Design of low molecular weight pectin and its nanoparticles through combination treatment of pectin by microwave and inorganic salts

Badrul Hisyam Zainudin[a,b,c,d], Tin Wui Wong[a,b,*], Halimaton Hamdan[d]

[a] Non-Destructive Biomedical and Pharmaceutical Research Centre, iPROMISE, Universiti Teknologi MARA, Puncak Alam, 42300, Selangor, Malaysia
[b] Particle Design Research Group, Faculty of Pharmacy, Universiti Teknologi MARA, Puncak Alam, 42300, Selangor, Malaysia
[c] Malaysian Cocoa Board, Cocoa Innovative and Technology Centre, Lot 12610, Kawasan Perindustrian Nilai, Nilai, 71800, Negeri Sembilan, Malaysia
[d] Razak School of Engineering and Advanced Technology, Universiti Teknologi Malaysia, 54100, Kuala Lumpur, Malaysia

ARTICLE INFO

ABSTRACT

This study examined primarily the molecular weight and degree of esterification profiles of pectin in response to treatment by microwave as a function of irradiation duration in combination with monovalent (sodium chloride) or divalent (calcium acetate) inorganic salt. The possibility of formation and physical attributes of nanoparticles prepared form these pectins were assessed against their suitability for use as nanocarrier in cancer therapy. The pectin was treated by microwave (900 W) with inorganic salts as the promoter of superheating at liquid state. Its molecular weight, degree of esterification, viscosity, particle size, zeta potential and elemental content were determined. The pectin was subjected to nanospray drying with the size, zeta potential and morphology of the formed nanoparticles examined. The use of calcium acetate translated to the formation of pectin with lower molecular weight and degree of esterification, but higher solution viscosity than that of sodium chloride. Fourier transform infrared spectroscopy, particle size and elemental content analysis indicated such pectin had its molecules crosslinked by soluble calcium at COO^- moiety in liquid phase. It experienced a higher heat transfer through salt bridges and chain breakdown propensity particularly with prolonged duration of treatment. The formed nanoparticles were characterized by a mean size smaller than 600 nm and were envisaged appropriate for use as nanocarrier of cancer therapeutics with respect to permeation and retention attributes of tumour vasculature. The combination of microwave with multivalent inorganic salt is a viable approach for use to convert the pectin into matrix material of nanoparticles.

1. Introduction

Pectin is a cell wall polysaccharide with polymeric chain consisting of repeating units of α-1,4-linked-D-galacturonic acid [1]. It is first discovered by a French chemist and pharmacist, Henry Braconnot in 1825 [2]. The primary sources of pectin for commercial applications are citrus peel and apple pomace, which are waste products from fruit juice manufacturing and are readily available at low costs [3]. Depending on their degree of esterification (DE), pectins are commonly categorized as high methoxyl pectin (HMP) or low methoxyl pectin (LMP), with DE > 50% and < 50% respectively [4]. Molecular weight and degree of esterification of pectin are important attributes that define its functional properties such as viscosity [5,6].

Pectin is biodegradable, biocompatible and safe for human consumption [7]. Pharmaceutically, pectin has been widely applied as the matrix or coat materials of drug delivery systems specifically in oral colon-specific carrier development [8–15]. Pectin, as a dietary fibre,

plays a role in preventing colon cancer [2,16]. Ginseng pectin, citrus pectin, and apple pectin, subjected to heat and/or physical treatments, have demonstrated apoptotic, cell cycle arrest, galectin-3 inhibitory activities that are beneficial in cancer prevention and treatment [17–21].

Polysaccharides such as pectin, chitosan and alginate have been advocated for use in design of nanoparticulate drug delivery system for the treatment of cancer [22–24]. The nanoparticles are considered as a favourable vehicle due to its small size and large specific surface area attributes. They can be decorated with ligands for specific cancer cell targeting and are suitable for receptor targeting at the cellular level [25–27]. The size of nanoparticles is known to be the governing parameter that dictates their interaction with the cell membrane and eventually cellular internalization into cytoplasm and nucleus where the drug targets reside [28].

The size of the polysaccharide nanoparticles is directly governed by the molecular weight and size of the matrix polymer [29]. Typically,

low molecular weight or small size polysaccharide is preferred in the development of nanoparticles. With reference to pectin, its physical constructs have been modified by means of chemical, enzymatic and heat approaches [18,30–32]. Lately, the dynamic high pressure microfluidization technique is used to reduce the molecular weight, size and viscosity of pectin [7]. The high pressure homogenization and ultrasound techniques are likewise used to reduce the chain length of pectin [3,33,34]. The modification of pectin via the physical approach could lead to minimal changes to the polysaccharide as a function of the source of pectin [33]. Using chemical and enzymatic approaches, it can however cause environmental pollution and is a costly process [3,35].

Microwave is an electromagnetic radiation with frequency ranges between 300 MHz and 300 GHz [36]. It is a green technique advocated in chemical reactions and polymer modifications for its evident advantages over conventional heating [37]. The microwave technology offers lower activation energies, higher reaction rates, reduced reaction times, extremely fast temperature increment and hazardous chemical-free operations [37,38]. It has been used to modify the physicochemical properties of polymers such as poly(alkyl methacrylates) [39], chitosan [35,40–47], and poly(ethylene oxide) [38]. The polymer modification by microwave is commonly assisted by inorganic salt in liquid state via its superheating action above normal boiling point. Thus far, the monovalent salt such as sodium chloride is primarily used as the promoter of superheating [41,42,47]. This study aims to modify the pectin by means of combination treatment using microwave and inorganic salt. The divalent salt, namely calcium acetate, is employed with the outcome of pectin modification compared against that of sodium chloride. It is hypothesised that the soluble dicationic calcium can crosslink with the polyanionic pectin chains. The microwave is inclined to interact with water and electrolytes [48]. The heat generated from the interaction between microwave, water and electrolytes could possibly transmit to pectin chains in a more effective manner if these polymeric backbones are held by electrolytes while surrounded by the water molecules in comparison to cases where monovalent salt is used. In the latter, the pectin chains are free from crosslinkages by the electrolyte. The heat transmission is not aided by pectin-electrolyte cross-linkages.

2. Experimental

2.1. Materials

Pectin from citrus peels (Herbstreith & Fox KG Pektin-Fabrik, Germany) was used as the polymer of interest. Calcium acetate (Sigma-Aldrich, USA) and sodium chloride (Merck, Germany) were used as the inorganic salts in pectin modification. Nitric acid 65%, ethanol 96%, acetone, sodium azide and sodium nitrate were obtained from Merck, Germany. Dextrans with molecular weights of 1000, 12000, 50000, 80000, 150000, 270000, 410000, 670000 and 1400000 Da (Sigma-Aldrich, Denmark) were used as the standard in molecular weight analysis of pectin.

2.2. Pectin modification

One gram of pectin was added into 49 g deionized water under continuous magnetic stirring to produce 2 %w/w pectin solution. Two ml of 0.9 %w/w sodium chloride solution or 2.4 %w/w calcium acetate solution, at an equivalent mole of sodium and calcium ionic species, were introduced into the pectin solution under stirring and further mixed for 1 h. The pectin-inorganic salt mixture was subjected to microwave treatment at 900 W for 5, 10, 20 and 40 min using a microwave oven (NN-CD997S, Panasonic, Japan). Following microwave irradiation, the mixture was cooled to 25 ± 1 °C. The pectin was precipitated using ethanol 96%, filtered, washed with acetone and left to dry in hot air oven (Memmert, Germany) at 40 °C for 24 h. The dried pectin was conditioned in silica gel desiccator at 25 ± 1 °C.

2.3. Nanoparticles preparation

Twenty five mg of pectin were dissolved in 50 ml deionized water under continuous magnetic stirring. It was subjected to nanospray drying process using the nanospray dryer B-90 (Büchi, Switzerland) by means of the following conditions: spray mesh size = 4 μm, pectin solution flow rate = 12 ml/min, inlet temperature = 90 °C, outlet temperature = 44 °C, drying air flow rate = 120 L/min, spray rate = 100%, atomization pressure = 44–52 mbar. The spray-dried nanoparticles were collected from the electrostatic particle collector. They were kept in amber glass vials and stored in a silica gel desiccator at 25 ± 1 °C.

2.4. Molecular weight

The molecular weight of pectin and its distribution were determined using the gel permeation chromatography technique equipped with a refractive index detector (1100 series, Agilent Technologies, Germany). A PL aquagel-OH mixed column (7.5 mm × 300 mm, 8 μm particle size; Agilent Technologies, USA) was used as the stationary phase with mobile phase constituting of 0.05 %w/w sodium azide and 50 mM sodium nitrate dissolved in deionized water. The flow rate of the mobile phase was 0.5 mL/min and the column temperature was set at 30 °C. The dextran standard solution (1 mg/ml) and pectin solution (1.5 mg/ml), dissolved in deionized water, were filtered through a nylon membrane (Whatman, UK) before analysis. At least triplicates were carried out for each sample and the results were averaged.

2.5. Degree of esterification

The pectin was mixed with potassium bromide powder (2.5 %w/w pectin; FTIR grade, Sigma-Aldrich, USA) and compressed into a pellet using a hydraulic press (Specac, UK). It was subjected to FTIR analysis using an IR spectrometer (Cary 630, Agilent Technologies, USA). The absorbance spectra of pectin were recorded in the wavenumber region of 400–4000 cm^{-1} at a resolution of 4 cm^{-1}. At least triplicates were conducted for each sample. The degree of esterification (DE) of pectin was calculated in accordance to the following equations [49]:

$$DE = 124.7R + 2.2013 \tag{1}$$

$$R = A_{1740}/[A_{1740} + A_{1630}] \tag{2}$$

where A_{1740} and A_{1630} were defined as the absorbance intensities of bands ascribing to esterified and non-esterified carboxyl groups of pectin at 1740 cm^{-1} and 1630 cm^{-1} respectively.

2.6. Specific viscosity

The specific viscosity of the pectin solution was measured via characterizing its flow time in an Ubbelohde dilution viscometer (size A, Poulten Selfe & Lee Ltd, UK) at 37 ± 0.5 °C in a temperature-controlled water bath (Memmert, Germany). The pectin was dissolved in deionized water to produce 0.1 %w/w pectin solution. The pectin solution was filtered using a nylon membrane filter (pore size = 0.45 μm; Whatman, UK) prior to viscosity measurements. The flow time of pectin solution (t) and deionized water (t_0) were determined. The specific viscosity (η_{sp}) of the pectin solution was calculated using the following equation:

$$\eta_{sp} = (t - t_0)/t_0 \tag{3}$$

Triplicates of experiment were conducted for each sample and the results were averaged.

2.7. Elemental analysis

The calcium level in pectin was quantified by inductively coupled