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Fabrication and fluorescent labeling of guar gum nanoparticles in a surfactant free aqueous environment

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ABSTRACT

Guar gum (GG) is a galactomannan obtained from the seeds of *Cyamopsis tetragonoloba*. GG polysaccharide is used in a range of technology arenas and its properties in water environment elicited a lot of interest. We report a simple acid hydrolysis technique to derive water dispersible spherical nanoparticles from GG for the first time without any stabilizing surfactant. The particles demonstrated colloidal stability and were observed to carry negative surface charges in aqueous environment. The TEM study of guar gum nanoparticles (GGn) indicated a size range of 30–80 nm with a mean at 48.8 nm. Mineral acid at apposite concentration have apparently solubilized the amorphous regions of the polymer chain leaving the crystalline parts. GGn were further covalently functionalized in aqueous environment with fluorescein isothiocyanate (FITC) using a hydroxy–propyl amine spacer group. Nanoparticle fluorescence intensity can be modulated with pH changes and a significant augmentation in the intensity was noticed at the physiologically relevant range of 6–8. New fluorescently labeled nanoparticles have potential for applications in bioimaging, biomolecular interaction studies and as a pH sensing probe in sub cellular environment.

1. Introduction

Nanoparticles elicit excellent properties that are known to add efficiency in different application areas [1–6]. Metal and hybrid nanomaterials for example are proposed as metamaterials, smart therapeutic tools, sensing devices and for imaging applications. Polysaccharide nanosystems are particularly interesting because of synthetic polymer reinforcing and printable paper appliances, moisture barrier effects and due to enormous possibilities in drug delivery applications [7,8]. Plant polysaccharides in addition, elicit some of the fundamental interests in material chemistry. These are the first polymer class that was created in nature through a highly organized template directed system [9]. Biopolymers like cellulose, chitins and some of the galactomannans associate specific intrinsic properties which suit them well for new material requirements in the current era [7,10–13]. Biopolymer nanoparticles on the other hand are also considered as greener tools in nanotechnology for low polluting effects, sustainability and low risk in environmental propagation. Some of these materials have also proved as very useful tools due to properties often akin to that of carbon based nanoparticles [14].

Nanomaterial extractions from renewable resources are significantly advantageous over particle synthesis techniques because of low energy cost involvement, process scalability parameters and simpler instrument requirement. Diverse nanotechnologies are currently experimented both for extraction and in post shaping of functional biopolymer materials [15]. In different earlier occasions large quantities of surfactants or hazardous stabilizers were essential to keep the biopolymer nanoparticle dispersions stable in water for final application [16]. Nanostructure stabilization in water environment due to colloidal propagation is facilitated when the particles are appropriately charged. Alternatively, hydrophobized biopolymer nanoparticles can be produced in water environment as a result of ouzo effects [15]. We intended to organize this work for extraction of freely water dispersible guar galactomannan nanostructures following a facile acid hydrolysis technique.

Guar gum (GG), is one of the most versatile engineering biopolymer. GG is a galactomannan available from the seeds of *Cyamopsis tetragonoloba*. Stable gels from GG are useful in a number of areas including textile sizing, printing of papers, additives, construction cement and in petroleum oil drilling as drilling mud to crack open rock pores for extraction of oils [17,18]. GG is also applied in food and cosmetic industry for excellent protein interactions and mouth filling quality. GG mannan chain is stable within the physiological environment and hydrolyzes only in the anaerobic colon microflora. This effect is applied extensively for different controlled colon centric drug delivery device designs [19]. GG nanoparticle (GGn) was further conceived for an early uptake in cellular systems like macrophages. We also perceived that the GG core nanostructures if achieved, can aid in understanding galactomannan structural chemistry. This can also help to realize some of the fundamental nano-bio-medicine applications.

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In the present study, extraction of nanoparticle GGn was carried out in a surfactant free technique and organic solvents were completely avoided. This enabled us to obtain the GG nanoparticles in a most environmental way possible. The particle formation was elaborately studied to realize the final structure morphology and physico-chemical properties. Covalent modification of the GGn surface was further performed following an epichlorhydrin reaction in liquor ammonia. Intense fluorescent labeling on highly mobile nanoparticles was facile in water environment. Fluorescently labeled nanoparticles, GGnF, were excellent tools in bioimaging and may further be applied as molecular sensors for biological, environmental and drug delivery applications.

2. Experimental

2.1. Materials

GG and analytical grade solvents like acetone, alcohol and ether were purchased from E. Merck (India). 5-Fluorescein isothiocyanate (FITC) was obtained from Koch-light Laboratories Ltd (England). Analytical grade sulfuric acid and 1-chloro-2,3-epoxypropane (epichlorohydrin) were procured from Spectrochem (India). Dialysis membrane tubes (D-9652-100FT), molecular weight cut-off 12.4 kDa was from Sigma Aldrich (USA) and was used after appropriate purifications. Unless otherwise mentioned, HPLC grade water from Spectrochem (India) was used throughout.

2.2. Purification of guar gum

Guar gum (GG) was purified following a method originally described by Wientjes et al. [20], with minor modifications. Briefly, 5 g of GG was refluxed for 10 min under magnetic stirring in 100 mL of 80% v/v boiling ethanol. The slurry was filtered on a glass filter and washed successively with 50 mL each of ethanol, acetone and solvent ether. The precipitate was further added in 500 mL of distilled water and allowed to soak under stirring for 1 h at room temperature. The solids were collected by centrifugation for 15 min at 1500 rpm. Precipitated biopolymer was dissolved in hot water at 70 °C and centrifuged at 6000 rpm for 1 h at 22 °C. The supernatant was added with ethanol under external cooling and the precipitate was collected on a glass filter, washed successively with 20 mL each of ethanol and acetone. Powdered GG thus obtained was dried in a current of air and preserved under nitrogen atmosphere in air tight containers.

2.3. Guar gum nanoparticles (GGn)

Nanoparticle GGn, were prepared following an acid hydrolysis reaction. Typically, 20 mL of aqueous sulfuric acid (64% w/w) was added to two gram of the purified GG powder (2.2) and the reaction mixture was stirred mechanically at 100 rpm for 60 min over a constant temperature bath maintained at 45 ± 2 °C. The reaction was terminated by pouring the reaction mixture into 200 mL of water and cooled externally at 4 °C. The resultant colloidal dispersion was centrifuged at 16,000 rpm, re-dispersed in water and re-centrifuged. A small amount of ammonia solution (0.5% w/v) was then added to remove adherent acids and the GGn were washed successively in water till neutrality. Final product was dialyzed for 72 h and the pH was checked to constant. Dialyzed aqueous suspension was added with 0.05 mL of chloroform to prevent microbial contaminations [21] and was preserved in desiccators until further applications.

2.4. Fluorescent labeling of guar gum nanoparticles (GGnF)

GGn 0.5 g, was taken in a round bottom flask and 1 M NaOH (70 mL) and 1-chloro-2,3-epoxypropane (1.5 mL) were added to it in a sequence. The reaction mixture was stirred under nitrogen purging at 60 °C for 2 h. Resultant solids were gathered after centrifugation at 16,000 rpm at 4 °C, washed with water and dialyzed against deionized water until the pH was <8. Following dialysis, the solution pH was adjusted to 12 using 50% w/v NaOH. 2.5 mL of Ammonium hydroxide (30% v/v) was added and the mixture was stirred at 60 °C for 2 h. The product was dialyzed against deionized water till pH 7 to obtain amine functionalized GGn. FITC (0.32 mmol/g), NaCl (0.15 M) and sucrose (0.3 M) were added to the aminated nanoparticles and were reacted overnight under magnetic stirring in the dark. The colored mixture was transferred into dialysis tubes and dialyzed against running deionized water for 5 days. The dialysis medium was routinely checked till no absorbance due to free FITC was observed. Final solution was sonicated for 15 min at 20 kHz and the nanoparticles GGnF, were gathered after centrifugation at 30,000 rpm for 30 min at 4 °C.

2.5. Particle analysis

The particle ζ potential and polydispersity in water for nanoparticles GGn and GGnF were determined in a Zetasizer Nano ZS (Malvern Instrument, Malvern, UK). Particles were diluted with water and analyzed at 25 °C against a 4 mw He-Ne laser beam, 633 nm and a back scattering angle of 173^0. The ζ potentials were calculated as the electrophoretic mobility values, at the interface of the particles under an applied electric field using Smoluchowski equation [22]. An average of ten measurements in each case was recorded. Different experiments were also run in order to understand different ionic concentrations effects on new nanoparticles.

2.6. Fluorescence and UV absorbance

The UV–vis absorption spectra were recorded in Shimadzu UV 2550 UV–vis scanning spectrophotometer using quartz cuvettes with an optical path of 1 cm. Diluted solution (10 μg/mL) of nanoparticles were used in UV absorption studies against water only as blank.

Fluorescence emission spectra for GGnF or FITC in water were recorded in a Perkin Elmer LS-55 fluorescence spectrophotometer (Perkin Elmer Instruments, USA). Separate experiments were run in case of GGnF at different pH levels adjusted with appropriate 100 mM buffers.

2.7. Nanoparticle yield and Degree of substitution

The hydrolysis yield (wt %) of the nanoparticles was calculated by recording the dry weight ratio of the nanoparticles in the final suspension as contrast to the initial weight of purified GG taken in analysis. All samples were dried in an air circulating oven maintained at 105 °C until a constant weight was achieved in each case.

The hydrolysis yield was calculated as Y % employing a formula [23]

$$Y\,\% = (\text{Weight of final nanoparticles}/\text{weight of guar gum taken initially}) \times 100.$$

Elemental composition of GGn, the aminated intermediate and the GGnF particles were analyzed in a CHNS analyzer (CHNS-932, Leco Corporation, USA). The Degree of substitution (DS) in all cases was calculated from the carbon percentage composition [24].

2.8. FT IR studies

FT–IR scans were carried out in pressed potassium bromide pellets, over the mid IR ranges of 4000–400 cm^{-1} in a Jasco-670Plus FT-IR Instrument (Jasco Corporation, Japan). Spectral acquisitions were recorded at a resolution of 4 cm^{-1} and an average of 256 scans for each sample was gathered and stacked in biorad software (Biorad, KnowItall, USA) for comparison of overlap regions and functional group studies.