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An augmented delivery of the anticancer agent, curcumin, to the colon

Rayan Sabra[a], Nashiru Billa[a,*], Clive J. Roberts[b]

[a] The School of Pharmacy, University of Nottingham, Malaysia Campus, Semenyih, Selangor, Malaysia
[b] The School of Pharmacy, University of Nottingham, Park Campus, Nottingham, United Kingdom

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ABSTRACT

This work describes the formulation aspects of an orally viable curcumin-containing mucoadhesive nanoparticulate system for management of colon cancer. Curcumin is documented to possess anticancer properties whilst modified citrus pectin yields a galactose functionality capable of inhibiting the growth and proliferation of colon cancer cells due to antagonism to galectin-3 (Gal-3). A successfully formulated curcumin containing chitosan-modified citrus pectinate nanoparticles (MCPCNPs) registered a z-average of 178 nm (± 0.896) and a positive surface charge of + 35.7 mV (± 1.41). The MCPCNPs presented high mucoadhesion propensity in the colonic region/media and minimal at pH 1.2 (stomach). There was approximately 18% curcumin release at pH 1.2 over 2 h and up to 68% release in the 33% (w/v) caecal medium over 24 h. The data obtained strongly suggests that the formulated MCPCNPs have the potential to be applied as an orally deliverable colon cancer formulation alternative in the treatment of colon cancer.

1. Introduction

Colon cancer is the fourth leading cause of cancer deaths worldwide, accounting for 8% of documented cancer deaths [1]. Due to the annual rise in the number of deaths resulting from colon cancer [2], several therapeutic procedures are in practice that addresses this trend, including surgical resection of afflicted tissue, radiotherapy or chemotherapy. Chemotherapy is the least invasive of the therapeutic options currently used to manage colon cancer. However, due to the side effects that manifests following chemotherapy, anticancer agents of plant origin, such as curcumin, are becoming serious contenders as chemotherapeutic alternatives. Curcumin is the major chemical constituent of turmeric and has received much attention in the past decade because of its 'acceptability' as it is derived from natural sources and perceived to manifest relatively fewer side effects [3]. Extensive research has elaborated the therapeutic potential of curcumin against a range of cancers and distinctly against colon cancer [4]. However, preclinical and clinical data from oral administration of curcumin have revealed its poor systemic bioavailability and high susceptibility to metabolic activity, where only 2.30 ± 0.26 $\mu g \cdot ml^{-1}$ of curcumin is registered in serum after oral administration of 10 g curcumin [5,6]. This shows that curcumin undergoes extensive metabolic changes in the intestine and liver, which hinder the systemic usefulness of curcumin in the treatment of cancer. To overcome this constraint, a variety of materials such as natural or synthetic polymers and lipids have been used to formulate delivery systems that traverse gastrointestinal epithelia effectively and therefore circumvent the metabolic constraints within the gastrointestinal tract.

Chitosan is a well-studied natural polymer that is biodegradable and possesses mucoadhesive properties [7]. It is derived from chitin and comprises of β- [1–4] -linked D glucosamine and *N*-acetylated units [8]. It is soluble in acidic media due to the formation of soluble complexes with the charged amine groups (NH_3^+). The amine moiety also promotes binding to negatively charged species such as mucin within the gastrointestinal tract [8]. Citrus pectin is also a widely studied natural polymer extracted from the cell wall of plants. It possesses a poly α-[1–4] - linked D-galacturonic acid units with varying degrees of methylation of the carboxylic acid moiety [9], which confers a negative charge to the molecule. Additionally, pectin shares very similar properties with chitosan and is resistant to degradation by the digestive enzymes in stomach [9]. Crucially, pH-thermal treatment of citrus pectin yields moieties that demonstrate chemo-preventive activities against cancers [10]. Modified citrus pectin (MCP) comprise of neutral sugar sequences with a low degree of branching and is rich in galactose, a constituent that is reported to inhibit the growth and migration of colon cancer cells due to its remarkable antagonism to galectin-3 (Gal-3) [11,12]. Gal-3 is a protein of the galectin super-family with a carbohydrate-binding domain that exhibits high affinity to β-galactosides [13] and is vastly expressed on tumour cell surfaces. There is evidence that Gal-3 binds to the colonic mucin with an altered carbohydrate structure causing tumour growth, cell-to-cell adhesion, and metastasis [14]. Therefore, the galactose-rich MCP is capable of binding to the β-

* Corresponding author.
E-mail address: Nashiru.Billa@nottingham.edu.my (N. Billa).

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galactoside protein of the Gal-3 and hence blocking the Gal-3 interactions with other proteins and peptides [12].

Earlier reports [15,16] have elaborated the potential of blending chitosan and pectin for colon-specific drug delivery. More recently, Andriani et al. [17] reported glutaraldehyde-cross linked chitosan-pectin nanoparticles as a promising carrier for effective delivery of curcumin to the small intestine following oral administration. In the present pursuit however, we aim to formulate mucoadhesive curcumin-loaded MCP-chitosan nanoparticles (MCPCNPs) for possible delivery to the colon following oral administration. In this regard, the MCP would serve the dual function of retaining the integrity of the nanoparticles within the acidic pH of the upper gut and also yielding the galactose moiety, which is known to manifest anticancer properties against galectin 3 (Gal-3) in the colon. Chitosan is destined to provide the required mucoadhesion within the colon mucosa in order to ensure longer residence time within the colon epithelia. It is envisaged that longer residence time in the colon coupled with augmented anticancer activity due to encapsulated curcumin and galactose moiety from MCP would be therapeutically cost-effective and attractive to stakeholders in the management of colon cancer. This manuscript describes *in vitro* work that supports the above hypothesis in which the proposed formulation has the potential to be delivered orally to the colon.

2. Materials and methods

2.1. Materials

Chitosan (low molecular weight), de-acetylated chitin, Poly (D-glucosamine) from Sigma Aldrich, Iceland and low methoxyl citrus pectin (DE lower than 50) from Genu, CPKelco® (Limeira- SP, Brazil) were utilised as coating polymers. Curcumin was bought from Fluka, U.S.A. Sodium tripolyphosphate (STPP) (Sigma Aldrich, USA) was used as the cross-linker with chitosan. Mucin type III from porcine stomach (Sigma Aldrich, USA) was utilised in mucoadhesive studies. Potassium dihydrogen phosphate (Sigma Aldrich, USA), acetic acid (R&M chemicals, UK), hydrochloric acid (R&M chemicals, UK), acetic acid (R&M chemicals, UK), ethanol (R&M chemicals, UK), acetone (R&M chemicals, UK), acetonitrile (RCI Labscan, Thailand), methanol (R&M chemicals, UK), sodium hydroxide and sodium acetate (Merck, Germany), were expended as solvents or pH modifiers.

2.2. Modification of citrus pectin

The low-methoxyl citrus pectin (LMP) was chemically modified as ascribed by Venzon et al. [12] with some modification. 1.5 g of the powdered pectin was dissolved in 100 ml ultra-pure water and the pH adjusted to 10.0 using 3 M NaOH followed by gentle stirring at 60 °C for an hour. The mixture was allowed to cool at room temperature; its pH was adjusted to 3.0 with 3 M HCl and then stored at 4 °C overnight. Subsequently, the modified citrus pectin (MCP) was precipitated in the sample with 95% ethanol and then washed with 100% acetone, followed by drying at 60 °C for an hour. Finally, the semi-dried MCP was frozen at − 20 °C and then lyophilized.

2.3. Formulation of loaded curcumin-modified citrus pectin -chitosan nanoparticles (MCPCNPs)

The MCPCNPs were prepared by ionic gelation in a one-step process. The stock solutions were made by dissolving: chitosan (2.5 mg/ml) in 2% (v/v) acetic acid and adjusting the pH to 5.0 with 2 M NaOH; sodium tripolyphosphate (STPP) was dissolved in ultra-pure water at 0.5 mg/ml; MCP was stirred in ultra-pure water at 0.5 mg/ml for 3 h at 60 °C and curcumin was dissolved in ethanol at 1 mg/ml. A 25 ml aliquot of the MCP solution was added to an amber-coloured beaker containing 300 µl of the curcumin solution with vigorous stirring, followed by drop-wise addition of 25 ml of chitosan solution and then drop-wise addition of 25 ml of STPP. The mixture was stirred for a further 20 min at 500 rpm at room temperature and then stored at 4 °C till further analyses.

2.4. Physical properties of the MCPCNPs

The hydrodynamic size distribution (expressed as average diameter) and surface charge (expressed as zeta- potential) of the MCPCNPs were determined after dilution using a Zeta Sizer Nano Series® (Malvern Instruments Ltd., UK) equipped with a 4 mW He-Ne laser at a wavelength of 633 nm. The average diameter and zeta-potential were measured by means of Dynamic Laser Scattering and Laser Doppler Anemometry (LDA), respectively. Samples were run in triplicates and presented as mean (± SD).

The morphology of the MCPCNPs was observed using a Field Emission Scanning Electron Microscope (FESEM), (Model Quanta 400F, FEI Company USA) equipped with a backscattered electron detector at 3 kV. 100 µl of the sample was placed on the SEM imaging stub with carbon layer and left under a drying chamber for 24 h at room temperature before viewing.

The Fourier transform infrared (FTIR) spectra of compacts from the MCPCNPs and excipients were reviewed after compressing them into KBr incorporated compact discs at a pressure of 5 t for 5 min. Spectra were obtained for chitosan, MCP, STPP, curcumin, MCP-chitosan nanoparticles and loaded- curcumin nanoparticles using a Perkin-Elmer FTIR spectrometer (Spectrum RX I) with scans run between 400 and 4000 cm^{-1} at a resolution of 4 cm^{-1} and interval 1 cm^{-1}.

The thermal responses of the MCPCNPs and components were assessed on a differential scanning calorimeter DSC-Q2000 (TA Instruments, USA). Accurately weighed samples were crimped into aluminium plates and the thermogram was conducted over a range of 0 to 300 °C, at a scanning rate of 10 °C/min and flushed with nitrogen gas at a rate of 20 ml/min.

2.5. Encapsulation efficiency and drug loading within MCPCNPs

The formed MCPCNPs were centrifuged at 8000 rpm for 10 min and the centrifuged pellet was rinsed twice with methanol. The curcumin content in the supernatant and the methanol rinse was analysed by injecting 10 µl onto an HPLC system (Series 200 pump, Agilent, USA) equipped with an Eclipse plus C18 (Agilent, USA) column (250 × 4.6 mm; 5 µm) and a UV detector (L-2485, Agilent, USA) set at 425 nm. The mobile phase (methanol: 0.01% acetic acid: acetonitrile (5: 43: 52, %)) was run isocratically at 1.5 ml/min. Responses obtained were compared to those from a standard curve of curcumin in methanol. The percentage encapsulation of curcumin and loading capacity of the MCPCNPs with respect to curcumin was calculated as follows:

$$\text{Encapsulation Efficiency (\%)} = \frac{\text{Amount of curcumin added} - \text{unbound curcumin}}{\text{Amount of curcumin added}} \times 100$$

$$\text{Loading Capacity (\%)} = \frac{\text{Entrapped curcumin}}{\text{Curcumin loaded NP weight}} \times 100$$

2.6. Mucoadhesion propensity of the MCPCNPs

2.6.1. Mucin adsorption studies

The propensity of the MCPCNPs to adhere to mucus was determined as the amount of mucin adsorbed by the MCPCNPs dispersed in standardised mucin solutions. The amount of mucin adsorbed was also studied as a function of pH representing relevant anatomical sections of the gastrointestinal tract: pH 1.2 (0.1 N HCl), pH 5.5 (0.1 M sodium acetate buffer), pH 6.25 and 7.0 (0.1 M phosphate buffer). Specifically, 2 ml of the MCPCNPs suspension was mixed in the mucin solutions, vortexed for few seconds and then rotated at 100 rpm maintained at