

# Gold Nanoparticles with Immobilized $\beta$ -cyclodextrin-capsaicin Inclusion Complex for Prolonged Capsaicin Release

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**Abstract.** Inclusion complexes of capsaicin with  $\beta$ -cyclodextrin (prepared at three different concentrations of  $\beta$ -cyclodextrin) were immobilized onto the surface of concentrated gold nanoparticles synthesized using the Frens method. Capsaicin release for 2 days into pure water and acetonitrile was recorded. The nanoparticles were characterized with absorbance spectra and transmission electron microscopy images. An inclusion complex without gold nanoparticles also displayed capsaicin release with a lag period of 1.5 days that suggested capsaicin release from gold nanoparticles was due to decomposition of the inclusion complex. Neither the inclusion complex nor the free capsaicin formed nanoparticles themselves. It was shown that physical immobilization of capsaicin onto the surface of gold nanoparticles without the aid of  $\beta$ -cyclodextrin did not lead to prolonged release. For comparison, inclusion complex encapsulation into chitosan nanoparticles was also carried out, but the release kinetics was less pronounced compared to that of gold nanoparticles.

## 1. Introduction

Capsaicin, a main pungent component in ‘hot’ chili peppers [1-3], can be used for treatment of obesity [2], osteoarthritis [3], diabetic neuropathy [3], post-therapy and postherpetic neuralgia [3]. Treatment with capsaicin causes sensory chemodenervation and thus improves myocardial function [4]. It was found to be a potent inhibitor of platelet aggregation [5], to induce apoptosis in cancer cells [6] and to have an analgesic effect [3, 7]. But its practical applications are limited by its very low water solubility [8-10] with unstable solutions [11] and strong gastrointestinal irritation [8]. Water solubility can be improved by the formation of an inclusion complex with  $\beta$ -cyclodextrin derivatives [8-10], even though the cavity diameter of  $\beta$ -cyclodextrin (0.6-0.65 nm) [12] is smaller than a capsaicin molecule (1.142 nm). This inclusion complex solution still contains free capsaicin at high concentrations [10] and only partially solves the problems mentioned above.

The aim of this study is to create nanoparticulated forms of capsaicin by means of the immobilization of its  $\beta$ -cyclodextrin inclusion complex onto the surface of gold nanoparticles. Nanostructured drug delivery architectures are promising candidates that will enable efficient and targeted delivery of drug compounds [13]. The advantages of gold nanoparticles include their ability to penetrate inside living cells, the possibility to synthesize stable particles with predefined size [14], and the possibility to produce multifunctional nanoparticles by binding chemically distinct moieties to the same nanoparticle [15]. For example, it would also be possible to immobilize antibodies on the



nanoparticle for targeted drug delivery [16]. To our knowledge, there is no report to date dealing with immobilization of capsaicin onto the surface of gold nanoparticles.

## 2. Materials and methods

### 2.1. Materials

Capsaicin, chitosan, glutaraldehyde, phosphotungstic acid hydrate, sodium citrate, sodium tripolyphosphate and tetrachloroauric acid ( $\text{HAuCl}_4$ ) were purchased from Sigma-Aldrich (USA). Cyclodextrin was a gift from Wacker Chemie AG (Germany). Glacial acetic acid was purchased from Karal, S. A. de C. V. (Mexico). Ethanol was purchased from Reasol (Mexico). Water used in all the experiments was distilled and subsequently purified by the Thermo Barnstead EASYpure II system, model No. D8611.

### 2.2. Preparation of $\beta$ -cyclodextrin-capsaicin inclusion complex

$\beta$ -cyclodextrin solution in water was mixed with 100  $\mu\text{l}$  of 15 mg/ml capsaicin solution in ethanol and with an additional amount of ethanol. The mixture was shaken at 250 rpm (BIOSAN PSU-20i, EU) at 4  $^\circ\text{C}$  for 2 days in the dark.

### 2.3. Synthesis of gold nanoparticles using the Frens method

1 ml of 1% sodium citrate was added to 50 ml of boiling 10<sup>-2</sup>% tetrachloroauric acid solution. The solution was boiled for 5 min [17]. The colorless solution soon turned light blue, then light red and finally dark red. Then the heater was turned off, and the solution was allowed to cool down to room temperature (about 27  $^\circ\text{C}$ ). The solution was stored at 4  $^\circ\text{C}$  in the dark.

### 2.4. Concentrating gold nanoparticles by centrifugation

The solution of gold nanoparticles was transferred to 8 plastic test tubes (1.5 ml for each tube) and centrifuged at 6000 rpm for 20 min using centrifuge 5430 with rotor FA-45-30-11 (Eppendorf, Germany). The precipitate was resuspended in fresh portions of solution by briefly vortexing at a maximal rate (Vortex-Genie 2 G-560, Scientific Industries, Inc., USA) and then by orbital shaking at 250 rpm for 10 minutes. Then 4 test tubes were centrifuged, and the precipitate was resuspended in solution from 4 remaining tubes. Two tubes were centrifuged again, and the precipitate was resuspended in a solution from the 2 remaining tubes. After each centrifugation step the supernatant was discarded [18].

### 2.5. Immobilization of the inclusion complex onto the surface of gold nanoparticles for capsaicin release in water

Concentrated gold nanoparticles were centrifuged at 6000 rpm for 20 min. The supernatant was discarded, but a small amount remained (about 50  $\mu\text{l}$ ). The precipitate was resuspended in this solution by briefly vortexing, and then 1.5 ml of the solution of the inclusion complex prepared with 900  $\mu\text{l}$  of 6.3 mg/ml  $\beta$ -cyclodextrin and with 700  $\mu\text{l}$  of ethanol was added. The solution was shaken at 250 rpm for 10 min in the dark. Then it was centrifuged at 8000 rpm for 20 min. After discarding the supernatant, the residual capsaicin concentration in the supernatant was determined by HPLC. The precipitate was resuspended in 1.5 ml of water by orbital shaking at 250 rpm for 10 min in the dark. In a control experiment, concentrated gold nanoparticles as prepared were resuspended in 900  $\mu\text{l}$  of water, then 700  $\mu\text{l}$  of ethanol was added. 100  $\mu\text{l}$  of 15 mg/ml capsaicin in ethanol was added after briefly vortexing, followed by orbital shaking at 250 rpm for 10 minutes in the dark. All of the experiments involving capsaicin were carried out at 4  $^\circ\text{C}$  as recommended [11] to enhance capsaicin stability.

### 2.6. The investigation of storage stability of gold nanoparticles with immobilized inclusion complex at different temperatures

500  $\mu\text{l}$  of the solution of gold nanoparticles with immobilized  $\beta$ -cyclodextrin-capsaicin inclusion complex in water were stored for 6 days in temperature regulated rooms at 4  $^{\circ}\text{C}$ , 28  $^{\circ}\text{C}$  and 37  $^{\circ}\text{C}$ , respectively. After that, the samples were analyzed by means of scanning electron microscopy (SEM).

### *2.7. Immobilization of inclusion complexes onto the surface of gold nanoparticles for capsaicin release in acetonitrile*

Concentrated gold nanoparticles were centrifuged at 6000 rpm for 20 min. The supernatant was discarded, but a small amount remained (about 50  $\mu\text{l}$ ). The precipitate was resuspended in this solution by briefly vortexing, and then 1.5 ml of the solutions of inclusion complexes prepared with 900  $\mu\text{l}$  of 3.1, 6.3 or 9.6 mg/ml  $\beta$ -cyclodextrin and with 700  $\mu\text{l}$  of ethanol were added. The solution was shaken at 250 rpm for 10 min in the dark and stored overnight at 4  $^{\circ}\text{C}$ . Then it was centrifuged at 8000 rpm for 20 min. The supernatant was discarded, and the precipitate was resuspended in 1.5 ml of acetonitrile by orbital shaking at 250 rpm for 10 min in the dark. All of the experiments involving capsaicin were carried out at 4  $^{\circ}\text{C}$ .

### *2.8. Preparation of chitosan particles crosslinked with tripolyphosphate*

The inclusion complex solution prepared with 1100  $\mu\text{l}$  of 3.93 mg/ml  $\beta$ -cyclodextrin and with 400  $\mu\text{l}$  of ethanol was added to 107  $\mu\text{l}$  of 0.5 mg/ml chitosan in 0.2%  $\text{CH}_3\text{COOH}$  followed by vortexing for 10 min. Then 400  $\mu\text{l}$  of 0.5 mg/ml sodium tripolyphosphate was added, and after vortexing for 10 min precipitate appeared. The solution was centrifuged at 7500 rpm for 5 min. The supernatant was carefully removed leaving the precipitate stuck to the wall of the tube. It was resuspended in 2 ml of water by vortexing for 10 min. All of the experiment was carried out at 4  $^{\circ}\text{C}$ .

### *2.9. Preparation of chitosan particles crosslinked with tripolyphosphate and glutaraldehyde*

The inclusion complex solution prepared with 1000  $\mu\text{l}$  of 5.8 mg/ml  $\beta$ -cyclodextrin and with 500  $\mu\text{l}$  of ethanol was added to 107  $\mu\text{l}$  of 0.5 mg/ml chitosan in 0.2%  $\text{CH}_3\text{COOH}$ . After vortexing for 10 min 300  $\mu\text{l}$  of 0.5 mg/ml sodium tripolyphosphate was added followed by vortexing for 10 min. 1500  $\mu\text{l}$  of the solution was centrifuged at 14000 rpm for 5 min. The precipitate was resuspended in 1.5 ml of 50% glutaraldehyde by vortexing for 10 min. Precipitate appeared after overnight shaking at 250 rpm in the dark. It was separated from glutaraldehyde solution by centrifugation at 5000 rpm for 5 min and resuspended into 2 ml of water by vortexing for 10 min. All of the experiment was carried out at 4  $^{\circ}\text{C}$ .

### *2.10. Characterization of nanoparticles*

Absorbance spectra were acquired by means of a microplate reader BioTek Synergy 2 (USA). Transmission electron microscopy (TEM) images were acquired by means of a Philips FEI Morgagni 268D microscope (USA). 7  $\mu\text{l}$  of the solutions were dropped onto 200 mesh formvar/carbon-coated copper grids (Ted Pella, Inc., USA), and the remaining liquid was collected by filter paper after 20 min of incubation under a Petri dish. In the case of chitosan nanoparticles, 7  $\mu\text{l}$  of 1% phosphotungstic acid was dropped onto the grids thereafter, and the remaining liquid was collected by filter paper after 15 min of incubation under a Petri dish. SEM images were acquired by means of a JSM-7800F microscope (JEOL, Japan). The solutions were dropped onto silicon and allowed to dry. The images were processed using ImageJ software bundled with Java 1.8.0\_112 operating under Linux. At first, the background was subtracted at a rolling ball radius of 50 pixels, and then the threshold was set using the default algorithm. The nanoparticles were approximated by ellipses, and the histograms of the minor axes length distribution of these ellipses were plotted.

### *2.11. The investigation of kinetics of capsaicin release*

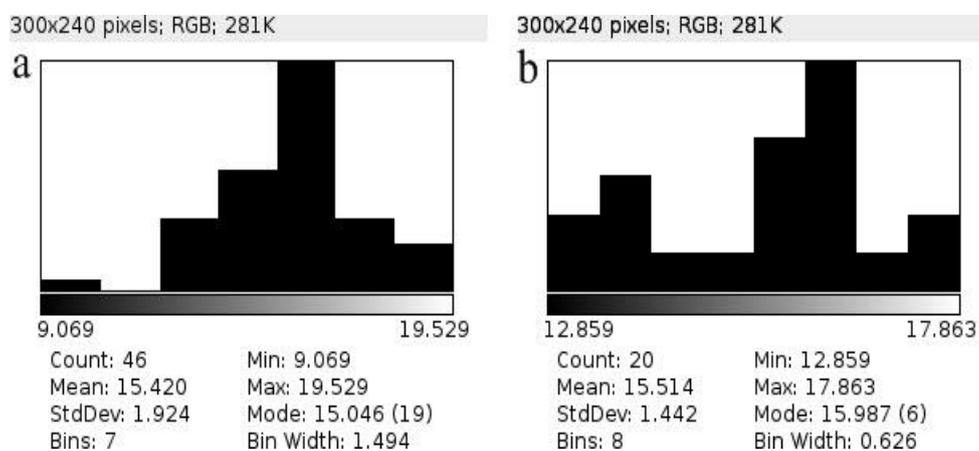
Samples having a volume of 350  $\mu\text{l}$  were taken from the solutions of nanoparticles with immobilized inclusion complexes at different time points, centrifuged at 14800 rpm for 30 min, and then the upper 300  $\mu\text{l}$  of supernatant was used for HPLC analysis. All of the experiment was carried out at 4  $^{\circ}\text{C}$  in the dark. Before HPLC analysis, the solutions were stored at -20  $^{\circ}\text{C}$  in the dark.

### 2.12. HPLC analysis

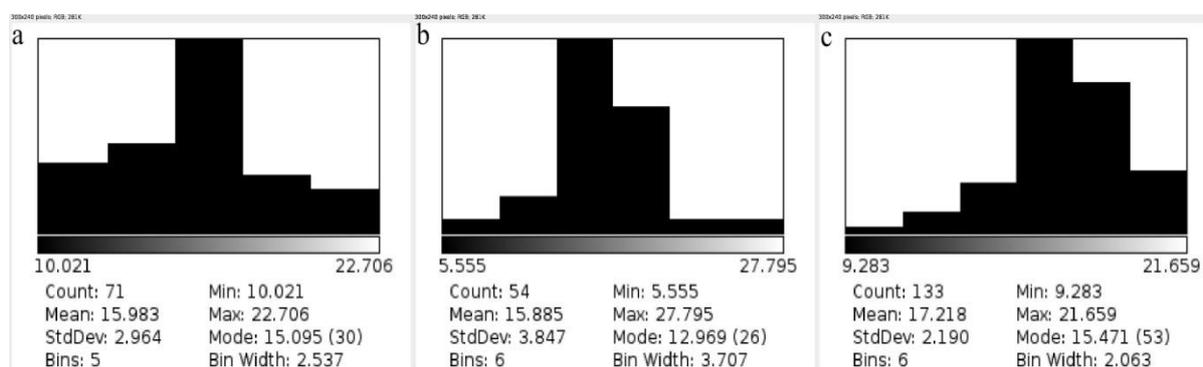
An Agilent Technologies 1200 Series chromatograph (USA) equipped with column Zorbax Eclipse XDB-C18 (4.6 × 150 mm, particle size 5 μm) and Diode Array Detector operating at 280 nm was used for HPLC analysis. Capsaicin was eluted with 60% acetonitrile in water at 25 °C using isocratic pump at 1 ml/min.

### 3. Results and discussion

Blank gold nanoparticles have an absorbance maximum at 518 nm. After the inclusion complex immobilization, the absorbance maximum shifts to 520.5 nm. Plasmon resonance shift to longer wavelengths corresponds to the presence of immobilized molecules [19]. The nanoparticles have a shell giving them an irregular form. This shell can be ascribed to the immobilized inclusion complex. The size of single nanoparticles has somewhat increased after inclusion complex immobilization (figure 1). The control experiment has not revealed any nanoparticles in the capsaicin solution and in the solution of the inclusion complex.



**Figure 1.** The histograms of length distribution of minor axes of ellipses of the gold nanoparticles before (a) and after (b)  $\beta$ -cyclodextrin-capsaicin inclusion complex immobilization.

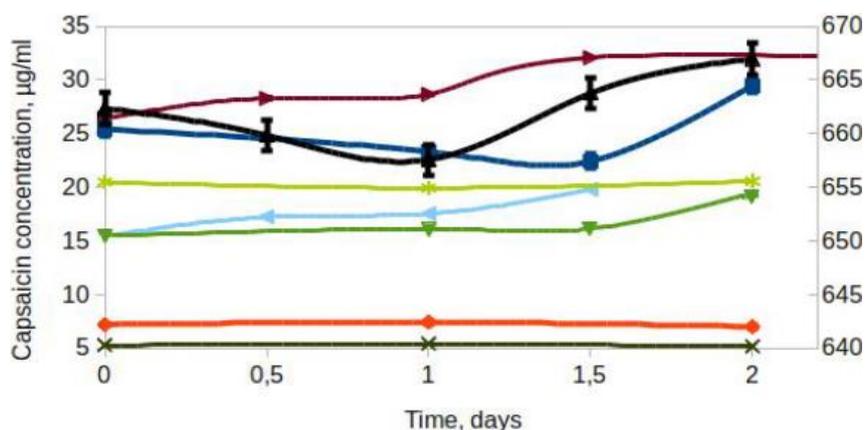


**Figure 2.** The histograms of length distribution of minor axes of ellipses of the gold nanoparticles with immobilized  $\beta$ -cyclodextrin-capsaicin inclusion complex after 6 days of storage at 4°C (a), 28°C (b) and 37°C (c). The Y axis represents frequency.

The investigation of the storage stability of the solution of gold nanoparticles with immobilized  $\beta$ -cyclodextrin-capsaicin inclusion complex in water at different temperatures has shown that the data calculated from SEM images for 4°C and 28°C (figure 2) is not significantly different from each other

and from those calculated from TEM images (figure 1(b)). However, at 37°C the size of the nanostructures is somewhat larger (figure 2(c)) because of an increased aggregation rate. Moreover, a temperature rise must destabilize capsaicin [11]. Therefore, the storage of the solutions of gold nanoparticles with immobilized  $\beta$ -cyclodextrin-capsaicin inclusion complexes at 4°C is preferred.

As can be seen from the release curves (figure 3), encapsulated capsaicin is liberated for at least 2 days. When glutathione was released from sodium tripolyphosphate-crosslinked chitosan nanoparticles with encapsulated  $\alpha$ -cyclodextrin-glutathione inclusion complex into simulated gastric and intestinal media for 1 hour, encapsulation efficiency was 6% [20]. Methotrexate and calcium folinate encapsulated in sodium tripolyphosphate-crosslinked chitosan nanoparticles containing their inclusion complexes with  $\beta$ -cyclodextrin were released in phosphate buffered saline for 24 hours with a burst release for 30 min [21]. One of the best results was obtained for the release of cisplatin from nanoparticles consisting of crosslinked  $\alpha$ -cyclodextrin — 28 hours of release in phosphate buffered saline with an encapsulation efficiency of 37.5% [22]. The nanoparticles with immobilized capsaicin (without  $\beta$ -cyclodextrin) have not shown any capsaicin release kinetics at all. An inclusion complex without nanoparticles has shown some capsaicin release with a lag period of 1.5 days. Therefore, the release of capsaicin from gold nanoparticles with an immobilized inclusion complex may be explained by decomposition of the complex. The encapsulation yield calculated by dividing the difference between residual capsaicin mass in the supernatant (see section 2.5) and initial capsaicin mass by initial capsaicin mass was 81%. In contrast, the ‘encapsulation yield’ calculated by dividing the highest capsaicin concentration registered in the solutions of gold nanoparticles (for those with an immobilized inclusion complex prepared at 6.3 mg/ml  $\beta$ -cyclodextrin and resuspended in acetonitrile) by capsaicin concentration registered in the solution of the inclusion complex after 2 days is 5%. This apparent inconsistency may be explained by significant amounts of capsaicin still being bound with nanoparticles even after 2 days of release. The results of the present work are comparable or even better than those reported for capsaicin immobilization in polymeric nanoparticles where release time into simulated gastric fluid and phosphate buffered saline in a dialysis bag was 60 hours with a burst release for 12 hours and an encapsulation efficiency of 81.5% [23]. The current results were also better than those reported for solid lipid nanoparticles with 14 hours of capsaicin release in a 1:1 ethanol-citrate-phosphate buffer in a diffusion cell with cellophane membrane [24, 25] and an encapsulation efficiency of 90.89% [24] or over 60% [25]. This superiority may be explained by dual immobilization: capsaicin is encapsulated in  $\beta$ -cyclodextrin, and the inclusion complex is immobilized onto the surface of gold nanoparticles giving additional steric hindrance due to the statistical orientation of immobilized inclusion complexes. For comparison, chitosan nanoparticles with a diameter about 50 nm and an encapsulated  $\beta$ -cyclodextrin-capsaicin inclusion complex were also prepared. As can be seen from figure 3, gold nanoparticles displayed much better capsaicin release kinetics that supports the conclusion above.



**Figure 3.** The dependence of capsaicin concentration on time for gold nanoparticles with an inclusion complex in water (triangles), for gold nanoparticles with capsaicin in water (rhombi), for gold nanoparticles with an inclusion complex at 3.1 (inverted triangles), 6.3 (triangles turned right) and 9.6 mg/ml  $\beta$ -cyclodextrin (triangles turned left) in acetonitrile; for chitosan particles crosslinked with tripolyphosphate (asterisks; the values are reduced by a factor of 4); for chitosan particles crosslinked with tripolyphosphate and glutaraldehyde (crosses); for an inclusion complex (squares, additional Y axis).

#### 4. Conclusions

$\beta$ -cyclodextrin-capsaicin inclusion complex can be physically adsorbed onto the surface of gold nanoparticles prepared by the Frens method. The nanoparticles show capsaicin release into water or acetonitrile for at least 2 days. This is better or comparable with the results reported previously for the nanoparticles releasing capsaicin or other low molecular weight drugs. This capsaicin release is caused by inclusion complex decomposition. The solutions of nanoparticles should not be stored at high temperatures, and 4 °C is recommended. Chitosan nanoparticles crosslinked with tripolyphosphate or glutaraldehyde and tripolyphosphate with an encapsulated  $\beta$ -cyclodextrin-capsaicin inclusion complex show a less pronounced capsaicin release when compared to gold nanoparticles.

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