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Encapsulation of Congo Red in carboxymethyl guar gum–alginate gel microspheres

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ABSTRACT

Congo Red (CR) is a hydrophobic dye commonly used for diagnosis and potentially useful as therapeutic agent of beta amyloid plaques in neurodegenerative diseases. CR, as drug model, was encapsulated on Alginate–Carboxy Methyl Guar Gum (Alg–CMGG) blend microspheres. Guar gum 18% carboxymethylated (CMGG) derivative was synthesized in order to improve aqueous solubility, polymer blending and help reduce surface tension. The derivative was confirmed by FTIR spectroscopy, and elemental analysis. Surface tension of the new CMGG is reduced in about 50% compared with the native polymer. Lowering of Guar Gum (GG) aqueous solutions viscosity from 30,000 cps to 350–400 cps in case of CMGG is indicating pseudoplastic fluid behavior modifications. Vibrational spectroscopy analysis confirmed interactions among CR molecules in alginate–CMGG matrices ascribed largely to the aromatic motif of the dye and the biopolymer a polar regions. CR was encapsulated on 68/32% alginate/CMGG blend microspheres as the best formulation tested. The release of CR from the microspheres was not detected at pH = 1.2 in 25 min, but 62% of CR was found in the supernatant when the pH was raised to 7.4 at 37 °C after 8 h incubation.

1. Introduction

Congo Red (CR) is a linear anionic secondary diazo dye used as an acid-base indicator. Additionally, CR was used to detect fibril proteins enriched in β sheet conformation useful in histological studies of some neurodegenerative pathologies such as Alzheimer's, Creutzfeldt–Jacob's, Huntington's and Parkinson's diseases [1]. Moreover, CR delays appearance of clinical signs on experimental prion trials [2]. The compound could also be used as a palliative in neurodegenerative disease therapies. CR is soluble in many organic solvents, but yielding red colloidal fluorescent solutions in aqueous media because of its hydrophobicity made by the presence of biphenyl and naphthalene groups in the molecule [3]. The postulated mechanism for CR aggregation is by hydrophobic interaction involving the π–π bonds of the aromatic rings making planar structures [4]. Based on the CR physicochemical properties, the dye is an excellent candidate to test the potential encapsulation of common hydrophobic drugs, e.g. anthracyclines, taxanes, fluoroquinolones, into biopolymeric gel matrices.

In the last decades, biopolymers have received increasing attention both in the academia and in industry. Remarkable biopolymer properties like structural diversity, biological specific properties over a range of molecular dimensions and favorable non-covalent physiological interactions were unearthed. Additionally, tailorability, biodegradability, mild environmental synthesis, and convenient rheological modulations have made biopolymers very attractive tools for a myriad of applications [5]. Ultrapure Alginates (Alg) produced under GMP/ISO 9000 guidelines are one of most common biopolymer gels currently used in food and pharmaceutical industries. Algs are linear biopolymers composed of β-mannuronic acid (M) and α-guluronic acid (G) linked by 1–4 bonds, purified from the seaweeds and some bacteria. Alg hydrogels are formed by ionic crosslinking in presence of divalent cations (e.g. calcium and zinc), which cooperatively interact with different carboxylate ions forming ionic bridges between different polymer chains. Alg gel structure is commonly named as "egg box" because of the analogy with the egg containers. Alginate gels have been

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used extensively as a matrix for entrapment of many molecules and cells, and also in tissue engineering applications [6]. However, Alg gels are showing some drawbacks such as high hydrophilicity and erodability at alkaline pH, poor mechanical strength, and gel instability in freeze–thaw cycles which are preventing extensive applications. Some alternative strategies were developed earlier in order to improve Alg gel quality for many applications. Prior attempts in similar applications relied upon polymer chemical modification and covalent linking with a wide range of molecules like polyoles, chitosan, to sorbitan esters, amongst others [7]. Exhaustive chemical alterations have rendered Algs untenable in physiological or biological applications. Biopolymeric blends in non-covalent interactions are immerging alternatives and are more clearly understood in very recent years. Furthermore, inherent biomolecular blend interactions are low energy processes that are industrially more acceptable and easy to prepare. Novel properties of individual polymer blends can also add advantages in pin point applications [8].

A very attractive molecule to develop polymer blends is Guar Gum (GG). GG is a biopolymer synthesized in the endosperm of *Cyamopsis tetragonolobus*, a seed legume commonly found in the north of India and Pakistan. Chemically, GG is a galactomannan composed by a linear chain of β 1,4-D-mannopyranoses to which D-galactopyranoses residues are α 1,6-linked at every second mannosyl residue forming short side-branches (2:1 ratio) with a molecular weight of about 200 kDa. GG is also a robust engineering biopolymer that has found applications in concretes, cement settings and in petroleum oil drilling as a drilling mud. Relevant GG properties include thixotropy (the decrease in viscosity over time at a constant shear rate) above 1.0% concentration in water, the ability to retard ice crystal growth non-specifically by slowing mass transfer across the solid/liquid interface, good stability during several freezing–thawing cycles, and water swelling activities pH-dependently. Additionally, GG has approximately 8-times the water-thickening potency of corn starch, and can be used in multi-phase formulations as an emulsifier preventing oil droplets from coalescing, and/or as a stabilizer in order to prevent molecular aggregation and particles from settling, and it can form gels in presence of calcium and other polyvalent cations [9]. GG is commonly used in cosmetics and foods as a thickener in the USA and in the EU (E412 additive code). In foods, GG is an additive in the dairy industry (yogurts, kefir, and liquid cheese products), also to prevent ice crystal formation on sauces and dressing, meats, baked goods, ice creams, and in dry foods (e.g. soups and deserts). However, GG standalone presents some problems to be handled, like low hydrophilicity and concomitantly slightly solubility in aqueous solutions. A typical strategy to increase the solubility and polymer compatibility of GG is by introducing polar groups in the main structure of polymer.

The GG trimethyl amine derivative is currently used in the hair conditioner Jaguar® under patent protection. Alternatively carboxymethylation of GG, is possible where in, the reactive primary hydroxyl groups of galactomannan could be substituted with carboxylate functions. Consequently, the Carboxy Methyl Guar Gum (CMGG) derivative can became more soluble in water and making clear and low viscosity solutions. Despite of many advantages, CMGG impede in standalone gel formation in presence of multivalent ions due to functional groups distance geometry and biopolymer structural constrains. Similar galactomannan biopolymers however can help shape polymer blends and develop interpenetrating smart materials networks for biological and environmental application [10].

In previous study, hundred percent encapsulation efficiency of BSA (bovine serum albumin) in Alg–GG matrix crosslinked with glutaraldehyde was reported. Also, the BSA release from the gel matrix was pH-dependent, and unsusceptible to freeze and thawing procedures [7]. The same research group described the entrapment of crosslinked subtilisin crystal aggregates in Alg–GG gel matrix increasing the enzyme stability under harsh environmental conditions [11]. Furthermore, a successful purification of the Jacalin lectin using Alg–CMGG in a fluidized bed technique was described [12]. Later, the swelling and degradation of Alg–CMGG cross-linked with divalent barium ion at different pHs and pretreatments was also reported [8]. On the other side, both Alg and GG biopolymers has been reported individually to have beneficial effect of human health reducing serum cholesterol, and having positive effects on blood glucose [13,14].

The aim of the present work is to develop alginate–carboxy methyl guar gum hydrogel blend microspheres containing CR as molecular cargo for drug delivery. That model could also be used further as a therapeutic agent to reduce abnormal protein β folding associated to some neuronal disorders.

In order to develop the biopolymer blend, Carboxy Methyl derivative of GG (CMGG) was synthesized and characterized by centesimal composition, derivatization degree, viscosity, surface tension studies, and FTIR. Alg–CMGG gel microsphere blend composition was optimized by controlled release kinetic and swelling studies *in vitro* under different experimental conditions. The studies were complemented by optical and scanning electronic microscopy (OM and SEM), and the interactions between the hydrogel components and cargo were analyzed by viscosimetry and correlated with FTIR and Raman spectroscopies.

2. Materials and methods

2.1. Materials

Congo Red (CR), the sodium salt of benzidinediazo-bis-1-naphthylamine-4-sulfonic acid (purity > 99%) was purchased from Merck (AG. Darmstadt, Germany). Low viscosity sodium Alginate (Alg) (average Mn 1.0×10^5 Da) was obtained from Biochem S.A. (Buenos Aires, Argentina). All other reagents used were of analytical grade purchased from Sigma (St. Louis, MO) or Merck (Darmstadt, Germany). Guar Gum (GG, average Mn = $(2.20 \pm 0.20) \times 10^5$ Da) was kindly provided by Hindustan Gums & Chemicals (India).

CR stock solutions (1.0 g/L) and its dilutions were made in miliQ water and/or proper buffers. Calibration curve of CR was made at λ_{max} 497 nm in a Beckman DU 640 UV–Vis spectrophotometer (Beckman-Coulter, CA, USA).

2.2. Synthesis of Carboxy Methyl Guar-gum (CMGG)

50.0 g of guar gum was taken in a three neck round bottom flask fitted with a condenser, a nitrogen purging set up and a mechanical stirrer. Isopropanol (100 mL) was added in the reaction mixture and the biopolymer was allowed to soak under stir for 30 min. Afterward, 30.0 ml of 0.25% (w/v) aqueous NaOH solution was added through the condenser and the resulting mixture stirred for additional 10 min at room temperature. A dropping funnel was then attached through a fork followed by the addition of 150 mL of 170 mg/mL aqueous chloroacetic acid solution to the flask. The solution pH was adjusted to 7.0 ± 0.2 with 1.0% (w/v) NaOH under nitrogen purging. The addition time altogether was 1 h. The stirring was continued further for three hours and the reaction temperature was raised to 55 °C. The reaction mixture was cooled and a light yellow solid separated by filtration through a Buckner funnel. The precipitate was washed twice with 80% (v/v) isopropanol in water, and 50% ethanol–water solution, and dried in a vacuum desiccator. The mass obtained was 43.0 g.