Chapter 1: Introduction

1.1. Background

The advancement of our civilization in this era has brought us a double-edged sword situation. For instance, the excellent accessibility to food resources is accompanied by the probability of dietary habit deviations. Consequently, gastrointestinal (GI) diseases, such as colorectal cancer (CRC), have risen in the past few decades. CRC has been the cause of around 10% of cancer-related mortality, yet in the mid-20th century, CRC incidence was infrequent. In 2012, more than six hundred new CRC cases emerged in women, making it the second-most common cancer in this sex category. It has been the third-most common cancer in men, with nearly 750,000 men diagnosed in 2012 (Kuipers *et al.*, 2015). In both sexes, global epidemiology data of 2018 stated that colon cancer placed fourth as cancer with the highest incidents and contributed to 5.8% of all cancer-related deaths (Bray *et al.*, 2018). The 5-year survival rates of the localized, regional, and distant metastatic CRC are 90%, 70%, and 10%, respectively (Haggar & Boushey, 2009). Numerous risk factors could lead to this condition, such as age, pre-existing GI diseases, unhealthy diet, and detrimental lifestyle.

One of the most commonly prescribed chemotherapeutic drugs for the management of CRC is 5-fluorouracil (5-FU). It has been used in clinical settings as the primary chemotherapeutic drug of choice for CRC since the 1950s (Healey *et al.*, 2013; Mármol *et al.*, 2017; Vodenkova *et al.*, 2020). It is a uracil-mimicking antimetabolite designed following the observations by Rutman, Cantarow, & Paschkis (1954) that rat hepatoma utilized uracil more exhaustively than healthy cells. The mechanisms of action of 5-FU involve DNA and RNA damage via, among others, misincorporations and deoxynucleotide pool imbalance via thymidylate synthase inhibition. The bioavailability of 5-FU, however, is considered short since more than 80% of the orally administered 5-FU will be degraded into dihydrofluorouracil in the liver following intestinal absorption (Longley, Harkin, & Johnston, 2003). Therefore, several strategies have been

developed, including 5-FU prodrug, capecitabine, which will be activated into 5-FU by thymidine phosphorylase or uridine phosphorylase in the cells. However, the administration of 5-FU and/or capecitabine still leads to numerous systemic adverse effects, such as alopecia, neutropenia, and stomatitis (Aoullay *et al.*, 2020), due to the non-specific systemic drug actions. Therefore, colorectal-targeting drug delivery systems (DDS) are sought to minimize the systemic adverse effects of chemotherapy.

In targeting the local colorectal area, there are two approaches, *i.e.* oral and rectal routes of administration (Arévalo-Pérez, Maderuelo, & Lanao, 2020). Oral administration introduces the drug first to the right side of the colon – cecum and ascending colon – while the latter enables the first contact with the left-colonic area to the rectum. Colorectal-targeting DDS have been developed with various materials that could aid the retention of the drug and maximize the release in the designated area. For the oral route, materials with selective solubility, *i.e.* neutral pH and biodegradable by colonic microflora, are favoured (Kumar & Mishra, 2008; Lee *et al.*, 2020). However, the most important feature to have in oral route is not to release the drug load in the upper GI tract. In comparison, the general considerations in rectal administration are the desired extent of the dosage form's spreadability, mucoadhesive characteristic, and the size of the formulation to ensure patient's compliance. The drug release profile is not a major determinant in rectal since compared to oral delivery, the distance to reach the colon is much shorter via the rectum (McFarlane, 1990; Raines *et al.*, 2014), and the liquid volume is also much lower in rectum (Hua, 2019; Mudie et al., 2014). Therefore, unlike in oral delivery, the secondary system in rectal delivery could still retain the drug-loaded primary system.

Several natural-based materials have been studied as the main matrices for the delivery of these chemotherapeutic agents, such as carbohydrates. An indigestible polysaccharide, pectin, has been involved in numerous DDS development (Sriamornsak, 2011). For instance, as a polymer base for

controlled release formulation, gastro-retentive, colon-specific, and mucoadhesive DDS. It can be found in almost all kinds of plants as a cell wall component, such as in orange, lemon, grapefruit, and apple. The 'indigestible' characteristic of pectin allows it to be resilient in the degrading environment of the stomach and small intestine without any occurrence of significant depolymerization; while degraded in the presence of colorectal microbes (Dongowski, Lorenz, & Anger, 2000). Also, the crosslinking of pectin by calcium further impairs the solubility in water (Muvva *et al.*, 2020). Given the abundance of thiol groups in the mucus (Duggan *et al.*, 2017), specifically the mucin, the thiolation of pectin was expected to enhance mucoadhesion of the beads and the intramolecular cohesion, integrity, and strength due to the disulfide cross-linking. The mucoadhesion is important in this study since it is the means to tether the drugs to the colorectal cancer site. The addition of positively charged natural polymer, chitosan (CS), was predicted to enhance the mucoadhesion of the produced beads (Morris, Kök, Harding, & Adams, 2010) and also to enhance intramolecular cohesion by partial polyelectrolyte complex gelation with negatively charged pectate (Zhu *et al.*, 2019). The addition of CS was also theorized to minimize drug leakage by further limiting the swelling of the beads (Günter & Popeyko, 2016).

Here, the definition of DDS is divided into two terms, primary and secondary. The primary system is the one designed in this study (beads); while the seconday system is defined as the common carrier, naïve or modified, that is used to contain a number of beads entering the body, *e.g.* capsule in oral delivery or suppository base in rectal delivery.

1.2. Objective

This study aims to develop a primary vehicle to facilitate colorectal cancer-targeting by maximizing 5-FU release in the target colorectal site.

1.3. Hypothesis

There are several hypotheses to be tested in this study, as listed below.

1. The thiolation of pectin is confirmed to be a success through analytical methods.
2. The content of the dried beads is the combination of all the previously mixed constituents without significant alterations.
3. There is no significant deviation of quantitative properties within a formulation group.
4. The swelling characteristics of thiolated and CS-complexed beads are lower than native pectin only beads.
5. The mucoadhesion properties of thiolated and CS-complexed beads are greater than native pectin only beads.
6. A minimum portion of the loaded 5-FU is released into the native phosphate buffer 7.4 solvent system, and less release percentage is expected in thiolated and CS-added groups.

Lastly, from all of the results gathered, the suitable route of administration, either oral or rectal, of the beads must be concluded.

1.4. Scope of Work

In this study, a set of experiments are conducted to test the hypotheses above. The scope of these experiments includes pectin thiolation, characterization of thiolated pectin, 5-FU loaded pectin-based

beads fabrication, and characterization of the beads products. The characterization of thiolated pectin comprises qualitative structural analysis with FTIR and quantitative determination of the free thiol group with Ellman's reagent. The characterization of the beads products consists of morphological observation, entrapment efficiency determination, particle size and circularity measurement, homogeneity test, swelling test, FTIR qualitative analysis, *ex vivo* mucoadhesion test, and *in vitro* drug release study.