International Journal of Biological Macromolecules 76 (2015) 181–187

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Preparation and characterisation of gelatin–gum arabic aldehyde nanogels via inverse miniemulsion technique

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ARTICLE INFO

Article history:
Received 7 October 2014
Received in revised form 18 February 2015
Accepted 23 February 2015
Available online 4 March 2015

Keywords:
Nanogel
Gelatin
Gum arabic
Inverse miniemulsion
Biomaterials
Schiff's base reaction

ABSTRACT

Gelatin–gum arabic aldehyde nanogels designed by a nanoreactor concept using inverse miniemulsion technique were reported. Stable separate miniemulsions were prepared from gelatin (Gel) and gum arabic aldehyde (GAA). These emulsions were intermixed under sonication to obtain cross-linked nanogels. During fusion, cross-linking occurred between aldehyde groups of GAA and amino groups of gelatin. The concentration of the surfactant and weight fraction of water in the inverse miniemulsion was optimised so as to yield nanogels with controlled particle size. Properties of the nanogels were studied by FT-IR spectroscopy, particle size analysis and XRD. Surface morphology of the nanogels was established by Scanning Electron Microscopy (SEM). SEM and particle size analysis confirmed that nanogels possess spherical morphology with an average diameter of 151 ± 6 nm. Hemolysis property of the nanogels was examined and the results indicated that the nanogels were hemocompatible. The *in vitro* cytotoxicity of the nanogels towards MCF-7 cells was evaluated by MTT assay and the nanogels showed nontoxic behaviour towards the cells. All these studies confirm that these nanogels are potential candidates in applications such as drug and gene delivery.

1. Introduction

The increasing interest on nanoscale materials with biocompatibility, biodegradability and nontoxicity has accelerated research on development of new nanomaterials and new synthetic routes. Nanogels are one such class of materials which bagged great attention from different areas of biology, chemistry, physics and medicine because of their unique properties offered by its nano size [1]. Nanogels are nanometer sized counterparts of hydrogels, possessing special properties such as huge surface area, colloidal stability and elevated drug loading ability offered by its small size in addition to the properties of hydrogels [2,3]. Because of their potential as versatile carriers for different therapeutic and diagnostic agents, nanogels find applications in biomedical fields, such as gene delivery, drug delivery and bioimaging [3–5]. Nanogels have been prepared using synthetic as well as natural polymers. Although synthetic polymers like poly(ethylene glycol) [6], poly(lactic acid) [7] and poly(ε-caprolactone) [8] proved their ability as suitable materials for development of nanogels, natural polymers like chitosan [9], dextran [10] and hyaluronic acid [11] received more importance owing to their nontoxicity, biocompatibility and biodegradability. Jayakumar and co-workers developed pH responsive chitin and chitosan nanogels and examined the viability for drug delivery to cancer cells, gene therapy, biosensing and bioimaging [12–15] applications. Pullulan, a highly hydrophilic polysaccharide was hydrophobically modified with cholesterol/spiropyran and self assembled nanogels were prepared from the resultant amphiphilic polysaccharide [16,17].

For the present work, natural polymers, namely, gelatin and gum arabic (GA) were selected. Gelatin is a well exploited natural protein with fascinating biomedical properties such as biocompatibility, biodegradability, non-immunogenicity and safety. It is extensively used in food industry, in pharmaceutical industry as drug carrier, and in biomedical field as tissue engineering matrix [18,19]. Koul et al. [20] prepared nanogels of interpenetrating polymer network of gelatin and polyacrylic acid by one pot inverse miniemulsion technique and this nanogels could be used as drug delivery vehicles for cancer targeting. Paclitaxel-loaded gelatin nanoparticles exhibited rapid release of the drug and showed significant activity towards human cancer bladder cells [21]. Tseng et al. [22] developed gelatin nanoparticles by desolvation method and its surface was modified with NeutrAvidin^{FITC}-biotinylated epidermal growth factor (GP-Av-bEGF) for targeting to lung cancer

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http://dx.doi.org/10.1016/j.ijbiomac.2015.02.038
0141-8130/

cells. Gelatin nanoparticles were also utilised for loading and delivery of protein and peptide drugs such as insulin [23], bovine serum albumin (BSA) [24], alkaline phosphatase (ALP), bone morphogenetic protein-2 (BMP-2), tissue-type plasminogen activator (t-PA) [25] and angiogenic basic fibroblast growth factor (bFGF) [26]. Other applications of gelatin nanoparticles include ocular drug delivery, nutraceutical delivery, pulmonary drug delivery, enzyme immobilization *etc.* [27].

For overcoming the poor mechanical properties and to improve strength and aqueous stability, gelatin based hydrogel systems were cross-linked with different cross-linking agents including glutaraldehyde, diisiocyanates, genipin, carbodiimide [28–31] and oxidised polysaccharides [32,33]. Cross-linking of gelatin with oxidised polysaccharides is a safer method to improve the properties compared to the cross-linking with toxic agents like glutaraldehyde, diisocyanates and carbodiimide. In the present work, a plant polysaccharide, namely gum arabic was selected to prepare the cross-linked nanogels. Gum arabic is obtained from the exudates of acacia tree. It has a complex branched structure with rhamnose, galactose and glucuronic acid residues. The back bone and side chains consist of 1,3 linked β-D-galactopyranosyl units with side chain joined to the main chain by 1,6 linkages [34]. High water solubility, biocompatibility and low cost are the main attractions for selecting this polysaccharide for this work. GA is widely used in the food industry as stabilizing, emulsifying and thickening agent. Even though GA was used for the preparation of microparticles and nanoparticles, biomedical applications of this potential polysaccharide were not explored in greater detail. Avadi et al. [35] developed gum arabic and chitosan based nanoparticle system for oral delivery of insulin. Release properties of gum arabic microparticles were investigated with vettiver essential oil and camphor oil as models [36,37]. Nishi et al. prepared oxidised GA and conjugated it with different drugs and evaluated its application in drug delivery [38–40]. The preparation, characterisation and *in vitro* biomedical applications of gum arabic aldehyde cross-linked gelatin hydrogels [41,42] have been reported by us recently. However, no research effort has been made to prepare GA based nanogels. In this work, nanogels from oxidised gum arabic (GA) and gelatin was prepared by fusion of two separate inverse miniemulsions.

Less energy intensive technique is the preferred choice of researchers to produce nanomaterials. One of the highly explored, such method is water-in-oil (w/o) mimiemulsion and it was used to prepare semiconductors [43], polymeric nanoparticles [44], drug nanocrystals [45] and magnetic particles [46]. The ease with which miniemulsion can be prepared makes it a favourable method for developing nanoparticles. For the preparation of Gel–GAA nanogels, fission and fusion of separate miniemulsions of GAA and gelatin was adopted. These two miniemulsions contain individual reactants in aqueous phase and during fusion, inter micellar exchange and cross-linking of individual reactants occur leading to the formation of nanogels. Processing parameters such as concentration of surfactant and aqueous fraction in the inverse miniemulsion were optimised to obtain Gel–GAA nanoparticles with controlled size. Physicochemical properties of the nanogels were analysed by particle size analysis, SEM, FT-IR, and XRD. Hemocompatibility studies and MTT assay were performed to assess the blood and cytocompatibility of the nanogels.

2. Materials and methods

2.1. Materials

Gum arabic (from acacia tree) of approximate molecular weight 250 kDa, trinitrobenzenesulfonic acid (TNBS) and gelatin (Type A) were obtained from Sigma–Aldrich, Saint Louis, USA. Sodium metaperiodate, sodium tetra borate (borax), Span 20, sodium chloride, disodium hydrogen phosphate, sodium dihydrogen phosphate, hydroxyl amine hydrochloride, sodium carbonate, methyl orange, minimum essential medium (MEM), isopropanol, sodium hydroxide, cyclohexane and acetone were obtained from Merck (Mumbai, India). Dialysis tubing (3500 MWCO) was procured from Spectrum Laboratories Inc., CA, USA.

2.2. Methods

2.2.1. Preparation of gum arabic aldehyde

Gum arabic aldehyde (GAA) was prepared from gum arabic using sodium metaperiodate by previously reported procedure [39]. Gum arabic (10 g, 0.058 mol) was dissolved in 100 ml distilled water and sodium periodate (1.24 g, 0.0058 mol) required for 10% oxidation was added to it. The reaction mixture was stirred magnetically under dark at 20 °C for 6 h. After the reaction, the mixture was purified by dialysis using dialysis tube of MWCO 6000–8000 for three days against distilled water. After purification, the dialysate was frozen and lyophilized. The yield of the product was in the range of 80–85%.

2.2.2. Preparation of gelatin–gum arabic aldehyde nanogels

Gelatin–gum arabic aldehyde (Gel–GAA) nanogels were prepared by an inverse miniemulsion technique [29]. The procedure involves preparation of two separate emulsions A and B, where A contains gum arabic aldehyde nanoparticles, while B contains nanoparticles of gelatin. Later, these two emulsions were mixed and sonicated to obtain Gel–GAA nanogel. For both the emulsions, 6 mg of span 20, dissolved in 5 ml of cyclohexane acted as the continuous organic phase. The procedure adopted in a typical experiment for the preparation of nanogel is as follows. For preparing inverse miniemulsion of GAA, a solution of GAA (10%, w/v) was prepared in 0.1 M borax. This solution (250 μl) was added to the continuous organic phase and sonicated for 5 min. The second miniemulsion (B) was prepared in a similar fashion. Gelatin (5 g) was dissolved in water (10 ml) by heating at 40 °C and 250 μl of the solution was added to the continuous organic phase and sonicated for 5 min. Later, these two emulsions were mixed and sonicated again for 5 min to obtain Gel–GAA nanogel. The nanogel emulsion was poured drop wise into 50 ml of acetone and was stirred magnetically. The nanoparticles were isolated by centrifugation at 6000 rpm for 5 min. This process was repeated for three times with acetone followed by two times with water in order to remove the surfactant and other impurities. The centrifugate was then dried under reduced pressure to obtain the nanogel powder. Nanogels were prepared by varying the surfactant concentration and total volume of the aqueous phase.

2.2.3. Determination of aldehyde content

Aldehyde contents in GAA and Gel–GAA nanogels were determined by titrimetric method [32]. GAA (0.1 g) and Gel–GAA nanogels (0.050 g) were dissolved in 25 ml, 0.25 N aqueous solution of hydroxyl ammonium chloride. Liberated hydrochloric acid resulting from the reaction of aldehyde groups with hydroxyl ammonium chloride was titrated against 0.1 N NaOH using methyl orange (0.05% solution, w/v) as indicator. The colour change from red to yellow is the endpoint. The number of moles of NaOH consumed is equivalent to the number of moles of aldehyde present in the sample.

2.2.4. Degree of cross-linking

The degree of cross-linking in Gel–GAA nanogel was estimated by TNBS assay. In this assay, free amino groups in gelatin was reacted with TNBS [47]. Gelatin (25 mg) and Gel–GAA nanogels (25 mg) were mixed with 1 ml of 4% sodium bicarbonate solution and 0.05% TNBS solution. The mixture was heated at 60 °C for 4 h