International Journal of Design & Nature and Ecodynamics
Vol. 19, No. 4, August, 2024, pp. 1261-1267
Journal homepage: http://iieta.org/journals/ijdne

Nanoencapsulation of Lycopene from Tomato Waste Using Chitosan and Alginate for Enhanced Stability and Antioxidant Activity

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https://doi.org/10.18280/ijdne.190417

Received: 2 April 2024
Revised: 18 July 2024
Accepted: 1 August 2024
Available online: 28 August 2024

Keywords:
lycopene, encapsulation, chitosan-alginate nanocapsules, antioxidant activity, nutraceuticals

ABSTRACT

Post-harvest and oversupply lead to significant tomato waste, which has potential for nutraceutical applications due to its rich antioxidant content. Lycopene, a light and heat-sensitive antioxidant found in tomatoes, loses its biological activity when degraded. This problem can be addressed by encapsulation using chitosan and alginate. This study aimed to extract and encapsulate lycopene from tomato waste to develop a cost-effective nutraceutical product. The lycopene content in dried tomato was 1.01±0.19mg/g. Treatments varied in the amount of lycopene added to the alginate solution (T1: 0.5mg, T2: 1.0mg, T3: 1.5mg). Treatment 3 exhibited the highest encapsulation efficiency (86.85±0.53%) and radical scavenging activity (2.51±0.02%). The particle size of the nanocapsules ranged from 148.7±8.7nm to 152.8±9.7nm. Lycopene-loaded nanocapsules showed FTIR peaks corresponding to the major functional groups of chitosan and alginate. SEM images revealed that Treatment 3 nanocapsules were clustered and quasi-spherical. Lycopene-loaded nanocapsules hold promise for applications in the nutraceutical industry as a cost-effective antioxidant with increased bioavailability and adsorption due to their smaller size.

1. INTRODUCTION

Tomato is the major fruit and vegetable crop produced worldwide [1]. The Philippines is one of the leading tomato-producing countries in the Asia Pacific with approximately 216.6 thousand metric tons produced in 2022 [2]. During oversupply, farmers in the Philippines particularly in Cordillera Administrative Region (CAR) were forced to dump tons of tomatoes amid oversupply and lack of buyers as farmgate price reached as low as Php5 per kilo. Utilization of these possible tomato waste and encapsulating its bioactive compounds can be a better option.

Tomato is a part of the nightshade family's flowering plant. It is a good source of vitamins and phytochemicals [3]. Humans can benefit from tomato intake due to its high content of bioactive compounds, mainly lycopene, β-carotene, phenolic compounds and ascorbic acid [4]. One of the notable substances present in tomato is lycopene. Lycopene is a member of the carotenoid compound family which is composed of carbon and hydrogen atoms. It absorbs all the visible light wavelength except the longest which gave its red coloration. It is composed of conjugated double bonds which reduces the energy required for the transition to the higher energy states of its electron, allowing it to absorb visible light [5]. Lycopene is responsible for the deep red pigmentation of tomato. Due to its natural antioxidant properties, it gained the attention of the public [6]. Many studies stated that lycopene can reduce renal damage [7], could lower the risk of many types of cancer especially in the breast and prostate [8] and has a potential role in the protection from cardiovascular diseases [9].

Most of the bioactive compound like lycopene are easily degraded when exposed to heat, light, oxygen and other environmental conditions. Lycopene is a heat and light sensitive substance [10], but this problem can be addressed by encapsulation [11, 12]. The use of green chemistry through nanotechnology is a more environment friendly method of minimizing hazardous chemicals while encapsulating the material [13, 14]. Encapsulation can protect lycopene from extreme conditions thus improving its stability and effectivity. Several studies have explored different encapsulation techniques for lycopene. For instance, lycopene-loaded nanofibers using whey protein isolate-*Tricholoma lobayense* polysaccharide complex stabilized emulsions was successfully developed [15]. This method resulted in enhanced photostability, thermostability, and controlled release of lycopene. In another study, α-lactalbumin nanotubes were used to encapsulate lycopene, improving its antioxidant

activity, viscosity, and colloidal stability in dairy products [16]. Additionally, lycopene encapsulation in emulsions and hydrogel beads using dual modified rice starch has been investigated, demonstrating its potential for controlled release during in-vitro digestion [17]. In this study, lycopene extracted from tomato was encapsulated with a chitosan-alginate nanocapsule system. We aimed to investigate the effectiveness of this method in protecting lycopene from degradation and evaluate its potential application for developing functional food products enriched with this valuable bioactive compound.

2. MATERIALS AND METHODS

2.1 Materials and reagents

Fresh tomatoes were purchased from Sangitan Market, Cabanatuan City, Nueva Ecija. Chitosan (≥95% deacetylation), sodium alginate (≥90% purity), and calcium chloride dihydrate (≥99% purity) were obtained from Sigma-Aldrich. Tween-20, a non-ionic surfactant, was used for emulsification process. Analytical grade solvents were used for extractions: n-Hexane (≥99.5% purity) from Ajax Finechem and ethyl acetate (≥99.8% purity) from Baker, J.T. All chemicals and reagents were used without further modifications.

2.2 Extraction of crude lycopene

Approximately 100 grams of tomatoes were washed, and the seeds were removed. The tomatoes were then homogenized using a blender and dehydrated by soaking them in 500 mL ethanol. The dehydrated tomatoes were filtered, and the collected residue was used for further processing. Crude lycopene was extracted by adding 200mL of a 1:1 hexane-ethyl acetate solvent mixture to 50 grams of the dehydrated tomato residue. The mixture was stirred for 2 hours, and then the solvent layer was decanted. This extraction step was repeated twice. The combined solvent fractions were concentrated by evaporation at 60-75℃ to a volume of approximately 5mL.

2.3 Purification of lycopene

Crude lycopene extract (50mL) was subjected to a purification process to remove impurities and enhance its purity. First, 20mL of ethanol was added to the extract, followed by homogenization to facilitate the re-dissolution of lipophilic lycopene molecules. The mixture was then heated to 50℃ in a water bath with constant stirring for 30 minutes to aid in the extraction and improve lycopene solubility in ethanol.

Next, a saponification step was performed to remove any remaining esterified carotenoids. Twenty milliliters of a 40% (w/w) potassium hydroxide (KOH) solution was added to the mixture, and the temperature was raised to 58-62℃ with continuous stirring for 2 hours. After saponification, the mixture was diluted with 100mL of distilled water while stirring continuously for 30 minutes. The diluted mixture was then filtered, and the filtrate containing dissolved impurities and some ethanol was discarded. The residue on the filter paper, containing the purified lycopene along with any undissolved impurities, was retained for further processing.

To remove residual salts and non-polar impurities, the residue on the filter paper was washed sequentially with distilled water and ethanol. Finally, the air-dried filter paper was soaked in 100mL of ethyl acetate to dissolve the purified lycopene. The presence and purity of the extracted lycopene in the final ethyl acetate solution were confirmed using a ultraviolet-visible (UV-Vis) spectrometer by comparing its absorption pattern with reference data.

2.4 Emulsification of lycopene

To quantify the lycopene content, the weight difference between the sample and the solvent after evaporation was measured. The lycopene emulsion preparation involved creating a target concentration of 0.085mg/mL. The required volume of the purified lycopene solution, calculated based on its initial concentration, was added to 80mL of a 0.7mg/mL Tween 20 solution to form a suspension. This suspension then underwent processing in three steps. First, heating at 60℃ with constant stirring for one hour facilitated the removal of the organic solvent. Next, the resulting emulsion was diluted to 100mL using a volumetric flask containing additional Tween 20 solution. Finally, to achieve a uniform dispersion, the diluted emulsion was sonicated for 180 seconds. This final solution served as the stock solution for the subsequent encapsulation process.

2.5 Encapsulation of lycopene

The encapsulation process involved varying treatments based on the amount of lycopene added to the alginate solution (0.5mg, 1.0mg, and 1.5mg). To achieve these targeted amounts, specific volumes of the lycopene emulsion were calculated and added (5.88mL, 11.76mL, and 17.65mL, respectively) to 40mL of a 2.25mg/mL sodium alginate solution adjusted to a pH of 4.9. For pre-gelation, 5mL of 5.29 mg/mL $CaCl_2$ solution was added dropwise with continuous stirring. Subsequently, 20 mL of 6.3mg/mL chitosan solution was also added dropwise while maintaining constant stirring to form a polyelectrolyte complex. The resulting suspension was stirred for an additional 10 minutes with the pH adjusted to 5.5. Finally, the suspension was equilibrated overnight under refrigeration.

To evaluate the encapsulation efficiency, the filtrate was collected. The remaining precipitate was then lyophilized using a freeze dryer for further analysis.

2.6 Encapsulation efficiency

The Encapsulation efficiency (%EE) was evaluated by measuring the absorbance of the solution before and after the encapsulation using Uv-Vis spectrophotometer at 472nm.

%EE was calculated as follows [18]:

$$\%EE = \frac{\text{abs.(before encap.)} - \text{abs.(after encap.)}}{\text{abs.(before encap.)}} \times 100 \quad (1)$$

2.7 Characterization of the encapsulated lycopene

The lycopene-loaded nanocapsules underwent physicochemical characterizations to assess their morphology, size distribution, and chemical composition. Scanning electron microscopy (SEM) revealed the surface morphology of the nanocapsules, while dynamic light scattering (DLS)

determined their average size Fourier-transform infrared (FTIR) spectroscopy was used to identify the major functional groups present, confirming the encapsulation matrix composition and potential interactions with lycopene.

2.8 Determination of antioxidant activity

A 0.1mM solution of DPPH was prepared by dissolving 6.9mg of DPPH in 100mL of methanol. To evaluate the antioxidant activity, 1.5mL of the filtrate from the treatment with the highest encapsulation efficiency (before encapsulation) was transferred to a test tube. Subsequently, 2.5mL of the prepared DPPH solution was added to the test tube. The mixture was vortexed and incubated in the dark for one hour. The absorbance of each sample was measured against a blank at 517nm using a UV-Vis spectrophotometer. Ascorbic acid was used as a standard for the calibration curve. The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\%Activity = \frac{abs.(\text{ blank }) - abs.(\text{ sample })}{abs.(\text{ blank })} \times 100 \qquad (2)$$

2.9 Statistical analysis

Treatments was carried out in triplicates. Data were reported using the mean and standard deviation. One-way analysis of variance (ANOVA) and LSD test was used to determine the significant differences among the treatment means at 95% confidence level.

3. RESULTS AND DISCUSSION

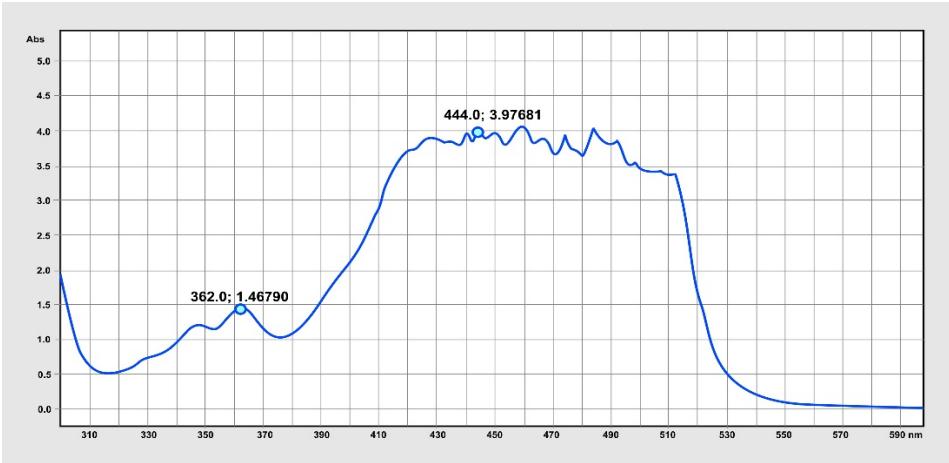
3.1 Extraction and purification of lycopene

Solid-liquid extraction using a hexane-ethyl acetate solvent mixture was employed to isolate lycopene. The extract exhibited a red color, and its intensity increased during solvent evaporation, indicating a rise in crude lycopene concentration. The average lycopene content in dried tomato was determined to be 1.01± 0.19mg/g.

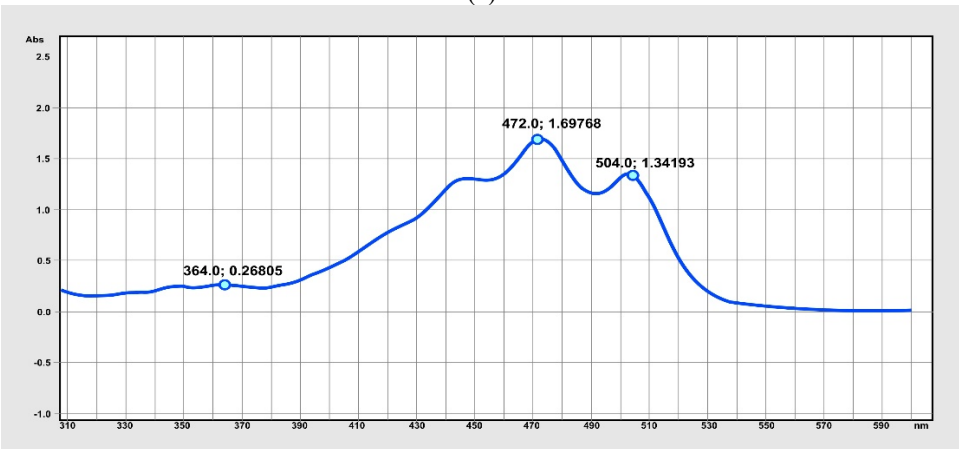
Saponification-crystallization was used for purification. Upon reaching 58-62°C, the formation of a dark red, glitter-like precipitate was observed. Saponification effectively removes impurities such as unwanted lipids and chlorophylls [19]. This process liberates lycopene trapped within cell debris by esterifying diglycerides, triglycerides, and phosphonates [20].

Confirmation of lycopene content was achieved through UV-Vis spectroscopy of the crude and purified extracts. The crude extract displayed no distinct peak (Figure 1(a)), likely due to the presence of interfering impurities. Their overlapping maximum absorption during the scan resulted in the undefined maximum observed in the graph.

In contrast, the purified lycopene (Figure 1(b)) exhibited a well-defined maximum absorption peak at 472nm, consistent with previous findings by Montesano et al. [21] and Berra [22].



(a)



(b)

Figure 1. Absorption spectra of (a) crude lycopene, and (b) purified lycopene

3.2 Encapsulation efficiency

Encapsulation efficiency, determined by the difference between free and encapsulated lycopene, is a crucial parameter for evaluating the effectiveness of the encapsulation process. In this study, three treatments were compared, with encapsulation efficiencies ranging from 52.92±2.21% to 86.85±0.53% (Table 1). Statistical analysis revealed a significant difference ($p \leq 0.05$) between Treatment 3 and the other two treatments, indicating a superior ability to encapsulate lycopene.

Table 1. Encapsulation efficiency of each treatment

Treatment	% Encapsulation Efficiency
1	53.39±2.49[a]
2	52.92±2.21[a]
3	86.85±0.53[b]

Means sharing the same superscript letter are not significantly different ($p \leq 0.05$).

The high encapsulation efficiency observed in Treatment 3 (86.85±0.53%) can be attributed to the presence of a surfactant. Surfactants act as interface modifiers, reducing interfacial tension and facilitating the formation of stable nanocapsules. These findings align with Abreu et al. [23], who reported that the encapsulation efficiency of drugs using the oil-in-water emulsion method, which often involves surfactants, is generally higher compared to methods using only aqueous media. They attributed this phenomenon to the reduced leaching of the encapsulated material. The surfactant likely played a similar role, minimizing lycopene loss during the encapsulation process.

The statistically similar encapsulation efficiencies observed in Treatments 1 and 2 (around 53%) suggest that these treatment conditions may not have significantly impacted lycopene encapsulation. However, further investigation is needed to determine the specific factors influencing the encapsulation efficiency in these treatments. One possibility could be the initial availability of lycopene in the solution. Treatment 3 might have involved a higher initial lycopene concentration, leading to a greater amount available for encapsulation compared to Treatments 1 and 2. These encapsulationresults however, are at par with other studies [24].

3.3 DPPH radical scavenging assay

The lycopene-loaded nanocapsules (Treatment 3) showed 2.51±0.016% Scavenging activity. Ascorbic acid was used as a primary standard of the analysis. The calibration curve was determined to be:

$$Y=-0.0648X+0.6665$$
$$r^2=0.9959$$

It was found that the lycopene-loaded nanocapsule (Treatment 3) has an equivalence of 0.40±0.01µg of ascorbic acid.

The structure of lycopene affects the scavenging ability to DPPH. The 11 conjugated double bonds of lycopene have a strong DPPH scavenging ability due to the fact that its overlapping orbitals in its chromophore can form a stable radical. However, the lycopene showed a low radical scavenging activity value because it is enclosed around with the surfactant (Tween 20). In the presence of lipid layer, Lycopene is expected to have a poor antioxidant activity due to lesser interaction with the aqueous radical [25].

3.4 FTIR analysis

Fourier transform infrared spectroscopy analysis was done to determine the major functional groups present on the nanocapsules. FTIR spectra of chitosan, alginate, blank and lycopene-loaded nanocapsules were presented in Figure 2. Both blank and lycopene-loaded nanocapsules have peaks on the major functional groups of chitosan and alginate.

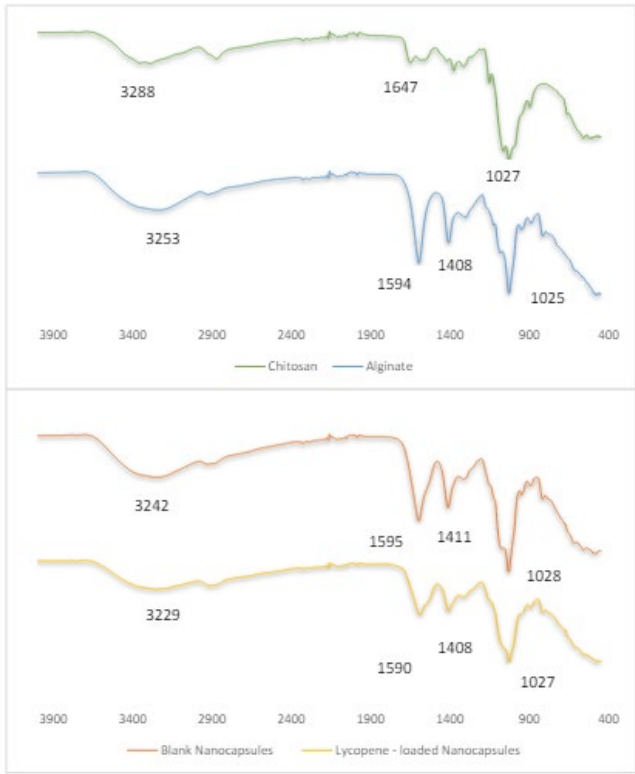


Figure 2. FTIR spectra of chitosan, alginate, blank and lycopene-loaded nanocapsules

All samples showed a strong broad peak at 3229cm^{-1} to 3288cm^{-1} which indicates the presence of amino and OH groups. It also showed a strong peak at 1025cm^{-1} to 1028cm^{-1} which indicates an ether group. Chitosan showed a peak at 1647cm^{-1} which indicates NH bending. Alginate, blank nanocapsules, and lycopene-loaded nanocapsules showed peak at 1408-1411cm^{-1} and 1590-1595cm^{-1} which indicates a carboxylate group asymmetric and symmetric stretching.

The presence of these peaks indicates the successful interaction between chitosan and alginate. However, blank and lycopene-loaded nanocapsules showed a very similar FTIR spectra that only varies on its intensities. During the gelation process, the loaded drug was embedded in the particle's matrix. This prevents the characteristic peaks of the drug loaded in the nanocapsules to be identified [26].

3.5 Particle size

Particle size is one of the advantages of nanocapsules. The small particle size plays an important role in nanocapsule systems. It increases the bioavailability of the loaded drug. Because of the small size of nanocapsule, it can travel and be

absorbed by the human body more efficiently compared to the conventional drug available in the market [27]. Table 2 shows the mean particle size diameter of the nanocapsules. In this study, the particle size of each treatment ranges from 148.7 to 152.8nm.

Table 2. Particle size of nanocapsules

Treatments	Particle Size Diameter
Blank nanocapsules	148.7±8.7nm
T1 nanocapsules	150.9±9.2nm
T2 nanocapsules	152.8±9.7nm
T3 nanocapsules	150.0±9.1nm

The presence of surfactant Tween 20 in the nanocapsule system can contribute to the larger particle size of the Lycopene-loaded nanocapsules. According to the study of Khoee and Yaghoobian [28], the particle size of nanocapsule increased when the Tween 20 content is increased.

3.6 Surface morphology

Scanning electron microscopy (SEM) was employed to characterize the surface morphology of the lycopene-loaded nanocapsules (Figure 3). Also shown is FSEM (Figure 3).

The micrographs at 30,000× and 70,000× magnification revealed clustered and quasi-spherical particles. Notably, some degree of particle aggregation was observed, consistent with the findings of Bulatao et al. [29]. This phenomenon is likely attributed to the absence of cryoprotectants during the freeze-drying process. Freeze-drying can induce various stresses that destabilize the nanocapsules. The use of cryoprotectants has been demonstrated to enhance the stability of nanocapsules during freeze-drying [30].

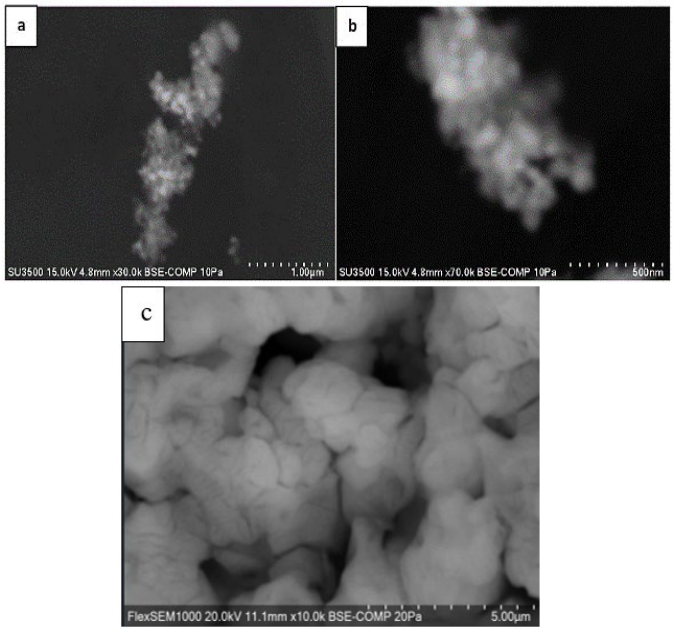


Figure 3. SEM image of lycopene-loaded nanocapsules at (a) 30,000× (b) 70,000× (c) FlexSEM magnification

4. CONCLUSION

In summary, solid-liquid extraction was used to extract crude lycopene from dried tomato then was purified using saponification-crystallization method. The recovered lycopene was found to be 8.07±1.48mg/kg of tomato. The purified lycopene was emulsified using 0.7mg/mL Tween 20 solution. The emulsified lycopene was encapsulated using 2.25mg/mL alginate, 36mM calcium chloride, and 6.3mg/mL Chitosan. Among encapsulation treatments, Treatment 3 showed the highest encapsulation efficiency of 86.85±0.53%.

The lycopene-loaded nanocapsule was characterized in terms of antioxidant activity, particle size, surface morphology, chemical properties. The antioxidant property was determined using DPPH scavenging assay. It was determined that the DPPH scavenging activity of Treatment 3 was 2.51±0.02% which is equivalent to 0.40±0.01µg ascorbic acid. The particle size of the nanocapsules was determined using zeta sizer. It was found that the lycopene-loaded nanocapsules has an average particle size ranging from 148.7±8.7nm to 152.8±9.7nm. Surface morphology was determined using scanning electron microscopy. It was found that the nanocapsules has a quasi-spherical shape.

The clumping of nanocapsules were also observed which is caused by absence of cryoprotectants during freeze drying. Lastly, major functional groups were determined using FTIR.

The peaks that were found in chitosan and alginate was also present on the spectra of both blank and lycopene-loaded nanocapsule which indicates the successful interaction and encapsulation of lycopene in chitosan-alginate nanocapsule. The synthesized ycopene-loaded nanocapsules hold promise for applications in the nutraceutical industry as a cost-effective antioxidant with increased bioavailability and adsorption due to their smaller size.

ACKNOWLEDGMENT

The authors would like to thank Dr. Nerissa C. Ramos for the good insight on the methodology. The author also would like to thank Central Luzon State University Nanotechnology R & D Facility for the fund support.

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NOMENCLATURE

A	Absorbance
Y	Equation derived
r^2	Curve constructed after samples

Greek symbols

β	Beta