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Development of Crystal Violet encapsulation in pectin - Arabic gum gel microspheres

Mariana V. Revuelta [a], M. Elizabeth Chacon Villalba [b], Alba S. Navarro [c,d], Jorge A. Güida [b,c,e], Guillermo R. Castro [a,*]

[a] Laboratorio de Nanobiomateriales, CINDEFI, Facultad de Ciencias Exactas, Universidad Nacional de La Plata - CONICET (CCT La Plata), 1900 La Plata, Argentina
[b] CEQUINOR, Facultad de Ciencias Exactas, Universidad Nacional de La Plata - CICPBA - CONICET (CCT La Plata), CC 962, 1900 La Plata, Argentina
[c] Facultad de Ingeniería, Universidad Nacional de La Plata, 1900 La Plata, Argentina
[d] CIDCA, Facultad de Ciencias Exactas, Universidad Nacional de La Plata - CONICET (CCT La Plata), 1900 La Plata, Argentina
[e] Departamento de Ciencias Básicas, Universidad Nacional de Luján, Luján, Argentina

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ABSTRACT

Six pectins with 35% to 91% methoxylation degree were purified and characterized to encapsulate Crystal Violet (CV). Amidated low methoxylated pectin (ALMP) was selected based on microsphere morphologies, aqueous solubility, viscosity and the effect of calcium concentration. Pectin microspheres were stabilized with Arabic gum (AG) and optimized according to the loading. Microspheres composed of 2.0% ALMP-1.0% AG crosslinked with 450 mM calcium(II) were able to encapsulate 217 ± 2 μM CV. Optical microscopy of the gels revealed spheroid microspheres with 250 ± 50 μm diameter containing homogenous CV distribution. Dried microspheres observed by SEM and epifluorescence showed a highly shrinkable matrix keeping the spheroidal morphology. Low relative viscosity of the ALMP-AG-CV solutions was found compared to ALMP and ALMP-AG. The Young moduli (60–80 Pa) of ALMP-AG microspheres by texturometric analysis indicated that the CV could interfere with the gel crosslinking. Raman spectroscopy analysis suggested some interaction of CV nucleophilic center within the matrix. FTIR of the matrix showed largest shifts in the carbonyl and carboxylate groups probably associated to H-bridges. CV stability studies performed on ALMP-AG microspheres, synthetized from polymer solutions with pH values above and below pectin pKa and showed faster CV release rates in presence of ionic strength.

1. Introduction

Microencapsulation is becoming popular in the food and pharmaceutical industries, because of preserving physicochemical and biological properties of the load from harsh environments, increasing the solubility of hydrophobic molecules, extending the half-life of the product and also providing molecular controlled release in a proper place and time [1,2]. Some biopolymers can be used to make smart matrices sensitive to diverse environmental conditions (e.g., pH, ionic strength, etc.). Polysaccharides such as alginates, pectins, and natural gums have excellent properties such as biocompatibility, biodegradability, muco-adhesiveness, and sensitivity to many environmental factors. Particularly, pectins and Arabic gums (AG) are considered GRAS (generally regarded as safe, FDA) and used in many industrial applications from foods to pharmaceuticals [2].

Pectins (Pecs) are plant biopolymers soluble in aqueous media and not hydrolysable by human intestinal enzymes but degradable by the microbial community present in the intestine. Pec backbone is composed of poly[α-(1,4)-D-galacturonic acid] partially methoxylated with a pKa in the range of 3.5–4.0 and could contains branches of neutral saccharides. The Pecs can be classified based on the methoxylation degree in: low (0–40%), medium (40 to 60%) and high (higher than 60%) methoxylation. Low and medium methoxylated Pecs can make reversible gels in the presence of multivalent cations, while high methoxylated Pecs are forming irreversible gels in presence of solutes (e.g., sugars) under acidic pH [2]. Amidated low methoxylated pectin (ALMP) are advantageous compared to low methoxylated pectins since can make gels in presence of low bivalent ion concentrations, are less sensitive to pH, and enhanced mucoadhesiveness because of the presence of amide groups [3].

Arabic gum (AG) is a complex biopolymer composed at least of three high-molecular weight fractions extracted from exudates of *Acacia* trees used in foods. The main fraction of AG is an arabinogalactan-protein complex backbone composed of poly[β-(1,3)-D-galactopyranose] with side chains of β-(1,6)-D-galactopyranose ending with β-D-glucuronic

* Corresponding author.
E-mail address: grcastro@gmail.com (G.R. Castro).

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acid and 4-*O*-methyl-β-D-glucuronic acid, also branched with α-(1,3)-L-rhamnofuranose and α-(1,4)-L-rhamnopyranose. The presence of glucuronic acids in AG is responsible of the polyanionic character with a pKa of about 2.0 [4]. The protein containts 18 amino acids with >50% composed of hydroxyproline, proline and serine [5]. The simultaneous presences of polar and hydrophobic amino acids are providing the amphiphilic character of the AG able to interact with both hydrophilic and hydrophobic chemical residues of different molecules [2]. The AG relative viscosity is pH-dependent and increases >100% from pH 2 to 6. Also, AG shows Newtonian behavior in aqueous solutions below 40% (W/V) but pseudoplastic comportment at higher concentrations (shear thinning). In fact, AG is commonly used as emulsifier-stabilizing agent of colloidal solutions, and it has low oxygen permeability. Additionally, AG is soluble in aqueous media but gelled at high concentrations [2].

Besides of pectin high solubility in aqueous media, the structural characteristics of the pectin gel matrices crosslinked with multivalent cations sometimes are compromised at pH above the pectin pKa, present in physiological environments (i.e., intestine). The simplest and greenest solution is to make coacervates, i.e., undefined chemical mixture of polymers. Arabic gum was selected for the coacervate formulation because of its gelling properties, biocompatibility, absence of toxicity in their degradation products and no-immunogenic response. However, the main requisite of coacervate synthesis is the chemical compatibility between the polymer components mainly established by short-term bond interactions (i.e., Hydrogen bonding, Van der Waals forces and others) which are stabilizing the matrix structure against different environments [6]. Examples of pectin coacervates showing proper molecular controlled release were previously reported [7].

Crystal Violet (CV) is a synthetic dye well known in the identification of bacteria by the Gram staining technique but also used in foods, cosmetic and fabrics (Fig. 1). More importantly, CV was pharmacologically employed for the treatment of fungi, parasites (i.e., leishmania, trypanosomes, etc.) and microbial infections in humans since the XIX century. However, many undesirable side effects were reported by therapeutic use of CV in biological systems such as mutagenic, carcinogenic, necrotic skin effects, irritant of mucosal membranes and depression of white blood cell lines because of the high doses of the dye [8]. One of the main problems associated with the administration of CV is related to the molecular aggregation in aqueous media that could probably be attributed to the π-π stacking of the aromatic rings. The CV aggregation is related to the concentration, at very low dye concentrations, below 1 mM, the dimerization is the main mechanism defined by a thermodynamic equilibrium constant of $K_d = 6 \times 10^{-2}\ 1 \cdot mol^{-1}$ at 20 °C, but at high CV concentrations multimeric aggregates were reported [9]. In previous work, CV aggregation in aqueous media was prevented by the addition of the poly(methacrylic acid) to the solution [10]. The complex formation was attributed to high hydrophobicity of poly(methacrylic acid) which provided a rigid matrix for CV exclusion from the aqueous polar medium and enhanced dye reactivity.

CV encapsulation and entrapment in matrices such as particles made of acrylamide and acrylates derivatives or in magnetic zeolite nanoparticles and in sol-gel silica clusters were reported [9,11,12]. However, the mentioned systems are requiring chemical synthetic precursors (i.e., acrylamide, tetraethoxysilane) or materials produced by coal combustion (i.e., fly ash) which are not environmentally friendly. Also, CV was encapsulated in liposomes made of phospholipids and dicetyl phosphate and SDS micelles, in both methods organic solvents are used [13,14]. Those lipid structures are thermodynamically unstable.

On the other hand, novel trends in matrix developments using green technologies are gaining more adepts in the academy and industry. For example, polysaccharides are no toxic, no immunogenic, biodegradable and biocompatible, can make 3-D structures by soft techniques like ionotropic gelation techniques in presence of divalent ions or by self-assembly make them very suitable hydrogels for medical applications such as drug delivery [15]. Hydrogel microencapsulation is an alternative to avoids adverse effects posed by the load and release of small molecules such as instability, bioavailability and toxicity. Biopolymeric microparticles are of special interest for oral drug delivery because of their stability, small size, porous control, tailoring, large surface area and safety, favoring their absorption and controlling the load release kinetic compared to other molecular carriers [16].

The aim of the work was to study and characterize the encapsulation of Crystal Violet in a biopolymeric matrix composed of pectin and Arabic gum. Pectins with different methoxylation degrees were purified followed by interaction studies between the dye and the biopolymers. Amidated low methoxylated pectins were chemically characterized and used to produce gel microspheres containing the dye. The microspheres were characterized by optical, epifluorescence and scanning electron microscopies (SEM). Mechanical, rheological and vibrational spectroscopies (FITR and Raman) were used to establish the hybrid matrix properties and the interactions between their components and the Crystal Violet. CV stability studies of microspheres synthetized from polymer solutions with pH values above and below pectin pKa, in presence or absence of ionic strength, were performed to examine the effect on CV encapsulation and its kinetics release.

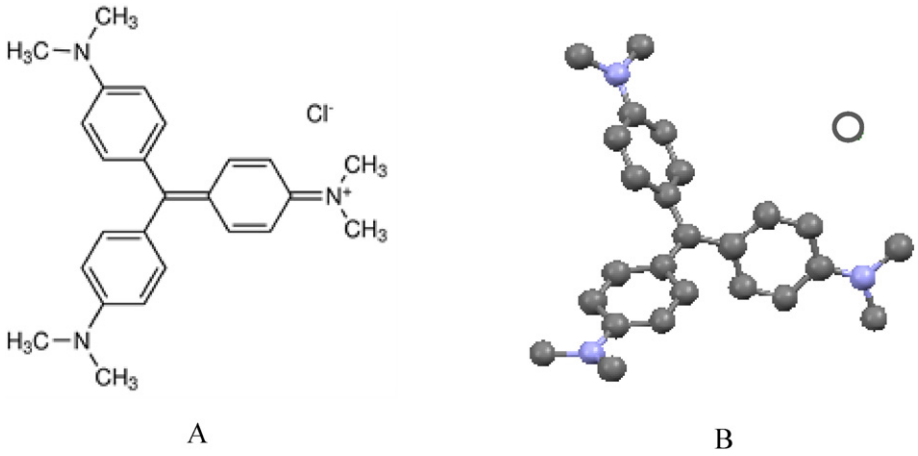

Fig. 1. Geometrical arrangement of Crystal Violet (A) and molecular structure showing the propeller geometry of the dye (B). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)