Ind. J. Pharm. Edu. Res., 2025; 59(3):995-1006.
https://www.ijper.org

Original Article

Investigation on the Effect of Alginate Concentration and Flow Rate on Production of Nanoparticle Loaded with Naringenin Using Electrospray Method

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ABSTRACT

Background: Naringenin, a polyphenolic phytochemical belonging to the flavanone class, exhibits notable anti-cancer, antioxidant, and anti-inflammatory properties. However, its therapeutic application is constrained by poor aqueous solubility and limited delivery to target sites. **Purpose:** This study aimed to address these challenges by encapsulating naringenin in alginate nanoparticles using an electrospray method. The effects of varying alginate concentrations and flow rates on nanoparticle production were investigated. **Materials and Methods:** Naringenin was mixed with sodium alginate and Tween 80, and the solution was electrosprayed into a calcium chloride cross-linking agent. Particle size, Polydispersity Index (PDI), zeta potential, and Encapsulation Efficiency were measured. **Results:** the research work indicated that an alginate concentration of 0.5% w/v and a flow rate of 0.2 mL/hr produced nanoparticles with optimal characteristics, including a particle size of 146 nm, a PDI of 0.331, a zeta potential of -1.10 mV, and an Encapsulation Efficiency of 92.80%. **Conclusion:** These findings highlight the significant influence of alginate concentration and flow rate on nanoparticle formation, providing insights into developing effective naringenin-loaded nanoparticles for therapeutic applications.

Keywords: Alginate, Electrospray, Encapsulation, Flow Rate, Nanoparticle, Naringenin.

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Received: 05-09-2024;
Revised: 15-11-2024;
Accepted: 06-04-2025.

INTRODUCTION

Flavonoids are natural occurring class of compounds as polyphenolic secondary plant metabolites that are mainly present in the food of plant origins such as vegetables, fruits, and nuts.[1] Naringenin is chemically named as 2,3-dihydro-5,7-dihydroxy-2-(4-hydroxyphenyl)- 4H-1-benzopyran-4-one with its molecular weight of 272.26 and the melting point of 251°C is one example of such a flavonoid compound that belongs to the flavanone subclass[2] as shown in the chemical structure in Figure 1.

Naringenin has been reported to exert a variety of pharmacological properties such as antioxidant, hepatoprotective, cardioprotective, anti-diabetic, anti-inflammatory, nephroprotective, anticancer, neuroprotective, gastrointestinal protective, and anti-atherogenic agent.[3] Despite possessing various pharmacological properties, naringenin found to be highly lipophilic in nature with poor aqueous solubility and low bioavailability, thus limiting its biological action and formulation development challenges.[4]

Nanotechnology or nanoparticle-based drug delivery systems has gained so much attention of recent, and it has been extensively studied for the treatment application of diseases especially cancer treatment.[5] This is due to the fact that nanoparticle-based drug delivery systems have many potential advantages in terms of the possibility to modify drug properties such as improving the solubility of hydrophobic drugs hence bioavailability owing to their unique characteristics physically and chemically which makes them superior compared to conventional drugs.[6] Generally, the nanoparticle can be defined as a tiny particle in which the size of the particle is within the range from 1 to 100 nanometers. Based on their physical characteristics such as

DOI: 10.5530/ijper.20256682

morphology and size as well as their chemical characteristics, nanoparticles can be classified into different classes which are lipid-based nanoparticles, polymeric nanoparticles which can be further classified into nanocapsules and nanospheres, as well as inorganic nanoparticles.[7-9]

Polymer-based nanoparticles are known as a colloidal system built of biocompatible and biodegradable natural or synthetic polymers in which the active ingredient is encapsulated and adsorbed onto a polymer matrix.[10] Alginate is one of the biodegradable polymers, which has been extensively studied for the development of drug delivery systems.[11] Alginate is a naturally occurring anionic polysaccharide made up of various arrangements of residues of a chain of biodegradable and biocompatible (1-4)-linked β-D-mannuronic acid and α-L-guluronic acid and it can be found abundantly distributed in the cell wall of a brown algae or Phaeophyceae species.[11] Alginate has unique biopharmaceutical properties including exhibiting pH sensitivity, biodegradability, biocompatibility as well as mucoadhesive nature and it does not appear to exhibit any toxicity and immunogenicity, to make this material interesting as a coating material and as a part of modified-release or controlled release drug formulation.[12] Furthermore, it has the capability to create gels through substitution of the sodium ions from guluronic acids for divalent cations like calcium ion, forming an "egg-box" shape.[13]

The electrospray method is one of the most effective methods for preparing nanoparticles as it has gain greater advantages including low cost, involve in one step, high reproducibility, and most importantly exhibit high Encapsulation Efficiency.[14] Furthermore, it has the capacity to create smaller particles, better size distribution, and less agglomeration. For this, the electrospray technique plays an important role in pharmaceutical applications.[15] The electrospray method works by applying a high voltage to a polymeric solution and forcing the polymer to emerge from the syringe as nanoparticles.[14] Despite this method is quick and simple, alginate polymer concentration could be one of the factors that could affect the production of nanoparticle using electrospray method. Therefore, this study was done to investigate the effect of different alginate polymer concentrations and flow rate on the production of nanoparticles loaded with naringenin using the electrospray method.

MATERIALS AND METHODS

Materials

Sodium Alginate (CAS no. 9005-38-3) was purchased from RandM Chemicals (Malaysia), Naringenin from grapeseed extract (CAS no. 67604-48-2, purity>98% HPLC) was purchased from Shaanxi Yuantai Biological Technology Co., Ltd (China), Ethanol (absolute ethanol, CAS no. 64-17-5), Tween 80 (CAS no. 9005-65-6) and Calcium Chloride (CAS no. 10043-52-4) were purchased from Sigma Aldrich (USA).

Preparation of sodium alginate loaded with naringenin formulation

General nanoparticle formulation preparation was adopted from Alallam *et al.*, (2020) with modification.[16] Nanoparticle formulation was prepared using materials composed of sodium alginate solution as a polymer, where naringenin was encapsulated with Tween 80 used as a surfactant that reduced the surface tension of the alginate solution. Sodium alginate solution (1% w/v) was prepared according to the procedure adopted from Cui *et al.*, (2022) with slight modification.[17] 5 g sodium alginate powder was weighed and dissolved in 500 mL of distilled water. The stock solution was then left on stir overnight at room temperature to achieve a homogenous state and to allow full hydration.[18]

Firstly, 0.1 g of naringenin powder was weighed to dissolve in 10 mL pure ethanol to produce naringenin solution with concentration of 10 mg/mL. The solution was kept in tight-closed container to prevent evaporation of naringenin solution. 10 mL of sodium alginate(1% w/v) was measured and taken into a beaker and while stirring, 10 mL of naringenin solution was added dropwise into the beaker containing sodium alginate. Then 0.1 mL of Tween 80 was added into the solution to produce alginate-naringenin solution up to the concentration of 0.5% v/v. These steps were repeated using the same amount and concentration of naringenin and Tween 80 but with different sodium alginate concentrations(0.5% w/v and 0.25% w/v) to produce alginate-naringenin solution to make up to final alginate concentrations of 0.25% w/v and 0.125% w/v respectively.

Nanoparticle production using electrospray method

The preparation of calcium chloride solution was adopted from Mehregan Nikoo *et al.*, (2016).[18] Firstly, 2 g of calcium chloride powder was weighed and then dissolved in 100 mL of distilled water to produce calcium chloride (2% w/v) solution that acts as a cross-linking agent. Electrospray system was set up according to the method adopted from Rutkowski *et al.*, (2018) with modification.[19] The set up consists of high voltage power supply unit, syringe pump, a syringe, plastic tube, a metal needle serving as nozzle, aluminum foil, positive and negative electrode, and collector as depicted in Figure 2 Briefly, 10 mL of alginate-naringenin solution with alginate final concentration of 0.5% w/v was loaded into 10 mL syringe. 10 mL of 2% w/v calcium chloride was put into petri dish that act as a collector. The electrospray was run for 1 hr. The parameters involved in the preparation of alginate nanoparticle loaded with naringenin using electrospray method were summarized in Table 1 and Table 2.

Electrospray mode identification

The effect of different sodium alginate concentrations on electrospray mode was investigated for alginate-naringenin

solution. The alginate-naringenin solution with sodium alginate with final concentrations of 0.5% w/v, 0.25% w/v and 0.125% w/v were studied. A camera (Industrial Microscope Camera with HDMI VGA two output) was set up and a laser was used to monitor the formation of the Taylor cone and spray formation.[20] Observation of the spray form under continuous light source is one of the simplest ways of mode identification.[21]

Particle size analysis and polydispersity index

Particle size and the Polydispersity Index (PDI) of nanoparticle were analyzed by dynamic light scattering using Malvern Zetasizer Nano series Nano-S (Model ZEN 1600) and Nano-Z (Model ZEN3600) (Malvern Instruments Ltd., Malvern, Worcestershire, UK). The median particle size (Di50) was evaluated to obtain the average particle size of nanoparticle, meanwhile Polydispersity Index was evaluated to determine the size distribution of the nanoparticle in terms of whether the sample is monodispersed or not.[22] Nanoparticle size and Polydispersity Index were measured at a detection angle of 173° and under the temperature of 25°C. The samples were diluted using distilled water at a 1:10 ratio to ensure optimal concentration.

Zeta potential

Zeta potential of nanoparticle was analyzed by dynamic light scattering using Malvern Zetasizer Nano series Nano-S and Nano-Z (Malvern Instruments Ltd., Malvern, Worcestershire, UK) under temperature of 25°C, where the nanoparticles were collected from Petri dish containing calcium chloride solution (2% w/v) and then transferred to 1-cm path length cuvette for the measurement.

Encapsulation Efficiency

The Encapsulation Efficiency of nanoparticles was measured. The method for determining Encapsulation Efficiency was adopted from Zare Kazemabadi *et al.*, (2019).[23] Alginate polymer loaded with naringenin nanoparticle was centrifuged at 14000 rpm for 30 min. After nanoparticle was subjected to centrifugation, a clear supernatant with a non-encapsulated naringenin in the nanocarrier was separated. The absorbance of this phase was measured using UV-visible spectrophotometer (Shimadzu/UV-1700, Kyoto, Japan) at 297 nm using a standard curve.

A standard curve was obtained by dissolving 50 mg of naringenin in 50 mL ethanol solution followed by a serial of dilution (0.5, 0.25, 0.125, 0.0625, 0.03125 and 0.015625 mg/mL). The absorbance of six standard samples were measured at $_{max}$ 297 nm. The percent Encapsulation Efficiency (EE) was calculated using the formula below (Equation 1).[24]

$$EE\ (\%) = \left[1 - \frac{Cf}{Ct}\right] x\ 100 \quad \text{..... Equation No. 1}$$

EE=Encapsulation Efficiency,

C_f=Free naringenin concentration,

C_t=Total naringenin concentration.

RESULTS

Effect of alginate concentrations on the electrospraying mode

In the absence of voltage, three samples of alginate-naringenin solutions were pumped at a flow rate of 0.5 mL/hr and exited the needle. The dripping mode could be clearly seen in Figure 3. When the applied voltage was increased slowly to 10 kV to the electro sprayed solutions of samples to create an electric field. It was depicted that the meniscus turned rapidly for sample C to a stable cone-jet spray mode known as Taylor cone, from which a jet of liquid emerged and became a stable spray.[25] On the other hand, the unstable multi-jet spraying mode of sample B and sample A respectively, when 10 kV voltage was applied as shown in Figure 4a, 4b and 4c below.

Particle size analysis

The results were obtained for the measurement of the particle size of nanoparticle for three samples (A, B, C) as shown in Table 3. In this analysis, Di50 and Polydispersity Index (PDI) of all the samples were compared. Sample C has the smallest average particle size (146 nm). Meanwhile, when lower sodium alginate concentration was used, sample A and sample B exhibit large average particle sizes of 900 nm and 1980 nm respectively.

Among these samples, sample B has the narrowest distribution as the sample has the largest average particle size, which might not be a good candidate for nanoparticle preparation. On the other hand, PDIs for sample A and sample C exhibit larger particle size distribution as 0.431 and 0.535 respectively compared to sample B.

Table 1: Parameters involved in the preparation of alginate nanoparticle loaded with naringenin using electrospray method.

Sample	Sodium Alginate final concentration (%w/v)	Voltage (kV)	Flow rate (mL/hr)	Needle size (Gauge)	Tip-to-collector distance (cm)	Calcium chloride concentration (%w/v)
A	0.125	10	0.5	27	5	2
B	0.25					
C	0.5					

Zeta potential

The results were obtained for the Zeta potential measurement of nanoparticles for different samples (A, B, C), in which sample C was slightly more anionic (-3.09 mV) compared to sample A and sample B (-1.10 mV and -1.43 mV) respectively based on the Zeta potential values as shown in Table 4 for different sodium alginate concentrations. It was interpreted as the concertation of the sodium alginate increased, there was an increasing trend of zeta potential of nanoparticles.

Encapsulation Efficiency

The results were tabulated for the measurement of Encapsulation Efficiency of nanoparticles for different samples (A, B, C), in which all three samples were exhibited with % good Encapsulation Efficiency in the range of 92.24-92.80 as shown in Table 5. Among three samples (A, B, C) sample C has shown highest percent Encapsulation Efficiency of 92.80 compared to encapsulation efficiencies of sample B and sample A (91.07 and 90.24) respectively. It was also noted that there was an increasing trend of Encapsulation Efficiency with increasing concentration of sodium alginate.

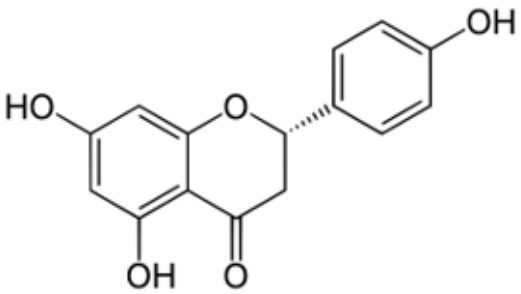

Figure 1: Chemical structure of Naringenin.

Effect of flow rate

All three formulations were made with the constant concentration of Alginate-Naringenin of 0.5% and 2% Calcium Chloride. Alginate solution (1% w/v) was prepared to maintain stable viscosity according to the procedure mentioned in the previous study done and it was left mixing for overnight to get a uniform formulation.[50] Further, Alginate solution was stirred for 3 min to allow fresh distilled water molecules to be in contact with Alginate particles resulting in a clump-free solution. While stirring, 10 mL of 10 mg/mL naringenin solution was mixed dropwise into the alginate solution to incorporate solution to produce Alginate-Naringenin nanoparticles (0.5% v/v) solution. 0.1 mL of Tween 80 was also added into Alginate-Naringenin solution as surfactant to reduce the surface tension producing a stable nanoparticles formulation.

The flow rate effect on Alginate nanoparticles loaded with Naringenin could be clearly seen and observations were tabulated in Table 6. The camera images in (a), (b) and (c) showed the spraying mode of spray solution of Alginate-Naringenin (0.5%

Table 2: Alginate nanoparticles loaded with Naringenin formulations (T1-T3).

Formulation	Process Parameter Flow rate (mL/hr)
T1	0.2
T2	0.3
T3	0.5

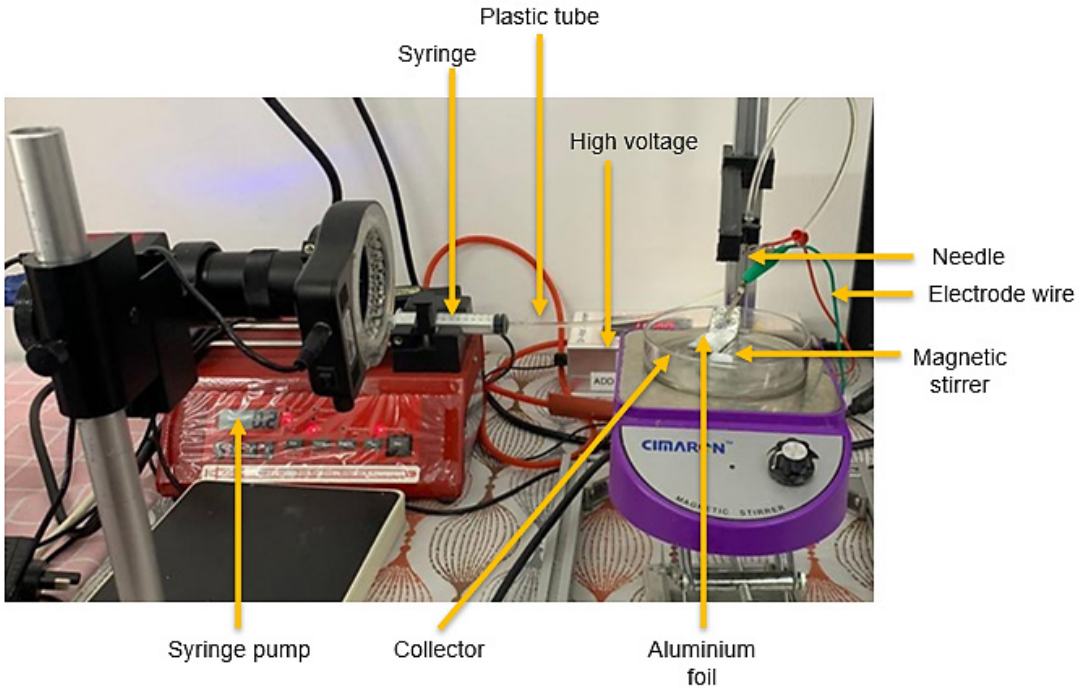

Figure 2: Electrospray setup.

Table 3: Particle size analysis of alginate nanoparticle loaded with naringenin.

Sample	Sodium Alginate final concentration (%w/v)	Di50 (nm)	PdI
A	0.125%	900	0.535
B	0.25%	1980	0.201
C	0.5%	146	0.431

Table 4: Zeta potential of alginate nanoparticle loaded with naringenin.

Sample	Sodium Alginate final concentration (%)	Zeta Potential (mV)
A	0.125	-1.10
B	0.25	-1.43
C	0.5	-3.09

w/v) solution with different flow rates employed as shown in Figure 5. Image (a) indicated with a stable Taylor's cone jet produced from T1 formulation with flow rate of 0.2 mL/hr, while image (b) showed an unstable cone jet produced from T2 formulation with flow rate of 0.3 mL/hr. An image (c) from T3 formulation with the flow rate of 0.5 mL/hr was produced with unstable cone jet and unstable multi jet.

Nanoparticle Size

Nanoparticle sizes were expressed in Di50 and PDI, known as nanoparticles with median diameter of 50% and polydispersity index. These two parameters are helpful to interpret the nanoparticles distribution within a given sample. Nanoparticle size analysis was done on three formulation samples (T1, T2, T3) with flow rates of 0.2, 0.3 and 0.5 mL/hr respectively as shown in Table 7.

It was clearly depicted from the results as the process parameter flow rate increased, both Di50 nanoparticles size and Polydispersity Index increased meanwhile T2 produced Di50 nanoparticles size of 215 nm with Polydispersity Index for formulations T1-T3. The small nanoparticle size (146 nm) was observed with PDI of 0.331 for formulation T1 at a low flow rate of 0.2 mL/hr.

Zeta Potential

Zeta potential analysis was done on all three formulation samples (T1-T3). T1 formulation with the flow rate of 0.2 mL/hr possessed a zeta potential of -1.10 mV, meanwhile as the flow rate increases for formulation (T2-T3) with flow rate of 0.3 mL/hr to 0.5 mL/hr, the zeta potential (-1.83 to -2.44 mV) value was shown increasing trend as shown in Table 8.

Encapsulation Efficiency

Encapsulation Efficiency, which is an indicator of drug loading efficiency, is one of the most significant parameters in developing nanoparticle drug delivery systems. Based on Table 9, the Encapsulation Efficiency has an increasing trend from

Figure 3: Formation of dripping mode under flow rate of 0.5 mL/hr in the absence of voltage of all samples.

T1-T3 formulation with T1 of 0.2 mL/hr flow rate has 92.80% Encapsulation Efficiency. On the other hand, formulation T2 of 0.3 mL/hr has 92.86% Encapsulation Efficiency meanwhile, T3 formulation with 0.5 mL/hr has the highest Encapsulation Efficiency value of 96.77%.

Calibration curve was done with different known concentrations pure Naringenin using UV-Visible Spectrophotometer at $_{max}$ of 297 nm. The linear regression equation of the standard curve was found to be y=2.591x-0.0942 with the correlation coefficient (R^2) of 0.9925. as shown in Figure 6.

DISCUSSION

Alginate nanoparticle loaded with naringenin using electrospray method

It has been reported that naringenin is soluble in the binary system that consists of ethanol and water.[26] 10 mg/mL of naringenin concentration was chosen to maximize the active ingredients being encapsulated in alginate nanoparticle. In this study, naringenin concentration higher than that resulted in cloudy solution.

Alginate-naringenin solution was prepared by employing different low sodium alginate solutions with three alginate final concentrations of 0.5%, 0.25% and 0.125%. In electrospray, the polymer solution is usually employed at low polymer concentrations and low polymer viscosity due to the relatively low electrostatic stretch over the surface tension during electrospray, which helps to form nanoparticles. When a very low polymer viscosity is used, it has resulted in the formation of whipping mode, due to instability of charged jet. Meanwhile, when a very

Table 5: **Encapsulation Efficiency of alginate nanoparticle loaded with naringenin.**

Sample	Sodium Alginate final concentration (%)	Absorbance	Unknown concentration of unencapsulated naringenin (mg/mL)	Total concentration of naringenin (mg/mL)	Encapsulation Efficiency (%)
A	0.125	2.4067	0.9760	10	90.24
B	0.25	2.2247	0.8929	10	91.07
C	0.5	1.7710	0.7182	10	92.80

Table 6: **Flow rate effect on Alginate nanoparticles loaded with Naringenin.**

Formulation	Process Parameter Flow rate (mL/hr)	Observation
T1	0.2	Stable Taylor's cone jet
T2	0.3	Unstable cone jet
T3	0.5	Unstable cone jet with unstable multi jet

high viscosity of polymers is used, there is a formation of fibers instead of nanoparticle formation.[27]

It has been reported that the surface tension of the alginate solution was significantly reduced when a nonionic surfactant was added to the preparation, thus resulting into stable cone-jet spray formation which is an important phenomenon to produce reduced particle size with narrow size distribution.[16] Tween 80 is a nonionic surfactant and considered to add to the mixture to prevent particles agglomeration in addition to surface tension reduction. In this study, it can be observed that addition of Tween 80 into sample C solution results in the formation of stable cone-jet spray mode (Figure 3) thus producing smaller particle size with narrow Polydispersity Index as depicted in Table 2.

After that, the charged droplets were collected in a petri dish containing a cross-linking solution with 2% w/v calcium chloride solution to facilitate the formation of alginate nanoparticle loaded with naringenin through ionotropic gelation reactions between calcium ion and alginate. Calcium alginate gels have a reputation for being an excellent drug carrier due to the ability of alginate's salts, such as sodium alginate, to be crosslinked by divalent calcium cations.[28] According to a study done by Mehregan Nikoo *et al.*, (2016), decrease in mean diameter of electrosprayed alginate has been observed with increasing calcium concentration up to 2% w/v.[18]

The process parameters including voltage, flow rate, nozzle diameter and distance between the tip of the nozzle and the collector were kept constant meanwhile the effect of different alginate concentration was studied. Flow rate of 0.5 mL/hr and voltage of 10 kV were chosen because it has been reported by Alallam *et al.*, (2020) that upon increasing the flow rate from 0.1 mL/hr to 0.5 mL/hr results in decrease particle size produced and when voltage above 8 kV was applied, the decrease in particle size has also been observed.[16]

A smaller needle (27G needle) was used as the nozzle to produce smaller particle size as increasing the needle diameter would lead to increased particle size. Besides that, the distance between the tip of the nozzle and the collector was kept at 5 cm because the distance further than that would lead to loss to the surrounding environment, hence reducing the yield of nanoparticle.[16]

Effect of alginate concentration on electrospray mode

Solution parameters such as polymer concentration have substantial impact on electrospray modes and nanoparticle production.[29] In this study, in the absence of voltage applied, the formation of dripping mode was observed for all the samples. When increasing voltage was applied until 10 kV, the formation of stable cone-jet spray mode was observed in sample C as shown in Figure 4a. Stable cone-jet spray mode is the most favorable electrospray condition for producing near-monodisperse polymeric nanoparticles compared to multi-jet spraying mode.[30] Another study by Noymer and Garel, (2000) reported that compared to the cone-jet, the multi-jet mode generates finer droplets but with a wider size distribution.[31]

However, when the lower alginate concentrations were employed, the formation of unstable multi-jet spraying mode of both sample A and B can be seen as depicted in Figure 4b and Figure 4c. This was due to very low polymer concentrations, where this was clearly supported in the literature, when very low polymer concentrations have been used, it resulted with weak viscosity of the solution thus making electrospray process unstable which in turn increases the particle size.[32]

On the other hand, it was emphasized clearly that by employing higher alginate concentrations (more than 1%) makes the solution too viscous to be exited through the nozzle tip due to higher alginate viscosity, occluding the nozzle tip, therefore preventing the stable cone jet formation.[16] Hence, it has been proven in this study the importance of alginate concentrations.

Particle size

A study has reported that 100 nm nanoparticles showed a greater uptake in comparison to 1 µm diameter particles and 10 µm particles. This is due to the larger surface area to volume ratio of nanoparticle as the particle size becomes smaller and thus, this will cause faster drug release.[33] Ales and Jeffrey M, (2008) has reported that particles with the size of 200 nm or larger have the tendency of getting cleared faster from the circulation because of lymphatic system activation.[34] Therefore, the optimal size for a nanoparticle appears to be around 100 nm since this size allows the particle to pass through the blood brain barrier, deliver sufficient amounts of drug due to the high surface area to volume ratio, and prevent quick clearance by the lymphatic systems.[33]

Sample C was exhibited with the best particle nanoparticle size (146 nm) compared to sample B and sample A in this study. This difference recorded in particle size were clearly because of different alginate concentrations. It was supported in a study conducted that the size of nanoparticle was reduced significantly when the concentration of polymer was reduced from 0.7% to 0.4%.[35] However, there was no further decreased observed in particle size and even small increase in nanoparticle size was observed when the polymer concentration was reduced from 0.4% to 0.1%. Furthermore, it was also reported that electrospray process becomes unstable when the polymer viscosity is reduced as a result of decrease in polymer concentration, hence this could explain the increase in observed nanoparticle size for the sample B and sample A in this study.

Table 7: Nanoparticle Size Analysis (Data expressed in Di50 and PDI).

Formulation	Process Parameter Flow rate (mL/r)	Nanoparticle Size Analysis	
		Di50 (nm)	PDI
T1	0.2	146	0.331
T2	0.3	215	0.664
T3	0.5	463	0.97

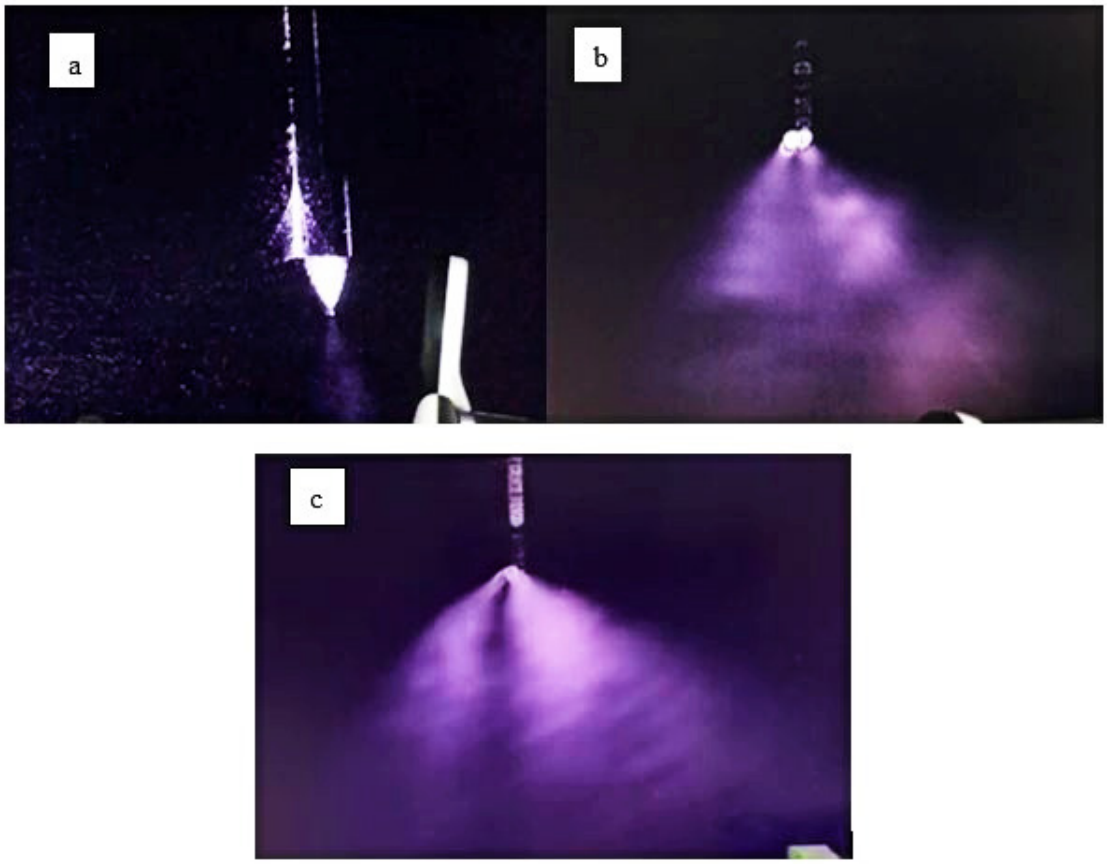

Figure 4: Formation of stable cone-jet spray mode of sample C (a) stable Taylor cone and (b) spray. (b) Unstable multi-jet spraying mode of sample B. (c) Unstable multi-jet spraying mode of sample A.

According to a study, when the concentration of alginate was increased from 0.5% to 1%, an increase in particle size from 512 nm to 4303 nm was observed due to an increase in the viscosity of polymer.[36] This resulted in wider cone thickness and longer duration of jet for the droplet to breakup as the viscosity acts in the opposite direction of gravity, hence producing large droplet size.[37]

Polydispersity Index (PDI)

On the other hand, Polydispersity Index (PDI) is an essential factor in determining nanoparticle size distribution dispersity ranging from 0 to 1, where PDI close to 0 indicates homogenous dispersion or monodisperse system and PDI close to 1 indicates high polydispersity.[38] PDI is also one of the important attribute as it determines the stability of nano delivery system.[39] According to Alallam *et al.*, (2020), majority consider PDI values of less than or equal to 0.3 to be optimal for a nanoparticle. However, PDI less than or equal to 0.5 is also reported to be acceptable for nanoparticle.[16] Sample C and sample B exhibited good Polydispersity Index. Despite good Polydispersity Index, sample B produced the largest particle size, therefore this concentration is not ideal to produce the ideal size of nanoparticle because particles with the size of 200 nm or larger have the tendency of getting cleared faster from the circulation.

Zeta potential

Zeta potential which refers to the electrical charge on the particle surface is one of the parameters assessed for nanoparticle produced as it determines the physical stability of colloidal systems.[40] In other words, the degree of electrostatic repulsion between similarly charged particles in dispersion is determined by the value of zeta potential.[41] As a result, samples with a high zeta potential which can be either negative or positive are electrically stable, thus reducing the likelihood of particle aggregation and floccule formation. Khalifa and Abdul Rasool, (2017) also reported that normally, particles that have zeta potentials that is more positive than +30 mV or more negative than -30 mV are considered strongly cationic and strongly anionic, respectively, and thus, are considered stable.[41]

In this study, all samples of alginate nanoparticle loaded with naringenin was found to be negatively charged. This could be explained by the usage of sodium alginate as it is anionic polymer in which it exhibits negative charge on its surface owing to the carboxylic acid groups.[42,43] As the sodium alginate concentration used increase, the result shows an increasing trend of zeta potential of nanoparticle from -1.10 to -3.09. One of the reasons would be due to polysaccharide concentration in which it has been reported that polysaccharide concentration was one of the most influential factors on zeta potential values where increase in the polysaccharide concentrations leads to more negative zeta potential values.[44] Alginate is one of the natural biopolymers that belongs to polysaccharide-based class.

However, according to Clogston and Patri, (2011), nanoparticles having a zeta potential between -10 mV and +10 mV are regarded to be approximately neutral.[45] The smaller magnitude of the zeta potential in this study could be due to an excess of positively charged calcium ions on the particle's surface, which then could impose a positive charge on nanoparticle.[46]

Encapsulation efficiency

Encapsulation Efficiency of the nanoparticle can be defined as amount of drug encapsulated in nanoparticle divided by initial amount of drug contained in polymer-solvent mixture.[47] Encapsulation Efficiency is one of the important parameters of nanoparticle as it determines the percentage of naringenin being successfully entrapped in the alginate nanoparticle. All the samples showed good Encapsulation Efficiency where increasing the sodium alginate concentration shows an increasing trend of Encapsulation Efficiency of nanoparticle.

It has been proven that electrospray has the main advantage over conventional encapsulating methods in terms of better Encapsulation Efficiency, narrow particle distribution and involves single-step processing which make it convenient.[48] According to the Huang *et al.*, (2018), when the polymer concentration was increased, the Encapsulation Efficiency significantly enhanced.

Table 8: Zeta Potential Analysis.

Formulation	Process Parameter Flow rate (mL/hr)	Zeta Potential (mV)
T1	0.2	-1.10
T2	0.3	-1.83
T3	0.5	-2.44

Table 9: Encapsulation Efficiency of Naringenin.

Formulation	Process Parameter Flow rate (mL/hr)	Absorbance (nm)	Unknown concentration of unencapsulated Naringenin (mg/mL)	Total concentration of Naringenin (mg/mL)	Encapsulation Efficiency(%)
T1	0.2	1.771	0.7182	10	92.80
T2	0.3	1.7605	0.7141	10	92.86
T3	0.5	0.7442	0.3228	10	96.77

This is due the risk of particle breaking was significantly lower in a more viscous polymer solution, which explains the observation that the drug encapsulations were higher at higher polymer concentrations.[49]

Flow rate effect on Alginate nanoparticles loaded with Naringenin

Based on Figure 6, camera images showed spray mode of different flow rate of 0.2, 0.3 and 0.5 mL/hr. Different flow rates produced different stability of cone jet. From (a) Stable Taylor's cone jet of T1 formulation was produced with the lowest flow rate of 0.2 mL/hr. From (b) had unstable cone jet from T2 formulation with flow rate of 0.3 mL/hr meanwhile (c) showed unstable cone jet with multijet from T3 Formulation with flow rate of 0.5 mL/hr.

Xu, Y. *et al.*, 2006 reported the suggested parameters with voltage supply of 12.5 kV and flow rate of 0.1- 0.2 mL/hr as the optimum values to produce smaller droplets size into the nanoparticles collector. This can be described by the uneven dispersion of the emulsion at the tip of the needle, which resulted in uncontrolled atomization and the formation of large particles as the flow rate increases.

It can be concluded with flow rate of 0.2 mL/hr and voltage supply of 10 kV produces smaller droplets size of nanoparticles as nanoparticles size may be reduced by increase of voltage that brings strong coulombic forces that increases the intensity of repulsion between adjacent molecules in producing small nanoparticles droplets size that is accompanied by the optimum flow rate.[50] Min Wang *et al.*, 2020 investigated that Taylor's cone is formed with other factors too such as liquid surface tension, electrostatic forces and gravity during electrospray mode.

Characterization of Alginate nanoparticles loaded with Naringenin

Nanoparticle Size

Particle size distribution is the most important parameter of characterization of nanoparticles. The primary use of nanoparticles is in drug release and drug targeting. It has been discovered that particle size influences drug release. Smaller particles have a greater surface area. Thus, this results in most of the drug loaded onto them is exposed to the particle surface, resulting in rapid drug release. Nanoparticles' small size and large surface area increase the dissolution rate and solubility of poorly soluble drugs.[50]

Media Diameter 50% (Di50)

The median diameter 50% tabulated in Table 9 ranging from 146-463 nm indicates increase of nanoparticle size with increase of flow rate. Based on the result written in Table 9, Di50 of T1

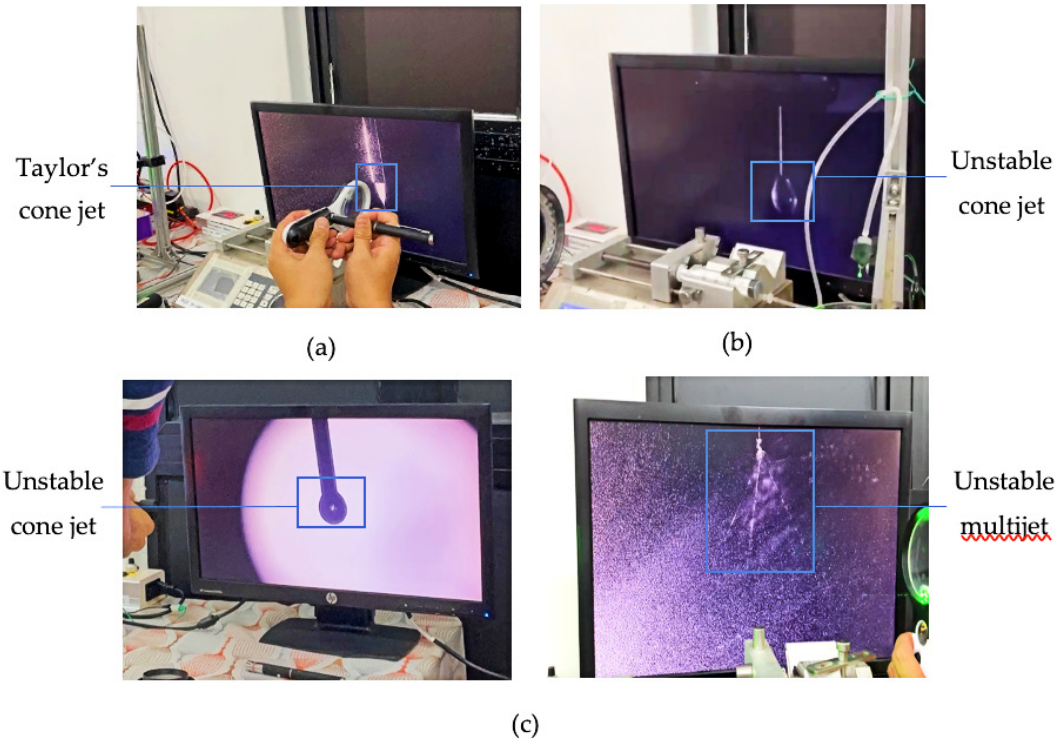

Figure 5: Camera images showing spray mode of Alginate-Naringenin 0.5% w/v solution with different flow rates: (a) Stable Taylor's cone jet; (b) Unstable cone jet and (c) Unstable cone jet with unstable multi jet. The constant process parameters were needle diameter of 27G, applied voltage of 10kV, Alginate-Naringenin concentration of 0.5% and distance between needle tip to collector of 5 cm.

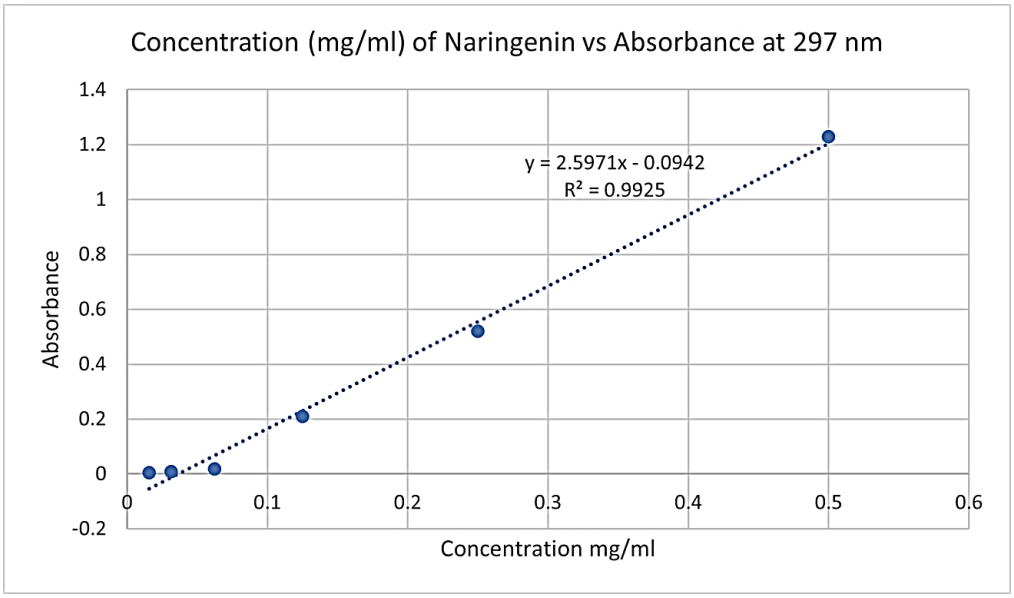

Figure 6: Calibration curve of Naringenin in Ethanol.

formulation of 146 nm is the only one that is within the acceptable range of 100-200 nm. It has been demonstrated that nanoparticles with sizes ranging from 100-200 nm have the ideal characteristics for cellular uptake. An optimal size range can result in improved cellular uptake.[51]

Polydispersity Index (PDI)

The PDI values were shown an increasing trend with the increase of flow rate from 0.2 to 0.5 mL/hr as per Table 9. The Polydispersity Index (PDI) is crucial in determining the dispersity of nanoparticles distribution within a given sample.[52] The range of the PDI numerical value is considered 0.0 (for a sample with uniform particle size) to 1.0. (For a highly polydisperse sample with multiple particle size populations). For polymer-based nanoparticle material, the PDI values 0.2 are most frequently considered acceptable in practice.[18] However, according to a recent study, the optimum value of PDI is ≤0.3.[16] In addition, PDI values are also acceptable ≤0.5.[53]

Zeta Potential

The surface charge-dependent zeta potential is important for the stability of nanoparticles in suspension and is also a major factor in the initial adsorption of nanoparticles onto the cell membrane. The rate of endocytic uptake after adsorption is determined by particle size. Nanoparticle toxicity is thus affected by zeta potential and size.[54]

Zeta potentials were shown for all 3 formulations as tabulated in Table 9 ranging from -1.10 mV to -1.83 mV. Zeta potential determination is a crucial method for characterizing nanocrystals that may be used to calculate the surface charge and determine the physical stability of nanosuspensions.[55] In general, it is believed that a zeta potential value outside of the range of -30 mV to +30 mV has enough repulsive force to improve physical colloidal stability.[56] Numerous investigations have demonstrated stable nanoparticles made using non-ionic surfactants that have low surface charges.

For zeta potential, pH is the most crucial variable. For instance, adding acid to a nanofluid will cause the pH to drop, which will increase the positive charges on the particle surface. The zeta potential will rise. An isoelectric point is the location where there is zero electrophoretic mobility.[57]

Thus, in this part of study, the zeta potential of all 3 formulations were within the range of -30 mV to +30 mV regardless of the low surface charges closely to neutral range as nanoparticles with non-ionic surfactants such as Tween 80 produced stable nanoparticles as it might be one of the factors that leads to the low surface charges.

Encapsulation Efficiency

Crosslinking is essential in the context of alginate polymer encapsulation. The degree of cross-linking at the surface of the extruded emulsion droplet, as well as emulsion stability, influenced Encapsulation Efficiency.[58] Encapsulation Efficiency all across 3 formulations in Table 9 shows ≥90% in which T1 has 92.80% efficiency, followed by T2 with 92.86% and the highest efficiency showed by T3 with 96.77%.

Based on Alallam, B. *et al.*, 2020, high EE was noted in electrospraying literature, where it was discovered to be greater than 80%. In addition, electrospray method provides an advantage of producing high EE which can be further explained by the strong crosslinking of nanoparticles outer shell. The crosslinking is important to evaluate the Encapsulation Efficiency of nanoparticles with alginate polymers.[58] The Encapsulation Efficiency of formulation depends on the type of API and resultant API-polymer interactions.[58]

Naringenin is highly soluble in methanol but has a relatively limited water solubility.[26] In Figure 6, Naringenin showed an absorbance maximum on 297 nm which was used in the UV spectrophotometric determinations with a concentration range R^2=0.9925. Based on a study done by Jha, D. K. *et al.*, 2020, it showed a linearity in the concentration range of R^2>0.99 using UV spectrophotometer which was chosen to be economical, and less time required in comparison with chromatographic analysis that is costly and time-consuming.

T1 has the lowest Encapsulation Efficiency with 92.80% in comparison to the T3 that has the highest Encapsulation Efficiency of 96.77% even though T1 formulation produced a stable Taylor's cone shape that represents the optimum nanoparticle formation. The possible attributing factor that may result in lower Encapsulation Efficiency in T1 was due to the slow flow rate contributing to the longer settlement time to encapsulate nanoparticles that may result in increased aggregation. Other than that, the friction force produced with higher flow rate in T3 with 0.5 mL/hr that produces nanoparticles that has greatest Encapsulation Efficiency.

CONCLUSION

This study was done to determine the best concentration of sodium alginate formulation for production of alginate nanoparticle loaded with naringenin using electrospray method. It can be concluded that sample C with alginate final concentration of 0.5% w/v as a polymer employed was chosen as the best concentration in producing alginate nanoparticle loaded with naringenin as the evaluation study done on the formulation meets all the criteria for nanoparticle. For example, the alginate nanoparticle loaded with naringenin produced forms stable cone-jet spray mode, has optimal particle size which is 146 nm, narrow polydispersity index, higher zeta potential and optimal Encapsulation Efficiency which accounts for 92.80% compared to other samples. Different sodium alginate concentrations significantly influenced the electrospray mode formation and nanoparticle production.

ACKNOWLEDGEMENT

We would like to express our sincere gratitude to Espray Sdn Bhd for generously providing us with the opportunity to utilize their facilities and equipment during the course of our research. We extend our heartfelt appreciation to the staff for their invaluable assistance and commitment to advancing scientific knowledge in our field.

FUNDING

UoC Research Grant Scheme (CRG/01/02/2021) from University of Cyberjaya, Malaysia.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

PDI: Polydispersity index; **EE:** Encapsulation Efficiency.

SUMMARY

Naringenin, a polyphenolic phytochemical with notable pharmacological properties, faces limitations in therapeutic application due to its hydrophobic nature, resulting in poor aqueous solubility and delivery to target sites. This study aimed to address these challenges by encapsulating naringenin in alginate nanoparticles using an electrospray method. Different concentrations of alginate and flow rates were investigated to optimize nanoparticle production. Results showed that a 0.5% w/v alginate concentration and a flow rate of 0.2 mL/hr yielded nanoparticles with optimal characteristics, including a particle size of 146 nm, narrow Polydispersity Index, high zeta potential, and Encapsulation Efficiency of 92.80%. These findings demonstrate the significant influence of alginate concentration and flow rate on nanoparticle production, providing insights for the development of high-quality naringenin nanoparticles for potential therapeutic applications.

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Cite this article: Razak FSA, Bin LK, Rahman SA, Rashid NIAA, Malek NAA, Lokesh BVS, *et al*. Investigation on the Effect of Alginate Concentration and Flow Rate on Production of Nanoparticle Loaded with Naringenin Using Electrospray Method. Indian J of Pharmaceutical Education and Research. 2025;59(3):995-1006.