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# Effect of pH and temperature on formation of curcumin-starch complex nanoparticles

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**Abstract**
Starch nanoparticles (SNPs) are recently developed as a carrier for curcumin to improve its solubility and bioavailability. In this study, the curcumin-starch complex nanoparticles were formed by loading curcumin (3%) to debranched starch paste at various pH levels (5.5, 6.0, 6.5) and temperatures (25ºC, 50ºC, 80ºC) before nanoprecipitation in ethanol with the ratio of 1:20 (v/v). The variation in pH, temperature significantly affected the formation of the curcumin-starch complex nanoparticles. The loading efficiency/loading ability of curcumin in the complex decreased from 35.62%, 10.69mg/g to 27.52%, 8.26mg/g as pH increased from 5.5 to 6.5. Likewise, these numbers also reduced from 37.97%, 11.39mg/ml to 20.66%, 6.20mg/ml with ascending order of fabricating temperature. Thus, the optimal condition for generation of nanoparticles was pH 5.5 and 25ºC.

**Keywords:** curcumin; nanoparticle; nanoprecipitation; debranched starch

## 1. Introduction
Starch nanoparticles (SNPs) are natural, abundant, biocompatible, and completely biodegradable carrier for bioactive compounds (Gong *et al*., 2016) [4]. In comparison with micro particles, nano-size substance displays an effective and sufficient encapsulation owing to their high surface-to-volume ratio which easily pass through biological barriers in living organism. Besides, due to the unique active endocytosis mechanism and highly specific targeting characteristic of nanoparticles in human body, the delivery, absorption as well as bioactivity in circulation of phenolic compound are intensive enhanced (Muller *et al*., 2000). Recently, debranched starch (DBS), a modified form carried out by debranching enzymes (pullulanase or isoamylase) is used to encapsulate various substances. Large amount of amylose in DBS is capable of forming helical inclusion complexes with lipids (Godet *et al*., 1993) [3], vitamins (Hasanvand *et al*., 2015) [6], flavors (Hausch *et al*., 2018) [7], ferulic (Hung *et al*., 2013) [8], tangeretin (Wang *et al*., 2019). In the presence of a ligand, amylose occurs a single left-handed helix with a hydrophobic inside helical channel, which is known as V-type crystalline structure (Godet *et al*., 1993) [3]. DBS also served as a powerful precursor for SNPs synthesis with high stability, homogenous nano-sized molecules (Qiu *et al*., 2016) [12].
Curcumin is a strong bioactive compound isolated from Turmeric (*C. longa*). Even though accounting for just 3% by weight, this polyphenol displays major therapeutic activities of tumeric including antioxidant, anti-mutagenic, anti-cancer, anti-microbial and anticardiovascular activities (Gupta *et al*., 2012) [5]. Despite of these beauties, the low solubility and bioavailability of curcumin in human system have challenged its medical applications. Curcumin possesses limited chemical stability in alkaline environment and fast metabolism physiological medium (Tønnesen & Karlsen, 1985) [13]. Researcher has found that water solubility and bioavailability of curcumin by loading of curcumin in SNPs improved their solubility and stability (Chin *et al*., 2014) [2]. Nevertheless, the formation of curcumin-starch complex nanoparticles depends on temperature, pH that consequently influenced on the recrystalization of starch and degradation rate of curcumin (Bhatia *et al*., 2016; Tønnesen & Karlsen, 1985) [1, 13]
Thus, to fill the knowledge, this project was performed to study the influence of pH, temperature on the formation of curcumin-starch complex nanoparticles by determining loading efficiency, loading ability of curcumin and the optimal condition was also clearly investigated.

## 2. Materials and Method
### 2.1 Materials
Cassava starch powder was obtained from a local company in Ho Chi Minh City, Vietnam with minimum starch purity of 90%, maximum moisture content of 13%. Curcumin for synthesis was purchased from Merck (Germany). Pullulanase enzyme (≥1000NPUN/ml) was purchased from Sigma-Aldrich (Germany). All materials and other chemicals were used as received.

### 2.2. Preparation of debranched starches
Native starch was debranched based on method previously described by Hung *et al*. (2013) [8] with some alterations. Starch solution was prepared by dissolving 1g of cassava starch in 100 mL of acetate buffer (0.1M and pH 5.2) to obtain starch suspension 1% (w/v). This mixture was then boiled with continuous stirring for 30 min for complete gelatinization and cooled to 50ºC. Then, 0.8µL of enzyme pullulanase were added and incubated at 50ºC for 3 h. Pullulanase was inactivated by placing sample in a water bath at 90ºC for 30 min. The debranched starch pastes were finally centrifuged at 8000 rpm for 10 min to remove any inactivated enzyme.

### 2.3 Formation of starch nanoparticle under various pH, and temperature
An amount (1 mL) of curcumin/ethanol solution (6 mg/mL) was added dropwise to 20 mL debranched starch paste, which was adjusted pH to various levels (pH5.5, pH6.0 and

pH6.5) at 50ºC. The mixture was then stirred at controlled temperature for 1 h followed the homogenization (5000 rpm, 2 min). Next, it was kept for equilibrated curcumin absorption for 24 h before every 1 mL of this mixture was added dropwise to 20 mL of absolute ethanol. Magnetic stirring with a speed of 10,000 rpm was applied during nano-starch formation for 1 h. The precipitation was separated by centrifugation with speed of 1500 rpm for 5 min. In order to obtained SNPs, supernatant was discarded and the residue was freeze dried to obtain dry nanoparticles. After that, the optimal pH level for studying effect of temperatures was selected by evaluating the loading efficiency, ability as described in the next section. The procedure for starch nanoparticles production was repeated at 25ºC, 50ºC, 80ºC and those standards for assessing pH levels were also used to obtain the most appropriate temperature for each debranched starch.

### 2.4 Determination of loading efficiency/loading ability of curcumin into curcumin-starch complex nanoparticles

Loading efficiency/ability of curcumin loaded starch nanocomposites were determined according to the method of Li *et al*. (2016) with slight modification. Each sample (2 mg) was mixed with 2 mL of distilled water and placed in a bath type sonicator for 30 min at 80ºC. Then, 10 mL of absolute ethanol was added before being centrifuged at 3000 rpm for 10 min. The extraction was continued with additional 10 mL of absolute ethanol for 2 times supernatant collection. Curcumin concentration was determined by UV-vis spectrophotometry at 419 nm according to the following calibration curve:

$Y=0.12107X+0.0073$, $R^2=0.999$

Where $X$ was the curcumin concentration in µg/mL and $Y$ is the absorbance at 419 nm

Loading efficiency was determined by the detected curcumin weight which was measured by aforementioned method in loading ability test and the initial curcumin weight, following the equation below:

Loading efficiency (%) = (Detected curcumin weight/ Initial curcumin weight) × 100

Likewise, loading ability was measured by the following the formula:

Loading ability (mg/g) = Detected curcumin weight/ Weight of starch

### 2.5 Statistical analysis

All experiments were performed at least in duplicate and expressed by means and standard deviations. P value of <0.05 was opted as statistically significant level as using analysis of variance (ANOVA) processed by SPSS (Version 16.0; SPSS Inc., Chicago, IL, USA).

## 3. Results and Discussion

### 3.1 Effect of pH levels on loading efficiency and loading ability of curcumin-starch complex nanoparticles

The loading efficiency and loading ability of curcumin into curcumin-starch complex nanoparticles at different pHs are shown in Table 1. There was a significant variation in obtaining data between samples, to be specific, pH 5.5 yielded highest curcumin loading with efficiency of 35.62%, followed by pH 6.0 and 6.5 which was 33.38% and 27.52%, respectively. Thus, the adequate pH for the highest loading curcumin capacity was pH5.5.

**Table 1:** Loading efficiency (%) and loading ability (mg/g) of curcumin-starch complex nanoparticles at 50ºC and different pH levels[1,2]

| pH | Loading efficiency (%) | Loading ability (mg/g) |
|---|---|---|
| 5.5 | 35.62 ± 0.28$^{d}$ | 10.69 ± 0.09$^{d}$ |
| 6.0 | 33.38 ± 0.40$^{e}$ | 10.01 ± 0.12$^{e}$ |
| 6.5 | 27.52 ± 0.24$^{g}$ | 8.26 ± 0.07$^{g}$ |

[1]All data are the means of triplicate experiments ± standard deviations.

[2]Data followed by the same letter in the same column are not significantly different (P≤0.05).

The low loading figures at higher pH values were attributed to the loss of curcumin. This polyphenol was not stable for long at neutral and alkaline pH and gets easily degraded into compounds like vanillin, ferulic acid, and so on. Regarding the pH lower than 7, the stability of curcumin was parallel with the decreasing pH values (Kunnumakkara *et al*., 2008, Tønnesen & Karlsen, 1985) [13]. However, in acidic environment, curcumin displayed sufficient low solubility due to its different aggregated forms. In this study, the curcumin was dissolved completely in ethanol to ensure the homogenous of solution before adding drop-wise to starch paste. Moreover, the continuous stirring operation applied through loading also supported the identical interaction of curcumin molecules and the DBS. The DBS with high proportion of amylose, molecule was responsible for entrapping hydrophobic molecules such as curcumin. Godet *et al*. (1993) [3] proved that amylose chain performed a folding in a helical conformation with six single glucose units per turn. The hydroxyl groups of these residues were positioned at the outer surface of the helix, while the outside cavity offered a hydrophobic characteristic. Therefore, the hydrophobic moiety of guest agents was able to lie within the amylose helix and stabilized by van der Waals force, while the hydrophilic ends of the ligands were external of the helix.

### 3.2 Effect of different temperature on loading efficiency and loading ability of curcumin into curcumin-starch complex nanoparticles

Table 2 reveals the loading efficiency and loading ability of samples loaded curcumin at 25ºC, 50ºC, and 80ºC. With the highest loading efficiency and ability of 37.97% and 11.39 mg/g, respectively, 25ºC was the optimal temperature for loading curcumin molecules to debranched starch nanoparticles.

**Table 2:** Loading efficiency (%) and loading ability (mg/g) of curcumin into curcumin-starch complex nanoparticles at pH 5.5 and different temperatures[1,2]

| Temperature (ºC) | Loading efficiency (%) | Loading ability (mg/g) |
|---|---|---|
| 25 | 37.97 ± 0.38$^{c}$ | 11.39 ± 0.12$^{c}$ |
| 50 | 35.62 ± 0.28$^{d}$ | 10.69 ± 0.09$^{d}$ |
| 80 | 20.66 ± 0.40$^{f}$ | 6.20 ± 0.12$^{f}$ |

[1]All data are the means of triplicate experiments ± standard deviations.

[2]Data followed by the same letter in the same column are not significantly different (P≤0.05).

For samples performed SNPs generation at 50ºC, and 80ºC, these high temperatures ruptured the intramolecular hydrogen bond which then caused curcumin aggregations, thus reduced the affinity of curcumin and DBS core (Bhatia

*et al*., 2016) [1]. As a result, ethanol used in nanoprecipitation dissolved untrapped curcumin and cause loss in loading efficiency.

## 4. Conclusion

In summary, high pH and temperature caused curcumin degradation and aggragation which resulting in low loading efficiency and loadind ability of curcumin-starch complex nanoparticles. The optimal condition for highest loading of curcumin to complex was pH 5.5 and temperature of 50$^{o}$C.

## 5. Acknowledgements

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