Preparation of Cassava Starch Nanoparticles and their Application as a Carrier System for Curcumin Delivery

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Abstract

Cassava is a tropical tuber crop useful in the production of high quality of starch economically. One interesting application of starch is in the preparation of starch nanocrystals and nanoparticles by acid hydrolysis. These nanoparticles possess a reactive surface covered with hydroxyl groups, providing the possibility of extensive chemical modification. Curcumin or diferuloylmethane, a polyphenol extracted from the rhizomes of turmeric (\textit{Curcuma longa}), is one of the most widely studied natural chemo preventive agents that allows suppression, retardation or inversion of carcinogenesis. It is also described as an anti-tumoral, anti-oxidant and anti-inflammatory agent capable of inducing apoptosis in numerous cellular systems. In the present work, the preparation of starch nanoparticles from cassava starch, their characterization using Transmission electron microscopy (TEM) and Atomic Force Microscopy (AFM) and their interaction with curcumin are attempted. The evidence for curcumin loading was provided by fluorescence and Fourier Transform Infrared (FTIR) spectroscopies.

Keywords: Cassava, starch nanoparticles, curcumin

Introduction

Great progress has been achieved in the development of starch as a renewable carbohydrate polymer, procurable at low cost from a great variety of crops. The low cost of this biopolymer and its biodegradability, are the major reasons leading to the
growing interest in the nonfood usage of starch-based products for applications in which synthetic polymers have traditionally been the materials of choice. One interesting application of this biopolymer is in the preparation of starch nanocrystals and nanoparticles by acid hydrolysis [1-4]. The starch nanoparticles, for their properties qualitatively different from those of native starch granules, could be utilized in new applications. Polysaccharide nanoparticles possess a reactive surface covered with hydroxyl groups, providing the possibility of extensive chemical modification [5]. Angellier and coworkers, [3] reported their work consisted of optimizing the preparation of nanocrystals from waxy maize starch granules using sulfuric acid hydrolysis. The use of starch nanoparticles currently receives much attention because of the abundant availability of starch, its low cost, renewability, biocompatibility, biodegradability, and nontoxicity. The latter properties make these nanoparticles excellent candidates for implant materials and drug carriers [6].

The tropical tuber crops contain starch as the major component and thus act as important source of starch. Cassava (Manihot esculenta Crantz) and to a small extent, sweet potato (Ipomoea batatas Lam.) are used for starch extraction in many regions of the world. Studies on different starches at Central Tuber Crops Research Institute (CTCRI, Thiruvananthapuram, India) and elsewhere have brought to light the wide diversity in the starch characteristics of tuber crops [7]. Extraction of starch from cassava is simple and the isolated starch is pure white in colour and relatively free from other chemical impurities. The total amylose content in cassava starch has been reported to range from 13.6-23.8% [8].

A variety of pharmacological properties of curcumin, such as antitumour and anticancer activities were reported. It has also been used as a photodynamic agent useful for the destruction of bacteria and tumor cells [9]. Despite of these pharmacological properties of curcumin, its drug value is low because of its reduced bioavailability when orally administered. This is due to the poor solubility of curcumin in water. The therapeutic effects are essentially limited to the tubular lower gastro intestinal tract [10, 11]. The development of a delivery system that can enable the administration of curcumin in an aqueous phase medium will significantly harness the potential of this promising anti-cancer agent [12]. Since the potential of starch nanoparticles as drug carriers has been extensively studied [6], the present work was undertaken to prepare and characterize starch nanoparticles from cassava and to develop it as a carrier system for curcumin which could possibly be used in pharmaceutical applications.

Materials and methods
Materials
Fresh cassava tubers immediately after harvesting were used for starch preparation. Curcumin was a kind gift from Synthite Industrial Chemicals, Kochi (Kerala, India). All other solvents used were of analytical grade. Hydrolysis of the starch was performed on the platform of New Brunswick orbital shaker (GMI, USA) kept at room temperature. For centrifugation of the hydrolyzed starch, a Hermle Z-36-6 refrigerated centrifuge (Hermle Labortechnik, Germany) was used. TEM images were
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observed using a Jeol 1001 Transmission Electron Microscope (Jeol Ltd., Japan) at 80 KV accelerating voltage. AFM was performed using a NT-MDT Digital Instrument (NT-MDT, Russia) operating in the tapping mode region. The FTIR analysis was performed using a Perkin Elmer FTIR spectrometer (Perkin Elmer, USA). Fluorescence measurements were performed on a FluoroLog-3 spectrofluorometer (Horiba Scientific, Japan).

Preparation of aqueous suspension of cassava starch nanoparticles

Starch was extracted from fresh cassava tubers as described elsewhere [13] and was dried in an air oven to make it moisture free, and submitted to acid hydrolysis as reported by Angellier and coworkers [3]. A given weight (7.0 g) of moisture free native cassava starch powder was mixed with 50 ml of 3.16 M sulphuric acid solution in a 100 ml Erlenmeyer flask. The reaction mixture was stirred using a shaker kept at 30 ± 2 ° C and continuously stirred for five days at 100 rpm speed with an orbital shaking action. After 5 days, the suspension was washed by successive centrifugations for 10 minutes at 10000 rpm, with distilled water until neutrality. The suspension was then submitted to a mechanical treatment with homogenizer Ultra Turrax for 2 minutes at 13000 rpm to disperse aggregates and obtain a “stable” suspension. The resulting suspension was stabilized by sulfate groups present at the surface of nanoparticles gained from the H2SO4 treatment [14]. To avoid bacterial growth during storage, a few drops of chloroform were added to the suspension that was kept in refrigerator.

The experiment was repeated with different weights of native starch, 5.0 g, 7.0g and 10.0 g. When 5.0 g was taken as the initial weight of native starch, practically no residue was obtained after 5 days hydrolysis, but solid unhydrolysed residue was obtained when the initial weight of the starch taken was 7.0g and 10.0 g.

Characterization of starch nanoparticles

Transmission electron microscopy (TEM)

After a brief sonication, a drop of a dilute starch nanoparticle suspension was deposited onto a glow discharged formvar-coated microscopy grid. After 1 min, the liquid in excess was blotted with filter paper and the remaining film was allowed to dry. Once positioned into a specimen holder, the grid was transferred into the microscope, and observed at room temperature. All specimens were observed using a Jeol 1001 Transmission Electron Microscope (Jeol Ltd., Japan) at 80 KV accelerating voltage. Micrograph was recorded.

Atomic Force Microscopy (AFM)

AFM was performed using a NT-MDT Digital Instrument (NT-MDT, Russia) operating in the tapping mode region. Micro- fabricated silicon Cantilever tips (MPP-1100-10) with a resonance frequency of 299 KHz and a spring constant of 20-80 nm\(^{-1}\) were used. The scan rate varied from 0.5 to 1.5 Hz. AFM analysis was done offline. Starch nanoparticles for the imaging were prepared by drop casting the suspension on freshly cleaned mica at the required concentration and examined under ambient conditions. In order to rule out the possibility of any artifacts, we have carried out
blank experiments with neat solvents (without test material) on mica. Scanning at various planes showed the neat surface of mica without the morphology of any objects.

**Aggregation of starch nanoparticle suspension**

The cassava starch nanoparticle suspension when refrigerated after adding a few drops of chloroform is stable for a long period. These particles have a tendency to unite with each other and to form aggregates. We have analyzed the particle size immediately after preparation and after two months of storage and found that aggregation of particles occurs on long standing. One interesting behavior of this suspension is that, when acetone is added in drops to the starch nanoparticle suspension after filtration through a Millex GP (Millipore) filter having a pore size of 0.22 μm (220 nm), the nanoparticles get aggregated as shown in Figure 3. The test at the right side of the figure clearly shows the aggregated nanoparticles. By centrifuging the mixture obtained after adding sufficient acetone for obtaining maximum aggregation, at 10,000 rpm for 10 minutes, the aggregated starch nanoparticles were recovered. This was then washed using methanol and vacuum dried. This solid easily becomes a gel, as soon as it comes in to contact with atmospheric air. This residue was used to record the solid fluorescence spectrum and FTIR spectrum.

**Loading of curcumin on starch nanoparticles**

Starch after hydrolysis as mentioned above, was diluted and filtered carefully through a Millipore filter having a pore size of 220 nm and the filtrate was collected. This filtrate was immediately treated with curcumin in acetone (0.005 molar) in the ratio 3:1 in a screw capped flask and mixed well and kept for 8 hours. To separate the modified starch particles after the reaction, sufficient amount of acetone was added to aggregate the starch particles and centrifuged. The residue was then washed with methanol to remove adhered curcumin till the filtrate is colorless, and dried in a vacuum dessicator. This residue was used to record the solid fluorescence spectrum and FTIR spectrum as briefed in section 2.6.2.

**Characterization of starch nanoparticles after loading with curcumin**

**Fluorescence measurements**

Fluorescence measurements were performed on a FluoroLog-3 spectrofluorometer (Horiba Scientific, Japan). A small amount of the sample was placed between the cover slips and placed in the sample holder directly and spectrum was recorded. An excitation wavelength of 390 was applied for curcumin and modified starch particles while taking the spectrum. The spectrum was recorded from 400nm to 600 nm.

**FTIR measurements**

The FTIR analysis was performed using a Perkin Elmer FTIR spectrometer (Perkin Elmer, USA). The samples were mixed with analytical grade KBr at a weight ratio of 5/200 mg. FTIR spectra of the starch nanoparticles before and after chemical modification were recorded in the wave number range 400-4000 cm⁻¹. Background scans were obtained using the KBr powder.
Results and discussion

TEM images
TEM micrograph of starch nanoparticles is shown in Figure 1. This micrograph clearly shows that the starch nanoparticles obtained after 5 days of sulphuric acid hydrolysis have the shape of aggregates of spherical nanoparticles with majority of them observed in the size range of 50-100 nanometers. In the figure there are white dots surrounded by a gray halo corresponding to individualized or aggregates of several starch nanoparticles.

AFM analysis
AFM images of starch nanoparticles are shown in Figure 2. From the figure it is clear that majority of particles are having a particle size around 100 nm. Results from TEM and AFM imaging clearly show that the particles produced are spherical with particle size in the nano scale; the structural integrity of the nanoparticles is confirmed. In contrast to the shape of nanoparticles produced from waxy maize, which were obtained as platelets of nanocrystals [3], these particles are spherical. The shape and particle size of granules depends strongly on its botanic origin [15]. The large surface area, inherent to the small size of nanoparticles, guarantees a large surface activity and a high grafting per unit mass of particles [14]. Szymonska and coworkers [16] prepared cassava and potato starch nanoparticles by grinding the starch-ethanol suspensions in a vibration mill. Mixture of the processed granules was separated by sedimentation into polysaccharide fractions and the fractions after 36 hours of sedimentation (about 15% of the mixture) were collected. They determined physicochemical properties of starch nanoparticles and found that these particles have a high aqueous solubility and swelling power compared to native starch which indicated that the particles fit the amyllopectin type short branched species. Disadvantages of this method are that the stabilization of the particles by the sulphate groups is not there, and that the mechanical processing of starch caused a severe damage to the granules.

Aggregation of starch nanoparticles
The aggregation of starch nanoparticles on adding acetone is shown in Figure 3. This behavior of starch nanosuspension on adding acetone can be explained on the basis of the agglomeration of the nanoparticles. Since these particles are not showing this behavior towards methanol, we can assume that, polarity of the solvent also plays an important role. The starch nanoparticles are believed to aggregate as a result of hydrogen bond interactions due to the surface hydroxyl groups [3]. Blocking these interactions by relatively large molecular weight molecules obviously improves the individualization of the nanoparticles [17]. Angellier et al [14] estimated the hydroxyl group content present at the surface of freeze-dried starch nanoparticles to be approximately 14% of the total amount available, i.e., in 1 g of freeze-dried starch nanocrystals, only 0.0025 mol of hydroxyl groups were reactive. This indicates that only the polar hydroxyl groups sitting at the surface of starch nanoparticles are available for chemical modification, and others remain intact within the particle, so the morphology of the starch nanoparticle skeleton can be kept unchanged, even after interaction.
Fluorescence spectra
Curcumin is naturally fluorescent in the visible green spectrum. From the fluorescence spectra (Figure 4), it can be seen that, both curcumin and starch nanoparticles interacted with curcumin exhibited a very similar spectra at the excitation wavelength of 390 nm. Since starch nanoparticles alone did not fluoresce at this excitation wavelength, it can be confirmed that there is considerable loading of curcumin on starch nanoparticles as a result of this treatment.

FTIR spectra
Figure 5 shows the FTIR spectra of starch nanoparticles; starch nanoparticles which have been interacted with curcumin [after aggregation by adding acetone] and curcumin respectively. The characteristic peak occurred around 1650 cm\(^{-1}\) for starch nanoparticles, is believed to be a feature of tightly bound water present in the starch [18]. The presence of curcumin in the curcumin loaded starch nanoparticle could be inferred from the observation of the peaks at 1250, 1165, 805 cm\(^{-1}\) which in the case of curcumin appear at 1275, 1164 and 817 cm\(^{-1}\). Similarly peaks at 3400, 2900, 2390 and 1680 cm\(^{-1}\) are all red shifted to 3530, 2970, 2360 and 1725 cm\(^{-1}\) respectively.

Figure 1: TEM of starch nanoparticles obtained after hydrolysis of cassava starch granules.

Figure 2: AFM image of cassava starch nanoparticles.
**Figure 3:** Aggregation of starch nanoparticles (left) after adding acetone (right).

**Figure 4:** Excitation and Fluorescence spectra of curcumin and starch nanoparticles treated with curcumin (after aggregation by adding acetone)

**Figure 5:** FTIR spectra of starch nanoparticles (B) and starch nanoparticles loaded with curcumin (A) (after aggregation by adding acetone) and pure curcumin (C).
Conclusion
Cassava starch nanoparticles were successfully produced by subjecting cassava starch to sulphuric acid hydrolysis. TEM and AFM images indicate particle size in the nano scale; with majority of particles having a size around 100 nanometers and these are spherical in shape. These starch nanoparticles are stable for several months, when refrigerated. The main advantage of this method over the mechanical grinding procedure [16] is that the nanoparticles attained inherent stability due to the presence of sulphate groups at their surface gained from the H2SO4 treatment [14]. Curcumin loading on the starch nanoparticles is confirmed by fluorescence and Fourier Transform infrared spectroscopies. Starch nanoparticles thus modified with curcumin could possibly be used in pharmaceutical applications since these particles possess excellent drug carrier properties [5].

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References


