CHAPTER 1

INTRODUCTION

1.1 Study Background

Colorectal cancer (CRC) is a malignancy affecting the colon and rectum. Being the third most prevalent cancer in Indonesia and worldwide, its incidence is projected to increase in the future. Unfortunately, it has the second highest fatality rate among cancers, reflecting the need to improve its management (Sayuti & Nauva, 2019; Sung *et al*., 2021). Current first-line therapy for CRC relies on surgery, radiotherapy, and chemotherapy, depending on the staging and patient condition. However, such treatment modalities still possess several challenges, such as complications and systemic adverse reactions, which may lead to a decline in quality of life, both physically and mentally.

Chemotherapy regimens, such as 5-fluorouracil, leucovorin, oxaliplatin, and irinotecan, are commonly used as adjuvant therapy for stage II-III CRC and first-line therapy of metastatic CRC. These drugs generally induce cancer cell death by blocking DNA synthesis and replication, which is essential in all living cells (Kuipers *et al*., 2015). Consequently, systemic side effects are commonly observed in patients. Moreover, despite having high potency *in vitro* and *in vivo*, these drugs have relatively poor bioavailability, thus requiring high doses to achieve significant responses. This highlights the urgency to develop targeted drug delivery systems (DDS) in order to tackle these issues. By delivering the drug specifically to the target organs, the drug bioavailability will be significantly enhanced, thus improving treatment response, while potentially resulting in lower dosages required. Not to mention, targeted drug delivery systems can be tailored for sustained drug release, which is highly implicated in cancer therapy, due to its lower risk of therapy resistance (Senapati, Mahanta, Kumar & Maiti, 2018).

Strategies to achieve targeted DDS in CRC may be pursued by two main administration routes, which are oral and rectal administrations. Gradually, conventional intravenous chemotherapy for CRC is being replaced by oral chemotherapy due to several advantages, such as better patient compliance, less hospitalization, and less systemic toxicity. These chemotherapeutics are primarily in the form of

prodrugs, which undergo biotransformation through CYP450-mediated oxidation or hydrolysis (Ikeda *et al*., 2000). However, current oral formulations still allow drug absorption to the systemic circulation, especially in the upper GI tract. This results in increased specific adverse reactions, such as hand-foot syndrome, stomatitis, and mucositis, which may negatively impact the quality of life of patients (van Beek, Roukens, Jacobs, Timmer-Bonte & Kramers, 2018; Kwakman & Punt, 2016). Rectal administration, on the other hand, such as suppositories or enemas, is preferred when oral administration is infeasible, such as GI symptoms, poor drug stability in upper GI tract, or inter-individual variations, as well as in conditions when fast-acting drugs are required (Hua, 2019). In fact, a multicentre clinical trial of 5-FU-based prodrug suppository administration as neoadjuvant chemotherapy in stage II/III CRC patients have been conducted, with generally positive outcomes, such as increased survival rates and decreased metastasis (Okabayashi *et al*., 2012; Ohwada *et al.*, 2006). Moreover, rectal administration may also exhibit minimum hepatic first-pass metabolism, thus may potentially increase the drug bioavailability (van Hoogdalem, de Boer & Breimer, 1991). Nonetheless, most development of rectally-administered chemotherapeutics for CRC are still limited due to less patient compliance compared to oral administration (Hua, 2019).

Besides the consideration of administration route, colorectal-targeted DDS can also be developed based on their prominent physiology, notably the thick mucus layer and diverse commensal microflora. This can be mediated by interaction with the mucus layer, known as mucoadhesion, to achieve higher drug bioavailability (Boddupalli, Mohammed, Nath & Banji, 2010). Another mechanism that can be tailored is the colorectal-sensitive release profile, which can be in the form of pH-responsive, redox-responsive, or microbial-responsive behaviors (Kadir & Lim, 2020). Apparently, numerous studies have proposed that microbially-triggered DDS possesses the highest efficiency due to the site-specific biological activity. This implies that in the context of oral administration, a colorectal-targeted DDS should be able to retain the majority of the drugs in the upper GI tract, while releasing it efficiently once reaching the target site.

Dietary fibers have been extensively exploited as potential microbially-triggered DDS, considering their indigestibility by human intestinal enzymes, while only being degraded by colonic microbiota (Khotimchenko, 2020). One such fibers is pectin, which is commonly found as plant cell wall components, notably fruit peels. Pectin has been commercially used in food industries as emulsifier, thickener, gelling agent, and stabilizer, due to its non-toxicity. Most importantly, pectin is potential as primary drug carriers due to its gel-forming ability with divalent metal ions or polyvalent ions, known as ionotropic gelation. This encapsulation method is often preferable due to its economical- and environmental-friendly reagents, as well as customizable functionalities (Sacco *et al.*, 2021). Pectin also exhibits mild mucoadhesion, which is mainly mediated by hydrogen bonding and hydrophobic interactions (Thirawong, Nunthanid, Puttipipatkhachorn & Sriamornsak, 2007). However, this property can be substantially enhanced by chemical modification known as thiolation, forming thiolated pectin (TP), via the formation of disulfide bridges with the mucus layer, thus potentially prolong its residence time, especially in CRC tissue, where the mucus layer is overproduced (Johansson, Sjövall & Hansson, 2013; Meldrum *et al*., 2018).

Furthermore, the usage of pectin as primary drug carriers can be combined with other dietary fibers, such as chitosan (CS), which is derived from crustacean shells and also exhibits mucoadhesive properties (Ways, Lau & Khutoryanskiy, 2018). Unlike pectin, CS contains positively-charged amine groups upon protonation, making it highly soluble in the upper GI tract and highly susceptible to gastric drug release (Shafabakhsh *et al*., 2020). Therefore, their combination in the form of polyelectrolyte complex (PEC) may possess beneficial properties. Due to their opposing charges in each monomer backbone, PEC may further strengthen the matrix structure by core crosslinking, resulting in a more stable drug carrier (Pandey *et al*., 2013). This may enhance drug entrapment and exhibit a more sustained release profile, which is implicated in CRC. Not to mention, given the potential of pectin and chitosan to exhibit chemopreventive activity *in vivo* as well as anticancer activity against CRC *in vitro* and *in vivo*, this supports their potential advantage as a primary CRC-targeted DDS (Zamorano-León *et al.,* 2019; Ogutu, Mu & Zhang, 2017; Adhikari & Yadav, 2019).

1.2 Study Objectives

Considering the unique physiology of CRC and chemistry of pectin and chitosan, this study objective was to synthesize and characterize TP, fabricate pectin-based drug delivery systems and compare the effect of pectin thiolation and addition of chitosan towards their physicochemical properties, evaluating the *in vitro* drug release profile, as well as evaluate its anticancer activity and specificity through an *in vitro* study.

1.3 Scope of the Study

The research scope included synthesis and characterization of TP (free thiol content and Fourier-transform infrared spectroscopy), fabrication and characterization of 5-FU-loaded pectin-based beads (entrapment efficiency, drug content, average circular diameter, individual weight, circularity index), *in vitro* drug release study of loaded beads, cell viability assay of free drug, empty beads, and drug-loaded beads on HT29 and HEK293 cell lines, and DNA fragmentation assay of empty and loaded beads on HT29 cell line.

1.4 Hypotheses

The author hypothesized that both thiolation of pectin and addition of chitosan may improve the drug entrapment and release profile, as well as improving the anticancer activity. Moreover, the presence of pectin and chitosan may have additive or synergistic effects with 5-FU in HT29 cells, whereas it may reduce the toxicity of 5-FU in HEK293 cells.