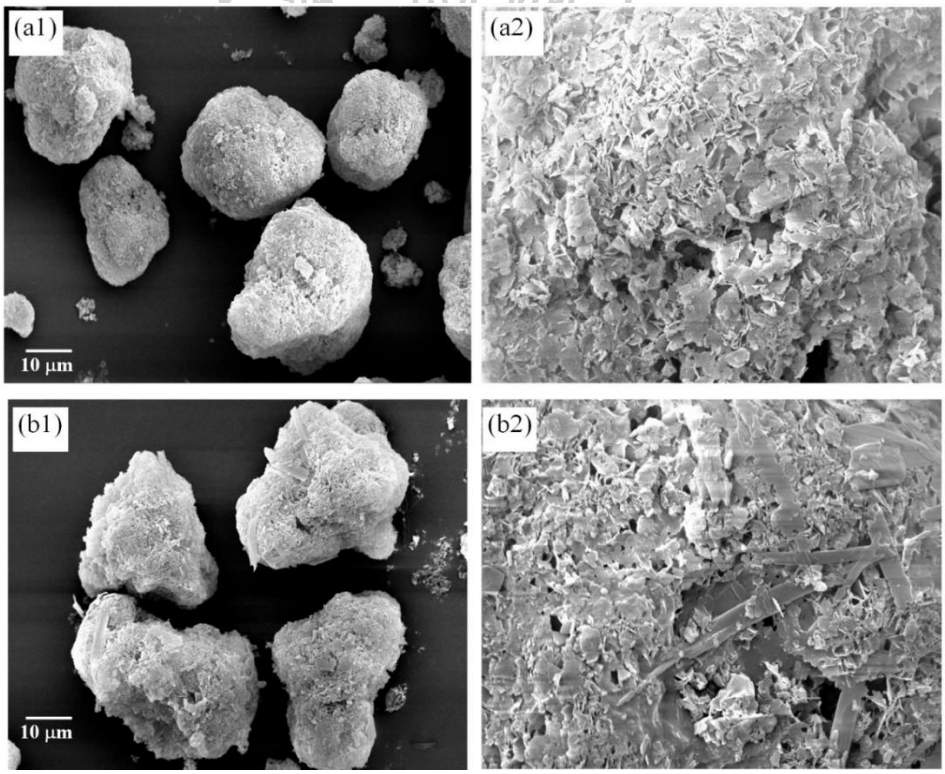

Figure 20. SEM images of intact PCS at magnifications of (a1) 1000× and (a2) 5000× and RVT/PCS powders standard order (S) 10 at magnifications of (b1) 1000× and (b2) 5000×.

4.3.2 Encapsulation efficiency of RVT/PCS powders

It was also necessary to study the factors affecting the encapsulation efficiency of RVT/PCS powders. A high encapsulation efficiency could show the effectiveness of the loading method of the RVT/PCS powders. The encapsulation efficiency of the RVT/PCS powders ranged from 60.34% ± 0.41% to 98.80% ± 0.81% (Table 16). A mathematical model used to describe the relationship between the various factors and the encapsulation efficiency of the RVT/PCS powders is shown below.

Encapsulation efficiency (%): $Y_2 = + 78.86 + 13.75X_2 - 3.27X_3 + 6.13X_3^2$(14)

From the ANOVA results (Table 17), the mathematical model for predicting the encapsulation efficiency of the RVT/PCS powders was significant (p<0.0001) and lack of fit was not significant. The model showed a high r^2 value of 0.7975. ANOVA showed that effect of EA amount (X_2) was the most significant. EA amount showed a positive association with the encapsulation efficiency of the RVT/PCS powders. From the contour plot (Figure 21), the high amount of EA in the emulsion led a relatively high encapsulation efficiency of the RVT/PCS powders, as compared with the formulation containing lower EA amount. EA containing dissolved RVT may have been adsorbed by the surface of the PCS powders. The increase in encapsulation efficiency of the formulation containing a high amount of EA may have been due to the viscosity difference between EA and the water phase in the emulsion. The viscosity of EA was 1.08 ± 0.03 mPa S at a shear rate of 3.13 S^{-1} at 25°C, whereas that of water was significantly higher, 2.50 ± 0.09 mPa S under the same conditions: such differences have been reported previously (158). In addition, the high amount of EA facilitated the adsorption of EA onto PCS powders owing to the increase in pressure gradient. The pressure gradient replaces capillary action when an adsorbent is completely submerged in an adsorbate (159). An increase in solvent volume may assist the migration of solvent-containing drugs deep inside the pores of an adsorbent (89). Therefore, the high amount of EA in the RVT/PCS powders may have induced the higher encapsulation efficiency, as compared with that containing the lower amount of EA.

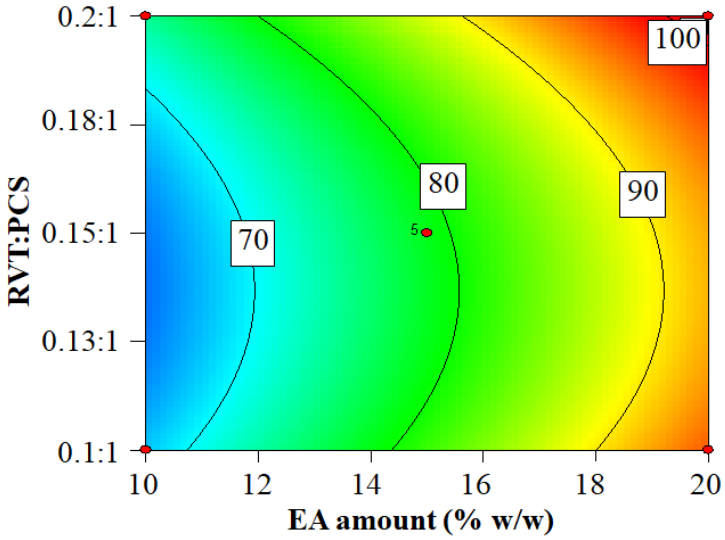

Figure 21. Contour plot showing the effect of PCS amount and EA amount on encapsulation efficiency of RVT/PCS powders.

4.3.3 *In vitro* dissolution study of RVT/PCS powders

The dissolution of the intact RVT was ~20% at 120 min, indicating the intrinsically poor water solubility of RVT as shown previously in the RVT spray-dried emulsions part. In contrast, the dissolution properties of RVT was improved in RVT/PCS powders, as shown in Table 16. The time required for 50% drug dissolution varied from 4 to 24 min. This suggests that the RVT/PCS powders significantly improved the dissolution properties of RVT. Ali et al. suggested that the dissolution of crystalline drugs loaded into porous powders is improved owing to a reduction in crystalline size, resulting in an increase in the surface area of the crystalline drug (160). Therefore, in this study, the improved dissolution of RVT when loaded into porous powders may have been due to the reduction of crystalline RVT size. A previous study reported that the volume of an RVT single crystal was 1075.5 $Å^3$ (161). From our results, the pore radius of PCS was 16.23 Å and the volume was 4275.2 $Å^3$. The pore size of PCS was only a few times larger than the size of an RVT single crystal. The formation of crystalline RVT after drying was restricted according to the limitation of the pore size of the PCS, which resulted in the reduction of the RVT crystal (to nano-sized) and an improvement in the dissolution properties of

RVT. Other studies have reported that the size reduction of poorly water-soluble drugs could be improved via adsorption onto porous powders (160,162).

A mathematical model used to describe the relationship between the various factors and $T_{50\%}$ of the RVT/PCS powders is shown below.

$$T_{50\%} \text{ (min): } Y_3 = + 6.33 - 2.1X_1 + 4.99X_3 - 3.15\, X_1\, X_3 - 3.45X_3^2 \quad (15)$$

From the ANOVA results reported in Table 17, the mathematical model for predicting the Q_5 of RVT/PCS powders was significant (p-value < 0.0019) and lack of fit was not significant. A high r^2 value was also obtained for this model (r^2 = 0.7337). ANOVA showed that effect of RVT:PCS (X_3) and its quadratic term were the most significant. RVT:PCS showed a positive association with $T_{50\%}$ of RVT/PCS powders. From the contour plot (Figure 22), an increase in RVT:PCS increased the time required for 50% dissolution of RVT when loaded onto the PCS powders. In other words, RVT/PCS powders containing high amount of RVT in the formulation showed slower drug dissolution than that in the formulation containing lower amount of RVT. Previous study reported that meloxicam-loaded onto PCS powders containing 1:3, meloxicam:PCS ratio showed faster drug dissolution than the formulation containing 1:1, meloxicam:PCS ratio (163). The dissolution rate was increased could be due to the increase in PCS amount leading large surface area to reduction in the size of crystalline form of drug. In addition, the high surface area of PCS powders may minimise the probability of drug agglomeration and could improve the wettability of the drug in aqueous fluids. Mellaerts *et al.* also reported that an increase in drug concentration adsorbed onto mesoporous SBA-15 silica materials beyond the optimum of loading limit could slow the dissolution rate of the drug (164). The high drug loading in the formulation could result in the presence of crystalline and amorphous form of the loaded drug led to slow dissolution rate of the drug. Therefore, an optimum formulation of RVT/PCS powders is necessary for achieving fast drug dissolution and other desire characteristics i.e. loading capacity and encapsulation efficiency.

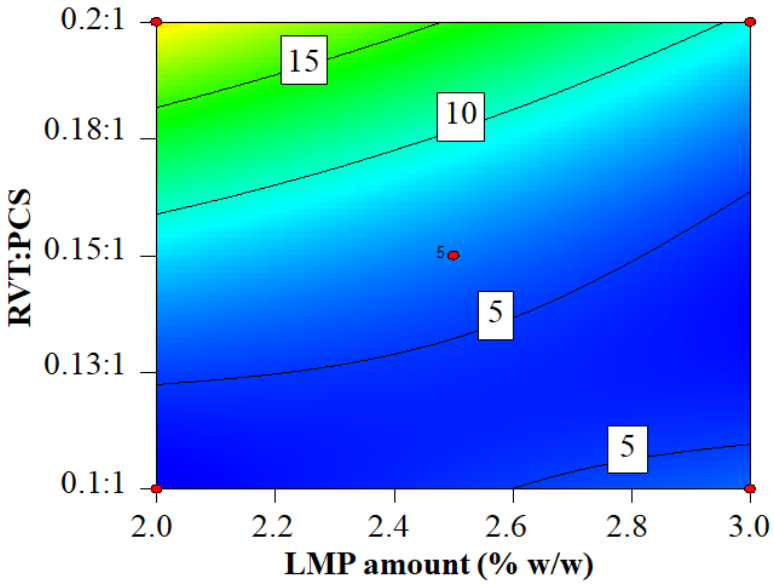

Figure 22. Contour plot showing the effect of RVT:PCS and LMP amount on $T_{50\%}$ in RVT/PCS powders.

4.3.4 Optimization of RVT/PCS powders formulation and verification of mathematical model

An optimization was carried out to find the optimal range of the RVT/PCS formulations that provided satisfactory RVT/PCS powder quality. The criteria for optimization of RVT/PCS powders characteristics included maximizing drug loading capacity (not less than 0.7%) and encapsulation efficiency (not less than 70%) and minimizing the $T_{50\%}$ (not more than 10 min). The goal and limit of each response were specified based on the desired properties of the product. The drug loading capacity and encapsulation efficiency should be high to ensure a high amount of RVT in the RVT/PCS powders and a low amount of RVT loss during the manufacturing process. In addition, rapid drug dissolution is also preferred to provide rapid absorption of RVT in the gastrointestinal tract. Figure 23 shows an established design space based on the criteria given being met. The optimized formulation could be produced with an LMP amount of 2.2% w/w. The amounts of EA and PCS used may be in the range shown in yellow in the design space (Figure 23); however, the optimized formulation was produced using 14% w/w EA and 0.15:1, RVT:PCS ratio.

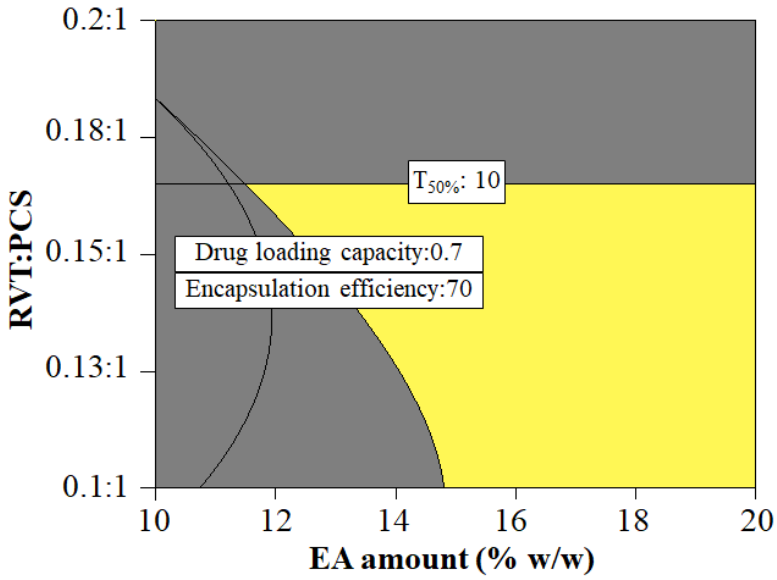

Figure 23. Overall design space to satisfy the criteria for all responses of the RVT/PCS powders. The design space is represented by the yellow area.

The results for the optimized RVT/PCS powders (V1) are shown in Table 18. The optimized formulation showed a drug loading capacity of 1.15% ± 0.01%. The encapsulation efficiency of the optimized formulation was 85.59% ± 1.28%, indicating the high amount of RVT encapsulated in the porous powders. The $T_{50\%}$ of the optimized RVT/PCS powders was 8.0 ± 3.1 min. The dissolution profile of the optimized formulation, as compared with intact RVT, is presented in Figure 24a. The dissolution properties of RVT in the optimized formulation were enhanced compared with intact RVT, as was observed in the Box–Behnken design experimental results. The optimized RVT/PCS powders were further evaluated for bulk density. LMP is a polymeric surfactant and is used to improve the bulk density of PCS powders. The bulk density of the intact PCS powders was 0.061 ± 0.001 g/cm^3. The PCS powders containing the same amount of RVT but without LMP showed similar bulk densities to those of intact PCS, i.e. 0.065 ± 0.003 g/cm^3, whereas the bulk densities of the optimized RVT/PCS powders showed a 40% increase, i.e. 0.085 ± 0.002 g/cm^3. The addition of LMP to the formulation increased the bulk density of PCS in the RVT/PCS powders. This increase facilitates certain manipulations during the manufacturing process, especially in relation to solid formulation.

The adequacy of the mathematical model was confirmed using the optimized RVT/PCS powders formulation and three additional batches of RVT/PCS powders. Characterization for drug loading capacity, encapsulation efficiency and $T_{50\%}$ were performed for these additional batches. The level of each factor, observed value (OV) and predicted value (PV) of the verification (V) batches are shown in Table 18. The RMSE was calculated to indicate the error of the model prediction (165). The model of drug loading capacity and $T_{50\%}$ responses showed low RMSE values, indicating that the models provided good predictability. However, the RMSE of the encapsulation prediction model was slightly higher than that observed for the other models. Nevertheless, the results of encapsulation efficiency obtained from each verification batch were found to be within 95% of the prediction interval of the PV (data not shown). Therefore, the models provided acceptable predictability and were suitable for prediction purposes.

Table 18. Verification experimental results for the model obtained from a Box–Behnken design of RVT/PCS powders

		Verification run (V)				
		V1	V2	V3	V4	
Independent factors	LMP amount (% w/w)	2.2	2.4	2.6	2.7	
	EA amount (% w/w)	14	16	19	19	
	RVT:PCS	0.15:1	0.1:1	0.2:1	0.2:1	
Responses						RMSE
Drug loading capacity (%)	OV	1.15 ± 0.01	0.72 ± 0.04	1.55 ± 0.02	1.61 ± 0.03	
	PV	0.83	0.78	1.99	1.99	0.39
Encapsulation efficiency (%)	OV	85.59 ± 1.28	72.51 ± 5.87	93.76 ± 1.08	83.5 ± 21.97	
	PV	76.10	84.47	99.26	99.26	11.30
$T_{50\%}$ (min)	OV	8.0 ± 3.1	7.2 ± 0.8	7.4 ± 1.2	8.9 ± 1.4	
	PV	7.6	4.6	13.7	12.68	3.92

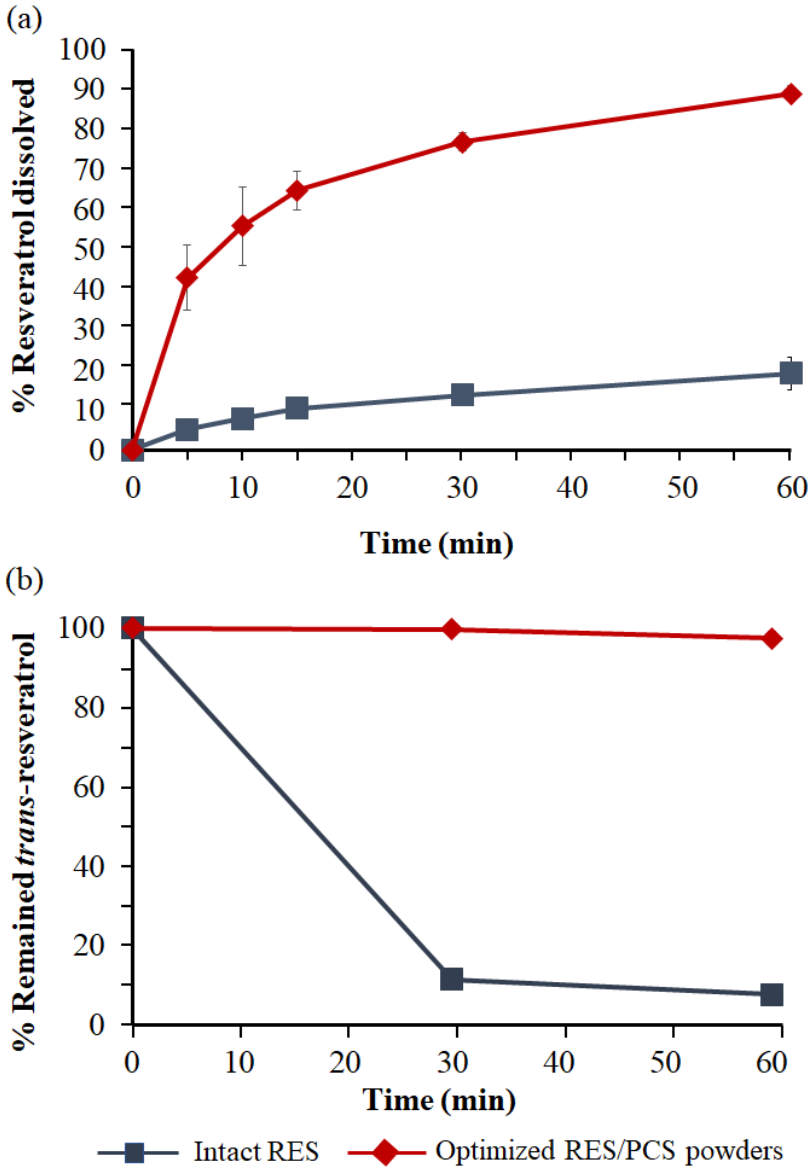

Figure 24. (a) Dissolution profile and (b) remaining trans-RVT after UV exposure of intact RVT and optimized RVT/PCS powders.

4.3.5 Chemical stability of RVT in the optimized RVT/PCS powders

The photostability of RVT in the optimized RVT/PCS powders and intact RVT is shown in Figure 24b. The *trans*-RVT isomer was transformed via UV irradiation to the *cis*-RVT isomer (166). Intact RVT in the *trans*-isomer was degraded rapidly and only 11.48% ± 0.04% and 7.67% ± 0.05% of the *trans*-RVT remained after UV irradiation for 30 min and 60 min, respectively. The optimized formulation showed remaining *trans*-RVT of 99.69% ± 1.21% and 97.48% ± 1.63% after UV irradiation for 30 min and 60 min, respectively. RVT could be effectively protected from UV irradiation when loaded onto the porous powders. Previous studies have reported that the stability of RVT is enhanced using techniques such as polymer matrix and acrylate microspheres (151,167,168). However, some chemical degradation of *trans*-RVT was observed after 60-min UV exposure. In this experiment, the relatively high amount of *trans*-RVT still remaining after UV irradiation for 60 min indicates the effectiveness of the PCS powders in protecting the UV-sensitive compound. The remained RVT in the optimized formulation of 1-year storage at 25ºC was 54.69% ± 2.05% of the freshly prepared sample. Some RVT in the porous powders could be degraded. The degradation observed from HPLC was not *cis*-isomer, which was the degraded product of *trans*-isomer from UV exposure (data not shown). The degradation of RVT could be due to an oxidation of RVT during storage. RVT possesses very strong antioxidant activity, hence it could be oxidized more rapidly (169).

4.4 Conclusion

The present study demonstrates that the dissolution properties of RVT were improved by RVT/PCS powder formulation. The effect of three independent factors, LMP amount, EA amount and RVT:PCS, on drug loading capacity, encapsulation efficiency and $T_{50\%}$ were studied using the Box–Behnken design. The experimental results showed that EA amount and RVT:PCS had significant effects on the drug loading capacity. The encapsulation efficiency was significantly influenced by EA amount. The RVT:PCS had a significant effect on $T_{50\%}$. The optimized RVT/PCS formulation showed that drug loading capacity, encapsulation efficiency and $T_{50\%}$ met all the satisfying criteria. Based on the dissolution profile, 50% of RVT was dissolved within 10-min intervals. In addition, the bulk density of PCS was improved by the addition of LMP to the formulation and RVT/PCS powder formulation could protect RVT from UV irradiation, resulting in the chemical stability of RVT in PCS powders. The development of RVT products using PCS powders could improve their dissolution properties, their stability after UV irradiation of RVT and the bulk density of PCS powders. Furthermore, the experimental design and optimization technique could be applied to the development of pharmaceutical products; thus providing an extensive understanding of the relationship between formulation and product quality.

CHAPTER 5

5.1 Summary and general conclusion

In recent years, an implementation of the HTS increases rate of drug discovery. However, HTS often results in the discovery of new drug candidates with poor water solubility and high lipophilicity. An enhancement of dissolution for poorly water-soluble drug such as RVT could improve drug adsorption and bioavailability. Emulsion is one of the efficient methods to increase dissolution rate and improve bioavailability of poorly water-soluble drug. However, the instability of an emulsion such as flocculation, creaming, cracking, or phase separation was often raised. The dry emulsion is one possible way to overcome such disadvantage of conventional emulsion. In this research, two techniques including spray-dried emulsions and RVT-loaded PCS powders were used for the enhancement of dissolution and photostability of RVT.

In the viewpoint of manufacturing process, multiple factors that were associated with the development of the RVT product could affect the its quality attributes. Identifying the product and process factors that considerably influenced the product quality, such as the process efficiency, drug dissolution, and drug loading, and so on was considered to be essential. QbD approach, which utilized both science- and risk-based was implemented in this study to determine effects of formulation and process on each RVT product quality attributes.

The RVT spray-dried emulsions was developed using a quality-by-design approach (Chapter 3). Further, the product and process factors that affected the quality of the spray-dried emulsions were analyzed and illustrated using an Ishikawa diagram. The product and process risks were prioritized using a risk-ranking system. The LMP amount, CCG amount, homogenization time, homogenization speed, inlet temperature, pump speed, drying airspeed, and de-blocking speed were observed to be the eight highest risk factors. Further, the criticality of these eight factors on the responses was determined using the Plackett–Burman design. Increasing the LMP amount increased the particle size, whereas increasing the CCG amount enhanced the drug loading capacity and Q_5 and decreased the moisture content. Q_5 was positively

affected by the homogenization speed and pump speed; however, it was negatively affected by the LMP amount. The spraying efficiency was affected by the pump speed and the LMP amount. Further, the risk level of the homogenization time, inlet temperature, drying airspeed, and de-blocking speed were reduced. However, the LMP amount, CCG amount, homogenization speed, and pump speed were observed to remain at high risk and require further investigation. The risk assessment and Plackett–Burman design mitigated the risks and identified the critical factors that affected the quality of the RVT spray-dried emulsions and the spray-drying process.

In the optimization study of RVT spray-dried emulsions (Chapter 3), effects of formulation and the pump speed of spray dryer on the resultant redispersed emulsion size, $T_{50\%}$, angle of repose, and spraying efficiency were investigated and optimized using the Box–Behnken design. Experimental results showed that the size of the redispersed emulsion was affected by LMP and CCG concentrations. An increase and decrease in LMP and CCG concentrations, respectively, resulted in smaller emulsion droplet size. The $T_{50\%}$ was influenced by CCG concentration. High CCG concentration could result in oil deposited at the spray-dried emulsions' surface and might result in fast drug dissolution. The angle of repose in RVT spray-dried emulsion was also influenced by LMP and CCG concentrations. RVT spray-dried emulsion generated using low LMP concertation and high CCG concentration showed a low angle of repose, indicating good flow property. The spraying efficiency enhanced following an increase in the pump speed of spray dryer. A design space was established based on the satisfying criteria for all RVT spray-dried emulsion responses. An optimized formulation containing 2.75% w/w of LMP and 7% w/w of CCG sprayed at a pump speed of 10.1 mL/min prepared within design space satisfied all criteria. The photostability of RVT in RVT spray-dried emulsion was significantly higher than that in intact RVT. Optimization study can elucidate the effects of formulation composition and pump speed on RVT spray-dried emulsion properties and process efficiency.

Another method was RVT-loaded onto PCS powders (Chapter 4), the effects of RVT/PCS powders that included varying amounts of LMP, EA and RVT:PCS on drug loading capacity, encapsulation efficiency and $T_{50\%}$ were investigated using a Box–Behnken design. The experimental results demonstrated that the EA amount and

RVT:PCS significantly influenced drug loading capacity. Encapsulation efficiency was affected by EA amount, whereas the RVT: PCS had a significant effect on $T_{50\%}$. Empirical experiments demonstrated the reliability of mathematical models. A design space was established based on the criteria set for maximizing each response of the RVT/PCS powders. An optimized formulation containing 2.2% w/w LMP, 14% w/w EA and RVT:PCS at 0.15:1 prepared within the design space satisfied all criteria. The dissolution and photostability of RVT in the RVT/PCS powders significantly improved, compared to intact RVT. Further, the bulk density of the PCS powders in RVT/PCS was increased by LMP. The Box–Behnken design used in this study provided an improved understanding of the effects of formulation factors on RVT/PCS powder characteristics as well as the optimization of RVT/PCS powder formulations with the desired properties.

In this research, both RVT products could improve dissolution property of RVT and also its photostability. The QbD approach could elucidate effect of formulation composition and process factors on the RVT spray-dried emulsions properties and process efficiency. In addition, the optimization study of RVT/PCS powders also provide an extensive understanding in effects of formulation factors on RVT/PCS powders responses and also optimization of RVT/PCS powders formulation with the desired properties.

5.2 Future directions of research

This study has developed the RVT spray-dried emulsions and RVT-loaded PCS powders, which can increase dissolution property and photostability of RVT in the formulations. The other lipid carriers, polymer-surfactant or porous powders could be applied for the RVT product to determine the possibility of the model prediction when using different ingredients. Even though this study suggested that the dissolution property of RVT was improved, however, bioavailability improvement of both RVT products should be studied.

APPENDIX

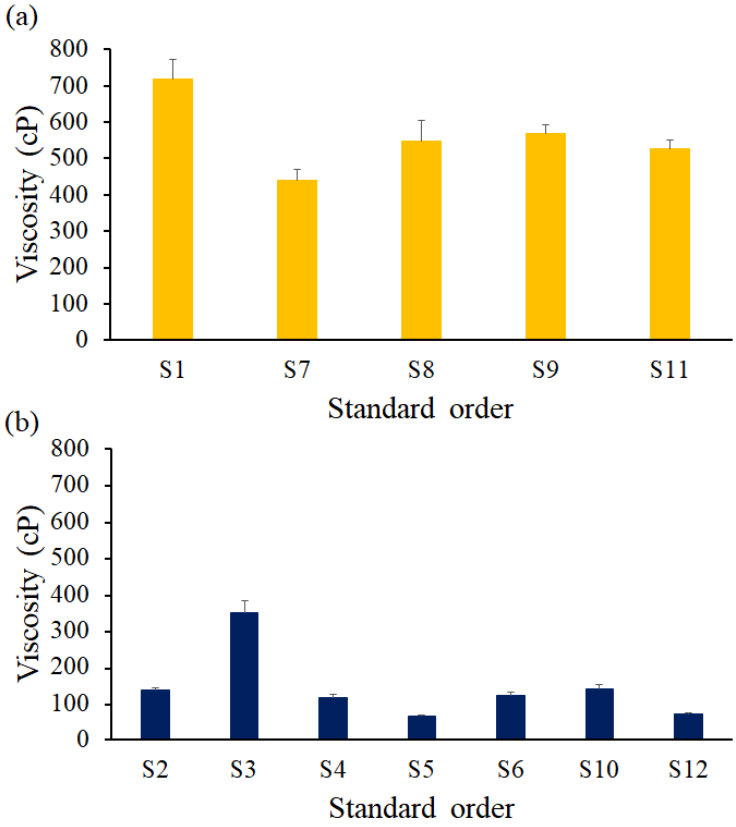

Figure A1. Viscosity value of RVT emulsion standard order (S) 1–12 containing (a) 3% w/w and (b) 2% w/w of LMP.

Table A1. r^2 of models obtained from the Plackett-Berman design experiment.

CQAs	r^2
Particle size	0.7622
Drug loading capacity	0.7980
Moisture content	0.6814
Q_5	0.8485
Spraying efficiency	0.9929

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VITA

NAME	Pontip Benjasirimongkol
DATE OF BIRTH	20 November 1980
PLACE OF BIRTH	Bangkok, Thailand
INSTITUTIONS ATTENDED	Doctor Degrees: Faculty of Pharmacy, Silpakorn University, Thailand and Department of Pharmaceutical Technology, Graduate School of Pharmaceutical Sciences, Chiba University, Japan Master Degree: Faculty of Life and Social Sciences, Swinburne University of Technology, Australia Bachelor Degree: Faculty of Pharmacy, Silpakorn University, Thailand
HOME ADDRESS	90/142 M.5 The Villa, Tha-it, Rattanatibeth Rd. Pakred, Nonthaburi, 11120
PUBLICATION	1. Benjasirimongkol P, Piriyaprasarth S, Moribe K, Sriamornsak P. Use of risk assessment and Plackett–Burman design for developing resveratrol spray-dried emulsions: A quality-by-design approach. AAPS PharmSciTech. 2019; in press. 2. Benjasirimongkol P, Ueda K, Higashi K, Sriamornsak P, and Moribe K. An insight into stabilization mechanism of a solid dispersion of indomethacin/partially hydrolyzed polyvinyl alcohol prepared by hot–melt extrusion. Chem. Pharm. Bull. 2018;66(9):859–865. 3. Benjasirimongkol P, Sriamornsak P. Stability study of resveratrol-loaded emulsions using pectin as an emulsifier. Asian J. Pharm. Sci. 2016;11(1):199–200. 4. Benjasirimongkol P, Sriamornsak P, Piriyaprasarth S. Design space development using risk assessment and design of experiments. TIPA J. 2016;4(1):5–17. 5. Benjasirimongkol P, Sriamornsak P, Piriyaprasarth S. Quality-by-Design (QbD): Approach for product and process development. TIPA J. 2015;3(1):40–50.