การเตรียมและการศึกษาคุณสมบัติของอนุภาคนาโนโบไวน์ซีรั่มอัลบูมินบรรจุเคอร์คิวมิน
Preparation and Characterization of Curcumin-loaded Bovine Serum Albumin Nanoparticles

นิพนธ์ต้นฉบับ

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บทคัดย่อ

วัตถุประสงค์: เพื่อศึกษาหาวิธีการเตรียมอนุภาคนาโนโบไวน์ซีรั่มอัลบูมินบรรจุเคอร์คิวมิน เพื่อให้มีคุณสมบัติทางเคมีฟิสิกส์และปริมาณการกักเก็บยาตามต้องการ **วิธีการศึกษา:** เตรียมอนุภาคนาโนของโบไวน์ซีรั่มอัลบูมินบรรจุเคอร์คิวมินด้วยวิธีดีโซลเวชั่น โดยไม่ใช้กลูตาร์อัลดีฮัยด์เป็นสารก่อครอสลิงค์ และศึกษาสภาวะต่าง ๆ ในการเตรียม ได้แก่ ความเข้มข้นของโบไวน์ซีรั่มอัลบูมิน และอัตราส่วนโดยปริมาตรของน้ำต่อเอทานอล ที่มีผลต่อขนาดอนุภาค การกระจายของขนาดอนุภาค และศักย์ไฟฟ้าบนผิวอนุภาค รวมทั้งวิธีการบรรจุเคอร์คิวมินที่แตกต่างกัน 3 วิธีลงในอนุภาคนาโนที่ส่งผลต่อประสิทธิภาพและปริมาณการกักเก็บยา **ผลการศึกษา:** การเพิ่มความเข้มข้นของโบไวน์ซีรั่มอัลบูมินรวมทั้งปริมาณน้ำในส่วนผสมน้ำ-เอทานอล ทำให้ขนาดอนุภาคนาโนใหญ่ขึ้น และประจุไฟฟ้าลบบนผิวอนุภาคลดลง สภาวะการเตรียมที่เหมาะสมคือ ความเข้มข้นของโบไวน์ซีรั่มอัลบูมินร้อยละ 20 โดยน้ำหนัก และอัตราส่วนโดยปริมาตรของน้ำต่อเอทานอล 1:6 อนุภาคนาโนของโบไวน์ซีรั่มอัลบูมินบรรจุเคอร์คิวมินที่เตรียมได้มีขนาดอยู่ในช่วง 195.14 ± 9.11 ถึง 256.80 ± 72.06 นาโนเมตร ค่าดัชนีการกระจายขนาดที่แคบมากอยู่ในช่วง 0.064 ± 0.024 ถึง 0.092 ± 0.049 และค่าศักย์ไฟฟ้าบนผิวอนุภาคอยู่ในช่วง -8.10 ± 0.30 ถึง -9.73 ± 0.63 มิลลิโวลต์ ประสิทธิภาพและปริมาณการกักเก็บยาอยู่ในช่วงร้อยละ 17.40 ± 8.23 ถึง 28.59 ± 3.52 และร้อยละ 1.77 ± 0.59 ถึง 2.46 ± 0.35 ตามลำดับ วิธีบรรจุเคอร์คิวมินในอนุภาคนาโน 3 วิธีไม่มีผลต่อคุณลักษณะทางเคมีฟิสิกส์ ประสิทธิภาพและปริมาณการกักเก็บยา **สรุป:** สามารถเตรียมอนุภาคนาโนโบไวน์ซีรั่มอัลบูมินบรรจุเคอร์คิวมินที่มีคุณสมบัติตามต้องการ โดยไม่ใช้กลูตาร์อัลดีฮัยด์ และวิธีการบรรจุเคอร์คิวมิน 3 วิธีไม่มีผลต่อคุณลักษณะทางเคมีฟิสิกส์ ประสิทธิภาพและปริมาณการกักเก็บยา

คำสำคัญ: เคอร์คิวมิน; อนุภาคนาโน; วิธีดีโซลเวชั่น; โบไวน์ซีรั่มอัลบูมิน

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Original Article

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Abstract

Objective: To explore various preparation by desolvation methods of curcumin-loaded bovine serum albumin nanoparticles (CUR-BSA-NPs) to obtain desired physicochemical properties and drug loading. **Method:** CUR-BSA-NPs were prepared by desolvation method without using glutaraldehyde as cross-linking agent. The influences of bovine serum albumin (BSA) concentration and water to ethanol volume ratio on size, size distribution, and particle surface charge were examined. The effects of 3 different loading methods of CUR into CUR-BSA-NPs on entrapment efficiency and loading capacity were also investigated. **Results:** The increase in BSA concentration and amount of water in water-ethanol mixture resulted in larger BSA-NPs with less negative surface charge. The optimum conditions from the screening results using 20 mg/mL BSA concentration and 1:6 water to ethanol volume ratio were chosen for preparation of CUR-BSA-NPs with 3 different loading methods. The sizes of CUR-BSA-NPs ranged from 195.14 ± 9.11 to 256.80 ± 72.06 nm and polydispersity indices of extremely narrow size distribution ranged from 0.064 ± 0.024 to 0.092 ± 0.049. The zeta potentials were in the range from -8.10 ± 0.30 to -9.73 ± 0.63 mV. The entrapment efficiency and loading capacity were in the range from 17.40 ± 8.23 to 28.59 ± 3.52 % and 1.77 ± 0.59 to 2.46 ± 0.35 %, respectively. The 3 different CUR loading methods into CUR-BSA-NPs were found to impose no significant effects on physicochemical characteristics, entrapment efficiency, and loading capacity. **Conclusion:** The CUR-BSA-NPs with desired properties could be developed by desolvation method without using glutaraldehyde. The 3 different loading methods of CUR into CUR-BSA-NPs had no significant effects on physicochemical characteristics, entrapment efficiency, and loading capacity of CUR-BSA-NPs.

Keywords: curcumin; nanoparticles; desolvation method; bovine serum albumin

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Introduction

It has previously been shown in a substantial number of studies that curcumin, a well-known yellow powder in category of polyphenols obtained from extraction of *Curcuma longa*, has bright prospects for many pharmacological effects such as anti-tumor, anti-inflammatory, antioxidant, and anti-cholesterol. Recently, curcumin has also been found to have lowering effects of total blood cholesterol levels, i.e., LDL-cholesterol and triglyceride as well as increasing blood HDL-cholesterol level.[1] However, curcumin has low permeability and gastrointestinal absorption due to its poor water solubility and less stability, leading to low oral bioavailability.[2,3]

Now-a-days, various types of nanocarriers could be developed in order to overcome low bioavailability problems of many drugs due to their extremely small particle sizes within

the range of 100 – 500 nm, which could be endocytosed by the cells.[4] Basically, these nanocarriers with different size ranges could be prepared by various methods using proper biodegradable materials such as proteins, polymers, or lipids. Among many types of nanocarriers, nanoparticles prepared from proteins such as bovine serum albumin (BSA) are of interest since they possess diversity of reactive sites on their surface.[5] As a result, high drug loading capacity along with nanosized range could be achieved by simple preparation methods.[6]

Basically, development of protein nanoparticles could be carried out by various methods such as desolvation, emulsification-solvent evaporation, nanoprecipitation, etc.[5] It has been reported that curcumin-entrapped albumin nanoparticles could be prepared by using desolvating agent to replace solvent in the system, followed by using cross-linking agent such as glutaraldehyde to harden and stabilize the nanoparticles.[6-8] However, glutaraldehyde was reported to be toxic to human body.[9] Interestingly, nanoparticles of BSA could be developed by desolvation method but using thermal curing instead of cross-linking agent in hardening process in order to avoid toxic effects to human body.[10,11] Preparation of nanoparticles by desolvation method could be prepared by addition of desolvating agent such as ethanol and acetone into aqueous solution of macromolecules to compete the solvent and allow nanoparticles to occur. Generally, process of desolvating agent addition is done by adding anti-solvent dropwise into solution of natural macromolecules to allow competition of anti-solvent. Moreover, there is another technique for addition of desolvating agent by submergingly pumping or injecting desolvating agent into solution of macromolecules such as BSA.[12,13] The objectives of this study were thus to prepare nanoparticles of BSA with loaded curcumin by using modified desolvation methods without using glutaraldehyde in hardening process as well as examine the effects of processing variables on their physicochemical properties and drug loading.

Methods

Chemicals

Bovine serum albumin (BSA) (Fraction V) (Lot No. K43146848 238) was purchased from Merck, Darmstadt, Germany. Curcumin (CUR) extract from *Curcuma longa* (Lot No. SLBH2403) was obtained from Sigma-Aldrich, St. Louis, Missouri, USA. Absolute ethanol anhydrous (Batch No. V8H029108H) was from Carlo Erba, Val-de-Reuil, France. Sterile water for injection (SWI) (Lot No. 0690817) was from Thai Nakorn Patana, Bangkok, Thailand. Bradford® dye reagent was from Bio-Rad, CA, USA. All chemicals used were of analytical or reagent grade.

Preparation of BSA nanoparticles (BSA-NPs)

Eight formulations of blank BSA-NPs were prepared by desolvation method as described previously with some modifications.[10,11,14] The concentration of BSA and volume ratio of water to ethanol were varied as shown in Table 1. Firstly, BSA was dissolved in 10 mL of SWI (pH 7.0) and incubated for 20 min at room temperature. Subsequently, ethanol was submergingly pumped through a peristaltic pump (Masterflex L/S Easy-load Model 7518-00, Cole-Parmer, Vernon Hills, IL, USA) into the mixture through a 0.5-mm spinal needle at 10 mL/min with continuous stirring at 500 rpm. The obtained BSA-NPs dispersions were then stirred for 10 min and subject to the hardening process by placing it on a hotplate stirrer (Dragon Lab Model MS7-H550-S, Ponpe, China) at 70 °C for 2 h. The BSA-NPs dispersions were kept at 4 °C in air-tight containers protected from light before characterization.

Table 1 Concentration of BSA and volume ratio of water to ethanol in preparation of blank BSA-NPs.

No.	Formulation	BSA concentration (mg/mL)	Water volume (mL)	Ethanol volume (mL)	Water to ethanol volume ratio
1	B1E6P10	10	10	60	1:6
2	B2E1P10	20	10	10	1:1
3	B2E2P10	20	10	20	1:2
4	B2E3P10	20	10	30	1:3
5	B2E6P10	20	10	60	1:6
6	B2E12P10	20	10	120	1:12
7	B4E6P10	40	10	60	1:6
8	B6E6P10	60	10	60	1:6

Preparation of CUR-loaded BSA-nanoparticles (CUR-BSA-NPs)

CUR-BSA-NPs were prepared by desolvation method with 3 different CUR loading methods. Firstly, BSA 200 mg was dissolved in 10 mL of SWI (pH 7.0) and then incubated for 20 min at room temperature. In Method A, CUR 10 mg was added with stirring in BSA solution, after which 60 mL of ethanol was pumped through a peristaltic pump submergingly into the mixture through a 0.5-mm spinal needle at 10 mL/min with continuous stirring to obtain NPs dispersion. In Method B,

CUR 10 mg was dissolved in 60 mL of ethanol which was subsequently pumped through a peristaltic pump submergingly into BSA solution through a 0.5-mm spinal needle at 10 mL/min with continuous stirring to obtain NPs dispersion. In Method C, CUR 10 mg was finally incorporated into the formerly obtained BSA-NPs dispersion. The mixture was then stirred for 20 min at room temperature and subject to the hardening process by placing it on a hotplate stirrer at 70 °C for 2 h. The volume was finally adjusted to 70 mL with ethanol.

The CUR-BSA-NPs dispersions were purified by using the ultrafiltration technique with centrifugal filter unit (Vivaspin®, 100,000 MWCO, GE Healthcare, Little Chalfont, Buckinghamshire, UK) and centrifugation (Ortoalresa Universal Centrifuge Model UNICEN 21, Ortoalresa, Madrid, Spain) at 1,000 g and 25 °C for 90 min. The samples were finally adjusted to initial volume with SWI and kept at 4 °C in air-tight and light-resistant containers until use.

Physicochemical characterizations of CUR-BSA-NPs

Particles size

Particle size and polydispersity index (PdI) of CUR-BSA-NPs were determined by dynamic light scattering technique (DLS) (Zetasizer®, Nanoseries, Malvern, Worcestershire, UK). The samples with no dilution were measured at 25 °C and a scattering angle of 173 °. The technique used was the scattering of light in order to detect the Brownian motion of particles in a liquid. The smaller the particles were, the faster they moved, inversely in line with the decline of the autocorrelation function. The mean particle size was calculated from triplications via Einstein equation.

Zeta potential

Zeta potential on the surface of CUR-BSA-NPs was determined with no dilution by measuring the direction and velocity that particles moved in the applied electric field of 150 V at 25 °C using particle electrophoresis (Zetasizer®, Nanoseries, Malvern, Worcestershire, UK). The mean zeta potential was calculated in triplications using Helmholtz-Smolsuchowski equation.

BSA-NPs recovery

The percentage recovery of BSA-NPs was determined by using Bradford assay, which involves the binding of Coomassie Brilliant Blue G-250 dye to proteins. The amount of BSA in both NPs and filtrates collected from ultrafiltration unit and centrifugation at 1000 g and 25 °C for 90 min during purification process were examined. Firstly, 250 µL of Bradford dye reagent was added into each well of 96-well plate followed by 5 µL of BSA-NPs dispersions or filtrates. Having incubated for 10 min at room temperature, the consequent increase in absorbance was measured at 595 nm by using a UV microplate reader (FLUOstar® Omega, Ortenberg, Germany). The BSA-NPs dispersion prior to purification process was diluted stepwise with SWI to the given concentrations in order to create BSA calibration curve. Calibration curve with the coefficient of determination (r^2) for linear regression of at least 0.999 was acceptable. The percentage yield was calculated as follows:

$$\% \text{ Yield} = \frac{\text{Amount of BSA recovered}}{\text{Initial amount of BSA added}} \times 100 \quad (1)$$

Drug entrapment

The amount of CUR entrapped in CUR-BSA-NPs was determined by subtracting the initial amount of CUR added into the system by the amount of unentrapped CUR. The dispersion of CUR-BSA-NPs was transferred to ultrafiltration unit and centrifuged at 1,000 rpm for 90 min. The filtrates were then collected, and the amount of unentrapped CUR was determined by UV/Visible spectrophotometer (Shimadzu Model UV-1800, Shimadzu, Kyoto, Japan) at wavelength of 429 nm. The entrapment efficiency and loading capacity were calculated respectively as follows:

$$\% \text{ Entrapment efficiency} = \frac{\text{Initial amount of CUR added into the system} - \text{amount of unentrapped CUR}}{\text{Initial amount of CUR added into the system}} \times 100 \quad (2)$$

$$\% \text{ Loading capacity} = \frac{\text{Initial amount of CUR added into the system} - \text{amount of unentrapped CUR}}{\text{Amount of carrier BSA recovered}} \times 100 \quad (3)$$

Data analysis

Results are reported as mean ± standard deviation. The statistically significant differences between means of sample groups were evaluated by using the non-parametric statistics with Kruskal-Wallis test and Dunnett's Multiple Comparison test from GraphPad Prism 5 software. A P-value of less than 0.05 was considered to be statistically significant.

Results and Discussions

Preparation of BSA-NPs

In this study, BSA-NPs were produced by desolvation method via addition of ethanol as desolvating agent into

aqueous solution of BSA.[10,11] On addition of ethanol, dissolved BSA became desolvated causing an increase in turbidity and the system reverted to a pre-coacervated state. Further addition led to a formation of protein aggregates as soon as a certain degree of desolvation was obtained. The initially clear solution gradually turned opalescent as the protein became less soluble in water and tended to form nanometric aggregates, leading to a milky suspension.[10]

Factors affecting physicochemical properties of BSA-NPs

Production parameters affecting nanoparticle characteristics previously reported were BSA concentration as natural macromolecule, ratio of water and desolvating agent, rate of desolvating agent addition, and also stabilization condition.[11]

BSA concentration

The effect of BSA concentration on size and size distribution as well as zeta potential of the obtained BSA-NPs are shown in Figure 1, which were in the ranges of 155.63 ± 1.38 to 449.00 ± 1.30 nm and -7.24 ± 1.38 to 0.15 ± 0.09 mV, respectively. The pH of obtained BSA-NPs were around 7.0 according to the SWI as dispersion medium. It was shown that the sizes were larger with increasing BSA concentrations while the size distributions were still narrow within the range of mono-disperse with PdI < 0.4.[15] It could be explained that high BSA concentrations could increase the chance of protein molecules to undergo electrostatic and hydrophobic interactions, leading to more extent of coagulation and larger particles thereafter.[16] Since the isoelectric point (pI) of BSA in water at 25 ºC is 4.7 [16], the surface charge of BSA-NPs would be slightly negative in neutral pH of the system. As a result, protein– protein interactions were enhanced along with an increase in coagulation among BSA molecules, resulting in larger particles. Surface hydrophobicity tends to let nonpolar amino acid groups interact with each other, leading to coagulations among protein molecules. Therefore, when hydrophobic interactions predominate electrostatic interactions as in the case of pH close to pI or at high BSA concentrations, large BSA-NPs with weak negative surface charge will be obtained.[16]

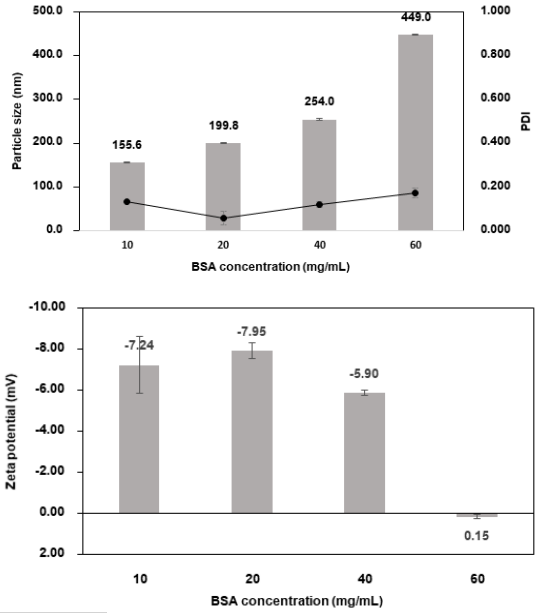

Figure 1 The effect of BSA concentration on size and size distribution (above) and zeta potential (below) of the obtained BSA-NPs. (Water to ethanol volume ratio = 1:6, ethanol pump rate = 10 mL/min, mean ± SD, n = 3).

Water to ethanol volume ratio

The effect of water to ethanol volume ratio on size and size distribution as well as zeta potential of the obtained BSA-NPs are shown in Figure 2, which were in the ranges of 199.83 ± 1.24 to 343.57 ± 1.40 nm and -7.95 ± 0.38 to 0.13 ± 0.16 mV, respectively. The size distributions were also narrow with PdI < 0.4[15], except that of 1:1 water of ethanol volume ratio with PdI of 0.86 and small mean size. Low amount of ethanol as desolvating agent might not be adequate to thoroughly precipitate the whole quantity of protein so that partially aggregated BSA-NPs with wide size distribution were obtained.[17] On the other hand, increasing the amount of ethanol in volume ratio of water to ethanol up to 1:2 resulted in significantly larger nanoparticles but abrupt decline in size distribution. Nevertheless, continuous increase in the amount of ethanol resumed decreasing nanoparticles with narrow size distribution again, which might be because of adequate amount of ethanol to precipitate BSA at higher extent. Besides, higher amount of ethanol would reduce the degree of water swelling around the protein matrix and hence even smaller particle size.[18] Such effect might result in predominating electrostatic interactions rather than hydrophobic interactions and hence higher surface charge of the obtained BSA-NPs.[11] It was mentioned elsewhere that higher BSA concentration or lower amount of ethanol will

result in a significant reduction of negative surface charge due to larger particle size and less surface area of BSA-NPs.[11]

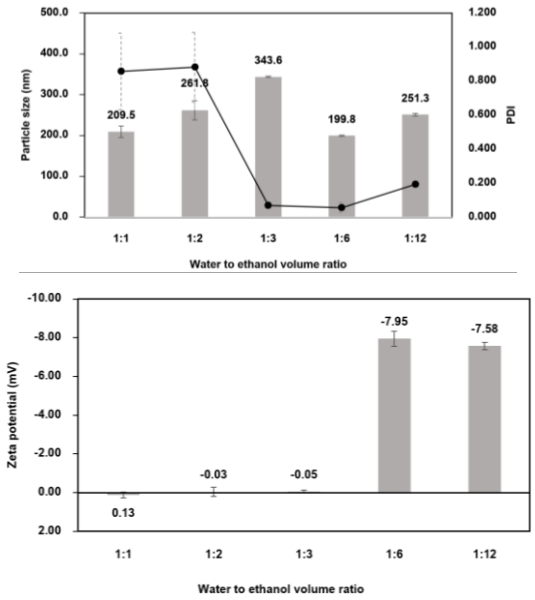

Figure 2 The effect of water to ethanol volume ratio on size and size distribution (above) and zeta potential (below) of the obtained BSA-NPs. (BSA concentration = 20 mg/mL, ethanol pump rate = 10 mL/min, mean ± SD, n = 3).

Preparation of CUR-BSA-NPs by various CUR loading methods

From the screening results above, the preparation conditions of 1:6 water to ethanol volume ratio, 20 mg/mL BSA concentration, and 10 mL/min ethanol pump rate were subsequently selected for further preparation of CUR-BSA-NPs by 3 different loading methods of CUR into nanoparticles due to their smaller sizes with narrow size distributions and acceptable values of zeta potential. The active ingredients can be incorporated both in the aqueous phase (solution of macromolecules) if they are sufficiently water soluble, or together with alcohols (desolvating agent) if they are soluble in alcohols.[11] In this study the active ingredient CUR was either dispersed in aqueous solution of BSA before desolvation (Method A), or dissolved in desolvating agent ethanol prior to desolvation (Method B), or directly incorporated into the obtained BSA-NPs dispersion after desolvation (Method C).

Physicochemical characterization of prepared CUR-BSA-NPs

The mean sizes and zeta potentials of CUR-BSA-NPs prepared by various CUR loading methods, i.e. Methods A, B, and C are shown in Figure 3, which were in the ranges of 195.17 ± 9.11 to 256.80 ± 72.07 nm and -8.10 ± 0.30 to -9.73 ± 0.63 mV, respectively. The size distributions were monodispersed with PdI values of 0.064 – 0.092.[15] Although the mean size of CUR-BSA-NPs loading by Method B seemed to be larger than the other 2 Methods, there were no statistically significant differences in their size among the 3 Methods (P-value > 0.05). There were no statistically significant differences in zeta potential among the 3 Methods as well (P-value > 0.05). The sizes of CUR-BSA-NPs obtained from all 3 loading methods in this study were within the range of 100 – 500 nm along with the monodisperse distribution so that they could readily be endocytosed by the cells.[4]

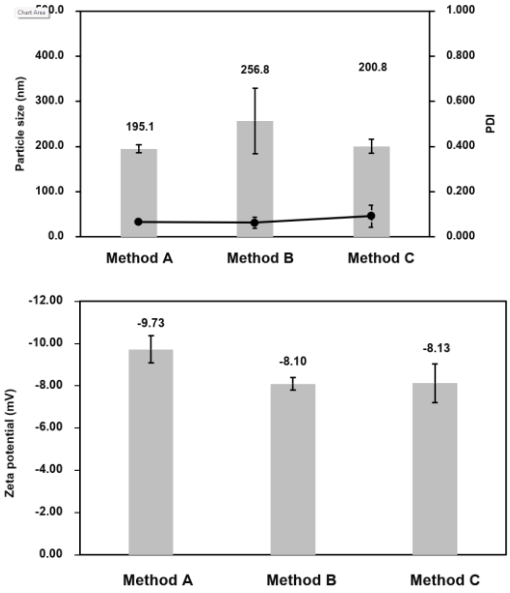

Figure 3 Size and size distribution (above) and zeta potential (below) of CUR-BSA-NPs prepared by desolvation method with 3 different CUR loading Methods. (Water to ethanol volume ratio = 1:6, BSA concentration = 20 mg/mL, ethanol pump rate = 10 mL/min, mean ± SD, n = 3).

The values of entrapment efficiency and loading capacity of CUR-BSA-NPs prepared by desolvation method with 3 different CUR loading methods as shown in Figure 4 were in the ranges of 17.40 ± 8.23 to 28.59 ± 3.52 % and 1.77 ± 0.59 to 2.46 ± 0.35 %, respectively. Among 3 CUR loading methods, it was found that both entrapment efficiency and loading capacity with Method B was lowest as compared to the other 2 Methods. However, there were no statistically significant differences in their values of entrapment efficiency and loading capacity among the 3 Methods (P-value > 0.05). Basically, the noncovalently reversible drug binding sites

present in the albumin molecule were major advantages of albumin nanoparticles for their high binding capacity of various drugs.[5] It was reported that CUR molecules bound to amino acid residues via hydrogen bonds and hydrophobic forces. If BSA molecules are in unfolding form, it will possess more hydrophobic cavities that could be ready to interact with CUR molecules.[19] Generally, the best chance to achieve efficient incorporation will occur when the interaction between drug and macromolecule is stronger than that between drug and solvent.[20]

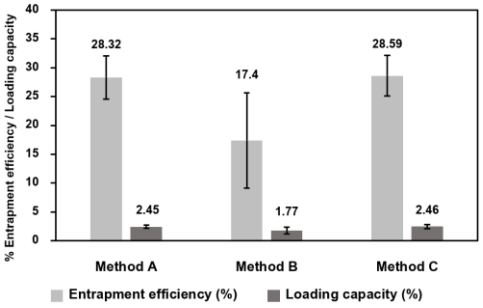

Figure 4 Entrapment efficiency and loading capacity of CUR-BSA-NPs prepared by desolvation method with different CUR loading Methods. (Water to ethanol volume ratio = 1:6, BSA concentration = 20 mg/mL, ethanol pump rate = 10 mL/min, mean ± SD, n = 3).

Loading of CUR with Method B, in which CUR was firstly dissolved in ethanol prior to denaturing BSA, appeared to result in low CUR entrapment with huge variation. This may be due to the limited amount of CUR dissolved in ethanol that was concurrently entrapped at molecular level at the time of protein aggregation. Because of stronger interaction with ethanol than with macromolecules, poor CUR entrapment into simultaneously aggregated protein took place. Besides, the rest unentrapped amount of CUR in ethanol was readily washed out from the nanoparticles during the purification step thereafter. On the other hand, loading of CUR by dispersing in aqueous phase either before (Method A) or after (Method C) protein aggregation yielded substantially higher entrapment with more reproducible loading amount. The reason behind this may be explained by the entrapment of CUR in particulate form rather than molecular form by BSA-NPs, leading to more amount of CUR entrapped in nanoparticles.

Since these prepared CUR-BSA-NPs were not cross-linked by using any chemical substance, they were prone to aggregation. To keep the dispersion system stable, hardening process by heating at 70 ^{o}C was used instead of the harmful glutaraldehyde generally used.[11] Cross-linking reaction with aldehyde promotes coupling and decreasing the number of surface amino groups, whereas the thermal cross-linking mechanism promotes a condensation reaction between intra-particulate amino groups and carboxylic groups without affecting functional groups on the surface. Basically, when using chemical stabilization, the number of positively charged amino groups is decreased, while the negative charge of carboxylic group became stronger with the net increase in negative zeta potential. The thermal stabilization technique could still preserve a higher number of available amino groups on the particle surface, which become beneficial for surface modification as targeting drug delivery systems. However, both thermal and chemical stabilization and the amount of cross-linking agent imposed no effect on particle size.[16] CUR release from CUR-BSA-NPs and effects of production parameters on CUR release suggest further studies to provide more information of CUR-BSA-NPs as a bright future of drug delivery system.

Conclusion

CUR-BSA-NPs were developed in this study by desolvation method without using glutaraldehyde as cross-linking agent and 3 different loading methods of CUR into nanoparticles were determined. The optimum preparation conditions used were 1:6 water to ethanol volume ratio, 20 mg/mL BSA concentration, and 10 mL/min ethanol pump rate. The sizes of nanoparticles were monodisperse in the order of magnitude of 200 nm, whereas zeta potentials were around -9 mV. The maximum entrapment efficiency and loading capacity were 28.59 % and 2.46 %, respectively. The 3 different loading methods of CUR into nanoparticles were found to have no statistically significant effect on physicochemical characteristics, entrapment efficiency, and loading capacity of CUR-BSA-NPs.

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