DEVELOPMENT OF NANOCELLULOSE INCORPORATED OLEOGEL MATRIX FOR THE ENCAPSULATION AND COLON-TARGETED DELIVERY OF THE 5-AMINOSALICYLIC ACID

by

ZIJIN QIN

(Under the Direction of Fanbin Kong)

ABSTRACT

5-Aminosalicylic acid (5-ASA) is a drug used to treat ulcerative colitis based on topical contact with colonic epithelium. However, due to gastric and intestinal digestion, orally administrated 5-ASA may be greatly degraded and may not reach the colon. Oleogel can be used as a carrier for the targeted delivery of active compounds with improved bioaccessibility. In this work, 5-ASA was successfully encapsulated in a nanocellulose incorporated coconut oil-sorbitan tristearate oleogel. The release of the 5-ASA from the matrix was tested in a static digestion model. The results showed that adding nanocellulose to the oleogel significantly improved the mechanical strength and thermal stability of oleogel. The oleogel encapsulation successfully delayed the release of 5-ASA in the gastrointestinal tract, and nanocellulose further modified the release properties of oleogel. The results indicated that the nanocellulose incorporated oleogel could serve as an effective delivery system for the targeted release of bioactives in digestive tract.

INDEX WORDS: Oleogel; Encapsulation; 5-Aminosalicylic acid; Nanocellulose; Sorbitan tristearate; *In vitro* digestion.

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DEDICATION

To my advisor and parents.

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CHAPTER 1

INTRODUCTION

The human colon is the last section of the gastrointestinal tract. It performs several functions, including storing, mixing, propulsion of the colonic fluids (Scott, 2003), and absorbing water, electrolytes, bacterial metabolites (Cook et al., 2000). However, colon functions may be disturbed by various diseases and disorders, including inflammatory bowel disease (IBD). IBD includes Crohn's disease and ulcerative colitis (UC) and can cause chronic inflammations in the human colon.

Mesalazine, also known as mesalamine or 5-aminosalicylic acid (5-ASA), is a medication for IBD and could be taken orally or rectally. Traditionally, oral administration is the predominant route for nutra-pharmaceuticals delivery due to its low cost, minor invasion, tolerable safety, and increased patient compliance (Maderuelo, Zarzuelo, & Lanao, 2011). However, the method also has some limitations which restrict its application. For example, oral administration is not suitable for people who have dysphagia, including coma. Additionally, the orally administrated active agents are exposed to gastrointestinal (GI) juice, which could diminish the active ingredient like insulin (Arbit & Kidron, 2009) and adrenalin (Giragossintz & Mackler, 1929). Also, many factors, including the motility of the GI tract, the component of the stomach and intraluminal content, and the environment pH, could make the transit time and release the location of the active agent uncontrollable. Thus, controlled release is highly preferred for the active ingredients to deliver a desirable effect, especially for the colon-targeted active

agents, like mesalazine, sulfasalazine, hydrocortisone, 5-flourouracil, typhoid, etc. (Philip & Philip, 2010). Different approaches were studied to allow controlled release property, and encapsulation is one of the methods that has been widely studied. Gelation as an approach of encapsulation method shows a promising effect on the modified release of the active agent (Andonova et al., 2017; Chloe, Davidovich-Pinhas, Wright, Barbut, & Marangoni, 2017; C.-C. Lin & Anseth, 2009). The gel system could be further divided into two groups depending on the polarity of the solvent: organogel and hydrogel (Marangoni, 2012). Both of them could be used in the encapsulation for the controlled release, and the choices depend on the polarity of the encapsulated material.

Oleogel, as a type of organogel, has shown a promising effect on bioactive delivery due to the dense network structure effectively immobilizing the active agent and reducing its diffusion to the outside media (Chloe et al., 2017). Gelator is a critical factor for the oleogel performance. Commonly used oleogelator include hydroxylated fatty acid (Hughes, Marangoni, Wright, Rogers, & Rush, 2009; Iwanaga, Sumizawa, Miyazaki, & Kakemi, 2010), sorbitan ester (Murdan, 2005; Singh, Pal, Pradhan, & Pramanik, 2013), sugar ester (Xu, 2014), monoacyl-glycerol (Yu, Shi, Liu, & Huang, 2012), fatty alcohol (Lupi et al., 2013), etc. Among them, sorbitan ester has been widely studied, and most studies applied the sorbitan monostearate as an oleogelator (Murdan, Gregoriadis, & Florence, 1999; Singh et al., 2013). Structurally similar to sorbitan monostearate, sorbitan tristearate also has the alkyl chains, which are the critical functional group to promote oleogelation. However, only a few research investigated the sorbitan tristearate as the oleogelator, especially for its application in encapsulation delivery systems. So far, its application in the food industry is limited.

Nanocellulose, including both cellulose nanofiber (CNF) and cellulose nanocrystal (CNC), is a promising wall material for encapsulation since it could form 3D-gel-networks not only by itself (Lin Huang et al., 2013) but also with other wall materials (Li et al., 2019). Nanocellulose was used in the preparation of oleogel based pickering emulsion, and exhibited a significant effect on improving the stability and bioaccessibility of the active agent (Qi, Zhang, & Wu, 2020). So far, no research has been conducted to investigate the influence of nanocellulose, when combined into the oleogel as a wall material, on the physicochemical properties of the gel matrix and the release profile of encapsulated bioactive agent.

The overall objective of this thesis research was to develop a sorbitan tristearate-gelated oleogel matrix incorporating nanocellulose for the encapsulation and colon-targeted delivery of the 5-aminosalicylic acid. To achieve this goal, the specific objectives and hypotheses were set as follows.

Objective 1: Develop a coconut oil oleogel system for encapsulation of 5-ASA using sorbitan tristearate as the gelator and nanocellulose as an additive to enhance its physicochemical property. The hypothesis is: The proposed method will result in a 5-ASA encapsulated oleogel matrix with relatively high encapsulation efficiency and improved stability.

Objective 2: Investigate the release of 5-ASA from the oleogel matrix using an *in vitro* static digestion model. The hypothesis is: The proposed encapsulation matrix will allow a delayed release of 5-ASA in the GI tract, and an increased amount of the drug will be delivered to the colon as compared with unencapsulated 5-ASA.

There are four chapters in this thesis. The first chapter introduces the topic rationales, hypotheses, and objectives of this research. The second chapter is a literature review of topics related to oleogel and nanocellulose and their applications in encapsulation delivery systems as well as detection of 5-ASA in bioanalytical samples. The third chapter reports the experiments and results, including the development of the proposed oleogel matrix and its properties, and the performance on *in vitro* gastrointestinal delivery of the encapsulated 5-ASA. The fourth chapter highlights the significance of this work, along with some suggestions for future work.

References

Andonova, V., Peneva, P., Georgiev, G. S., Toncheva, V. T., Apostolova, E., Peychev, Z., Dimitrova, S., Katsarova ., M., Petrova, N., Kassarova, M. (2017). Ketoprofen-loaded polymer carriers in bigel formulation: an approach to enhancing drug photostability in topical application forms. *International Journal of Nanomedicine, 12,* 6221.

Arbit, E., & Kidron, M. (2009). Oral insulin: the rationale for this approach and current developments. *Journal of Diabetes Science and Technology, 3*(3), 562-567.

Chloe, M., Davidovich-Pinhas, M., Wright, A. J., Barbut, S., & Marangoni, A. G. (2017). Ethylcellulose oleogels for lipophilic bioactive delivery–effect of oleogelation on in vitro bioaccessibility and stability of beta-carotene. *Food & Function, 8*(4), 1438-1451.

Giragossintz, G., & Mackler, H. (1929). The oral administration of adrenalin. *Endocrinology, 13*(1), 88-92.

Cook, I. J., Furukawa, Y., Panagopoulos, V., Collins, P. J., & Dent, J. (2000). Relationships between spatial patterns of colonic pressure and individual movements of content. *American Journal of Physiology-Gastrointestinal and Liver Physiology, 278*(2), G329-G341.

Gibson, T. B., Ng, E., Ozminkowski, R. J., Wang, S., Burton, W. N., Goetzel, R. Z., & Maclean, R. (2008). The direct and indirect cost burden of Crohn's disease and ulcerative colitis. *Journal of Occupational and Environmental Medicine,* 50(11), 1261-1272.

Huang, L., Chen, X., Nguyen, T. X., Tang, H., Zhang, L., & Yang, G. (2013). Nano-cellulose 3D-networks as controlled-release drug carriers. *Journal of Materials Chemistry B, 1*(23), 2976-2984.

Hughes, N. E., Marangoni, A. G., Wright, A. J., Rogers, M. A., & Rush, J. W. (2009). Potential food applications of edible oil organogels. *Trends in Food Science & Technology, 20*(10), 470-480.

Iwanaga, K., Sumizawa, T., Miyazaki, M., & Kakemi, M. (2010). Characterization of organogel as a novel oral controlled release formulation for lipophilic compounds. *International journal of pharmaceutics, 388*(1-2), 123-128.

Kappelman, M. D., Rifas–Shiman, S. L., Porter, C. Q., Ollendorf, D. A., Sandler, R. S., Galanko, J. A., & Finkelstein, J. A. (2008). Direct health care costs of Crohn's disease and ulcerative colitis in US children and adults. *Gastroenterology, 135*(6), 1907-1913.

Li, J., Wang, Y., Zhang, L., Xu, Z., Dai, H., & Wu, W. (2019). Nanocellulose/gelatin composite cryogels for controlled drug release. *ACS Sustainable Chemistry & Engineering, 7*(6), 6381-6389.

Lin, C.-C., & Anseth, K. S. (2009). PEG hydrogels for the controlled release of biomolecules in regenerative medicine. *Pharmaceutical Research, 26*(3), 631-643.

Longobardi, T., Jacobs, P., Wu, L., & Bernstein, C. N. (2003). Work losses related to inflammatory bowel disease in Canada: results from a National Population Health Survey. *The American Journal of Gastroenterology, 98*(4), 844-849.

Lupi, F. R., Gabriele, D., Baldino, N., Mijovic, P., Parisi, O. I., & Puoci, F. (2013). Olive oil/policosanol organogels for nutraceutical and drug delivery purposes. *Food & Function, 4*(10), 1512-1520.

Maderuelo, C., Zarzuelo, A., & Lanao, J. M. (2011). Critical factors in the release of drugs from sustained release hydrophilic matrices. *Journal of Controlled Release, 154*(1), 2-19.

Marangoni, A. G. (2012). Organogels: an alternative edible oil-structuring method. *Journal of the American Oil Chemists' Society, 89*(5), 749-780.

Murdan, S. (2005). Organogels in drug delivery. *Expert Opinion on Drug Delivery, 2*(3), 489-505.

Murdan, S., Gregoriadis, G., & Florence, A. T. (1999). Inverse toroidal vesicles: precursors of tubules in sorbitan monostearate organogels. *International Journal of Pharmaceutics, 183*(1), 47-49.

Philip, A. K., & Philip, B. (2010). Colon targeted drug delivery systems: a review on primary and novel approaches: *Oman Medical Journal, 25*(2), 79.

Qi, W., Zhang, Z., & Wu, T. (2020). Encapsulation of beta-carotene in oleogel-in-water Pickering emulsion with improved stability and bioaccessibility. *Int J Biol Macromol, 164*, 1432-1442.

Scott, S. (2003). Manometric techniques for the evaluation of colonic motor activity: current status. *Neurogastroenterology & Motility, 15*(5), 483-513.

Singh, V. K., Pal, K., Pradhan, D. K., & Pramanik, K. (2013). Castor oil and sorbitan monopalmitate based organogel as a probable matrix for controlled drug delivery. *Journal of Applied Polymer Science, 130*(3), 1503-1515.

Xu, X. (2014). Improve bioaccessibility of quercetin using pseudo-organogel based nanoemulsions. Rutgers University-Graduate School-New Brunswick,

Yu, H., Shi, K., Liu, D., & Huang, Q. (2012). Development of a food-grade organogel with high bioaccessibility and loading of curcuminoids. *Food Chemistry, 131*(1), 48-54.

CHAPTER 2

LITERATURE REVIEW

Human digestive system and simulation

Digestive system is a crucial part for maintaining metabolic homeostasis in the human body. The digestive system is composed of digestive glands and digestive tract. The digestive glands secrete digestive juice into the digestive tract, where the food is digested physiochemically. The digestive tract is started with the mouth, where food is administrated. The food then arrives to the stomach through the esophagus, driven by the swallowing process. In the stomach, the food is mashed and mixed with gastric juice, and the protein is digested to peptides. Small extent of water, nutrients (water-soluble vitamins, amino acids, simple sugar, etc.), and some medicine (like aspirin) are absorbed during gastric digestion. After the gastric phase, the chyme passes to the small intestine via a pyloric sphincter. In the small intestine, carbohydrates, proteins, fat, vitamins, and minerals are further digested and absorbed. After small-intestinal digestion, chyme enters the colon, where the water, electrolytes, short-chain fatty acids are further absorbed.

In vitro digestion model is commonly used in the study of the behavior of food and nonfood material in the human GI system. Both dynamic and static *in vitro* models are developed. The dynamic digestion models are more sophisticated and could mimic the complicated physiological process such as the motility of the GI tract in addition to the chemical digestion. These models generally showed good *in vitro - in vivo* correlations (Li, Fortner, & Kong, 2019; Wright, Kong, Williams, & Fortner, 2016).

However, the model development and validation are time and money-consuming. Compared with dynamic models, static in vitro digestion models are simple and easy to construct. Minekus et al.(2014) developed a standardized static *in vitro* digestion method suitable for food digestion study. The model consists of oral, gastric, and small intestine phases with conditions for each phase defined similarly to that of *in vivo*. Recommendations are given on the composition and pH of the digestive juice, and mass ratios of food to digestion juices in different digestion phases. The physical digestion (breakdown of food structure by mechanical or hydrodynamic forces) is mimicked by a stirrer, shaker, or impeller, while the chemical digestion (enzymatic or acidic hydrolysis) is obtained by adding the corresponding digestive juices containing enzymes (pepsin, α-amylase, pancreatin, and lipase) in each stage. The method has shown broad applicability.

5-Aminosalicylic acid and its delivery in the gastrointestinal tract

Inflammatory bowel disease (IBS) is a common colonic disorder leading to diarrhea, rectal bleeding, abdominal pain, fatigue, weight loss, and sometimes life-threatening complications. It includes Crohn's disease and ulcerative colitis (UC) and is characterized by inflammations of the large intestine (Cohen & Weisshof, 2020). In the US, the total annual financial burden of IBD was $14.6 to $31.6 billion in 2014, and this number is increasing due to the increasing number of people suffering from IBD (Gibson et al., 2008; Kappelman et al., 2008; Longobardi, Jacobs, Wu, & Bernstein, 2003).

5-Aminosalicylic acid (5-ASA), also known as mesalamine, is a type of aminosalicylate compound used to treat UC due to its anti-inflammatory activities. The chemical structure of 5-ASA is shown in Figure 2.1. It can inhibit production of interleukin, prostaglandins, and leukotrienes (Peskar, Dreyling, May, Schaarschmidt, &

Goebell, 1987), acting as a free radical scavenger and an antioxidant (Ahnfelt-Rønne et al., 1990). The detailed mechanism of 5-ASA's action on UC is unknown. However, it is believed that the pharmacological effects of 5-ASA are based on its topical contact with the colonic epithelium, so the efficacy of the 5-ASA on UC depends on its concentration in the lumen instead of the blood (Ham & Moss, 2012).

Currently, two forms of 5-ASA are frequently used to treat ulcerative colitis: suppository or enema for the rectal administration and pill for oral administration. The pill form of 5-ASA has been frequently used due to its advantage of the extensive treatment area (Ham & Moss, 2012), increased patient compliance, and simple administration method (Nokhodchi, Raja, Patel, & Asare-Addo, 2012). However, the major limitation for oral administration is the instability of 5-ASA in the acidic gastric phase. Moreover, it can be also inactivated in the small intestine via acetylation to N-acetyl -5-ASA by the N-acetyltransferase, leading to a low bioavailability (20–30%) of 5-ASA in the colon (Desreumaux & Ghosh, 2006; Schroeder, 2002; Vishwakarma, Ganeshpurkar, Pandey, Dubey, & Bansal, 2015). So, encapsulation has become an important method to deliver the active agent to the colon successively. The transit and release of encapsulated 5-ASA are affected by the motility and pH of the stomach and small intestine as well as the wall material of the capsules (Ham & Moss, 2012). Various wall materials were studied. For example, zein (Lau et al., 2013), ethylcellulose (Rasmussen et al., 1982), chitosan (Mladenovska et al., 2007; Quinteros, Manzo, & Allemandi, 2010), clay (Hong, Jeong, Roh, & Kang, 2018), gum acacia (Sharma et al., 2019) were used to encapsulate 5-ASA and have shown to effectively delay the release of 5-ASA in the GI tract.

Application of oleogel in encapsulation drug delivery system

Gels, including hydrogel and organogel, are frequently used to encapsulate nutreints and drugs. Oleogel, as one type of organogel, is composed of edible oil trapped within a three-dimensional, cross-linked network formed by a gelator. Oleogel is made by adding a low concentration of gelator molecule to oil which is then subjected to appropriate processing (e.g., heating, stirring, and cooling) to allow the formation of 3-D networks by the gelator molecules trapping and structuring the liquid oil. The oleogel system could be divided into self-assembly system and crystal particles system based on the oleogelation process (Dassanayake, Kodali, & Ueno, 2011). In the self-assembly system, the 3D network of oleogel is formed due to molecular-level self-organization. The oleogelators that could cause the self-assembly system are sorbitan esters (Murdan, Gregoriadis, & Florence, 1999), monoacylglycerols (Larsson, Quinn, Sato, & Tiberg, 2006), phytosterol (Bot, Veldhuizen, den Adel, & Roijers, 2009), etc. For example, sorbitan monostearate has been widely studied for oleogel formation. At the gelation temperature, toroidal vesicles are formed consisting of sorbitan monostearate molecules that further develop into tubules during cooling. The tubules associate with each other to form a 3D network that could immobilize the liquid oil phase in the oleogel (Murdan et al., 1999). In comparison, the network in crystal particles systems is formed by nucleation, followed by the growth of crystals (Dassanayake et al., 2011). The oleogelators that could cause the crystal particles systems are fatty acid (Hughes, Marangoni, Wright, Rogers, & Rush, 2009; Iwanaga, Sumizawa, Miyazaki, & Kakemi, 2010), fatty alcohol (Lupi et al., 2013), wax ester (Daniel & Rajasekharan, 2003), etc. The physical properties (hardness, brightness, melting point, etc.) of oleogel depend on

the gelator type and concentration, cooling rate, etc. (Ojijo, Neeman, Eger, & Shimoni, 2004). So it could be designed for different applications.

Stortz et al. (2012) first proposed the use of oleogel for the controlled delivery of nutraceuticals and pharmaceuticals. Chloe et al. (2017) conducted the digestion experiment and reported that oloegel encapsulated beta- carotene showed a lower extent of lipolysis compared to liquid oil due to oloegel's dense 3D network, which trapped the liquid oil and created a physical barrier preventing access of the lipase. As the digestion time increased, the structure of the 3D network was broken down, the liquid oil and beta-carotene diffused out, and its diffusion extent was related to the extent of lipolysis. Additionally, they found the bioavailability and stability of beta-carotene were improved in the oleogel compared with the control group, due to the protection of the oleogel's 3D network. This study revealed the advantage of oleogel as an encapsulation carrier for the controlled release and protection of bioactive components. Similarly, past researches showed that oleogel could effectively encapsulate and deliver other bioactive components like fish oil (M. C. Lee, Tan, & Abbaspourrad, 2019; Perez et al., 2019), lutein and neoxanthin (Pérez-Monterroza, Chaux-Gutiérrez, & Nicoletti, 2018), etc. In a recent study, oleogel was combined with hydrogel to form a bigel system for the controlled release of nurta- and pharmaceuticals (Behera, Sagiri, Singh, Pal, & Anis, 2014; Sagiri et al., 2015; Soradech, Petchtubtim, Thongdon-A, & Muangman, 2016; Zheng, Mao, Cui, Liu, & Gao, 2020).

Application of nanocellulose in the encapsulation delivery system

Nanocellulose is obtained from cellulose, the most abundant biopolymer on Earth. It is an emerging renewable polymeric nanomaterial that holds promise in many different

applications, including food and pharmaceuticals (Peng, Dhar, Liu, & Tam, 2011). Nanocellulose is classified into cellulose nanofibril (CNF), cellulose nanocrystal (CNC), and bacterial nanocellulose (BC) depends on the production process. CNF, with the high aspect ratio, is produced by applying mechanical force on the cellulose fibers. CNC is produced by acid hydrolysis of cellulose. Compared with CNF, the aspect ratio is lower in CNC because hydrolysis usually causes transversely cleavage of cellulose fibers. BC is generated by several species of bacteria. Due to the excellent biocompatibility and biodegradability, low ecological toxicity risk, and low cytotoxicity (Domingues, Gomes, & Reis, 2014), nanocellulose is currently a subject of great interest for interdisciplinary research. For example, it was used as a food additive to improve the texture of the product. A previous study showed that the incorporation of bacteria nanocellulose into meat products could promote the hardness, springiness, and chewiness of the product (Marchetti & Andrés, 2020). Additionally, nanocellulose has strong mechanical properties and thermostability, which made it an ideal additive for reinforcing polymer structure (Dufresne, 2013; Han, Yu, & Wang, 2018).

It has been shown that nanocellulose could be applied for the encapsulation of bioactive components with controlled release properties (N. Lin, Huang, Chang, Feng, & Yu, 2011). Huang et al. (2013) conducted a study to evaluate the effect of the 3D network of bacterial nanocellulose as a drug carrier on the release of hydrochloride and berberine sulphate, and found that the bacterial nanocellulose significantly extended the drug release time. Additionally, nanocellulose showed a promising effect on the controlled release of active components when combined into other hydrogel systems. For example, Lin et al. (2011) evaluated the performance of CNC incorporated alginate-based

microspheres on the release of theophylline, and found that the matrix allowed a sustained release of the active agent as the diffusion of molecules was inhibited by the crosslinked network structure. Nanocellulose derivatives are also used in the design of drug-controlled release systems. Recent research also demonstrated that carboxymethylated nanocellulose incorporated montmorillonite is highly effective in prolonging the ciprofloxacine release (Hong et al., 2019).

Quantification of 5-aminosalicylic acid in the bioanalytical sample

In this study, a new method was developed to measure the content of 5-ASA in the chime samples. A brief review of the existing methods is presented here. Chromatography, spectrophotometry, spectrofluorimetry, and electrochemistry are the four frequently used analytical methods to quantify 5-ASA in the bioanalytical samples. As the most commonly used method, liquid chromatography has the advantage of high selectivity and low sample cost (Tavares Junior, de Araújo, Meneguin, & Chorilli, 2020). Before chromatography is conducted, the bioanalytical sample like plasma (Gu et al., 2011; Qin, Di, Wang, & Liu, 2015) and rectal tissue (Hussain, Ajjan, Moustafa, Anderson, & Riley, 1998) were usually added methanol followed by the centrifuge to precipitate protein. The detector coupled to the liquid chromatography could be mass spectrometer, photodiode-array detector, and refractive index detector, etc. (Banda et al., 2016; Elmasry et al., 2011; Hartzell, Maldonado-Gómez, Yang, Hutkins, & Rose, 2013). However, its drawbacks like the high cost of the equipment and HPLC grade solvent as well as the long testing time are also considered when choosing the analytic method for 5-ASA. Spectrophotometry is also a common choice for the detection of 5-ASA; its selectivity and sensitivity are related to the concentration and the chemical properties of

the sample. From 5ASA's UV-Vis spectra, the pure 5-ASA has the maximum absorbance at the wavelength of 297 nm (Lau et al., 2013). Compared to the HPLC method, spectrophotometry can conduct the quantification of 5-ASA with minimal cost as well as simple sample preparation, so it is also a good choice for the detection. However, due to the relatively low sensitivity and presence of salt and protein in the bioanalytical samples, various derivatization methods have been applied for spectrophotometric detection of 5-ASA to increase sensitivity and lower the effects of impurities. For example, the 5-ASA could react with potassium iodide (Sloka, Gurupadayya, & Kumar, 2010), Bratton-Marshall reagent (N-1-naphthyl ethylene diamine dihydrochloride), paradimethylaminobenzaldehyde, Gibb's reagent (2,6-dichloroquinone-4-chloroimide) (Patel, Patel, Panigrahi, Parikh, & Patel, 2010) to form products with high sensitivity in spectrophotometric detection. Also, 5-ASA is a type of phenol acid (p-aminophenol), which could react with ferric chloride to form a brown product and can be detected using a spectrophotometer (Soloway & Wilen, 1952). Although less frequently, spectrofluorometric and electrochemical methods are also used for the quantification of 5-ASA with minimal cost, low detection limit, high reproductivity as well as simple sample preparation (Cui, Qin, Li, & Luo, 2008). The selection of quantification methods should depend on analyte concentration and matrix, cost, and quality of labor.

References

Ahnfelt-Rønne, I., Nielsen, O. H., Christensen, A., Langholz, E., Binder, V., & Riis, P. (1990). Clinical evidence supporting the radical scavenger mechanism of 5-aminosalicylic acid. *Gastroenterology, 98*(5), 1162-1169.

Banda, J., Lakshmanan, R., Katepalli, R. B., Venati, U. K. R., Koppula, R., & Prasad, V. S. (2016). Determination of mesalazine, a low bioavailability olsalazine metabolite in human plasma by UHPLC–MS/MS: Application to a pharmacokinetic study. *Journal of Chromatography B, 1008*, 1-10.

Behera, B., Sagiri, S. S., Singh, V. K., Pal, K., & Anis, A. (2014). Mechanical properties and delivery of drug/probiotics from starch and non-starch based novel bigels: A comparative study. *Starch-Stärke, 66*(9-10), 865-879.

Bot, A., Veldhuizen, Y. S., den Adel, R., & Roijers, E. C. (2009). Non-TAG structuring of edible oils and emulsions. *Food Hydrocolloids, 23*(4), 1184-1189.

Bruschi, M. L. (2015). Strategies to modify the drug release from pharmaceutical systems: Woodhead Publishing.

Chloe, M., Davidovich-Pinhas, M., Wright, A. J., Barbut, S., & Marangoni, A. G. (2017). Ethylcellulose oleogels for lipophilic bioactive delivery–effect of oleogelation on in vitro bioaccessibility and stability of beta-carotene. *Food & Function, 8*(4), 1438-1451.

Cohen, R. D., & Weisshof, R. (2020). A comprehensive review of topical therapies for distal ulcerative colitis. *Gastroenterol Hepatol (NY), 16*, 21-27.

Cui, F.-l., Qin, L.-x., Li, F., & Luo, H.-x. (2008). Synchronous fluorescence determination and molecular modeling of 5-aminosalicylic acid (5-ASA)

interacted with human serum albumin. *Journal of Molecular Modeling, 14*(12), 1111-1117.

Daniel, J., & Rajasekharan, R. (2003). Organogelation of plant oils and hydrocarbons by long-chain saturated FA, fatty alcohols, wax esters, and dicarboxylic acids. *Journal of the American Oil Chemists' Society, 80*(5), 417-421.

Dassanayake, L. S. K., Kodali, D. R., & Ueno, S. (2011). Formation of oleogels based on edible lipid materials. *Current Opinion in Colloid & Interface Science, 16*(5), 432-439.

Desreumaux, P., & Ghosh, S. (2006). mode of action and delivery of 5-aminosalicylic acid–new evidence. *Alimentary Pharmacology & Therapeutics, 24*, 2-9.

Domingues, R. M., Gomes, M. E., & Reis, R. L. (2014). The potential of cellulose nanocrystals in tissue engineering strategies. *Biomacromolecules, 15*(7), 2327-2346.

Dufresne, A. (2013). Nanocellulose: a new ageless bionanomaterial. *Materials Today, 16*(6), 220-227.

Elmasry, M. S., Blagbrough, I. S., Rowan, M. G., Saleh, H. M., Kheir, A. A., & Rogers, P. J. (2011). Quantitative HPLC analysis of mebeverine, mesalazine, sulphasalazine and dispersible aspirin stored in a Venalink monitored dosage system with co-prescribed medicines. *Journal of Pharmaceutical and Biomedical Analysis, 54*(4), 646-652.

Gu, G.-Z., Xia, H.-M., Pang, Z.-Q., Liu, Z.-Y., Jiang, X.-G., & Chen, J. (2011). Determination of sulphasalazine and its main metabolite sulphapyridine and 5-aminosalicylic acid in human plasma by liquid chromatography/tandem mass

spectrometry and its application to a pharmacokinetic study. *Journal of Chromatography B, 879*(5-6), 449-456.

Ham, M., & Moss, A. C. (2012). Mesalamine in the treatment and maintenance of remission of ulcerative colitis. *Expert review of Clinical Pharmacology*, *5*(2), 113-123.

Han, Y., Yu, M., & Wang, L. (2018). Soy protein isolate nanocomposites reinforced with nanocellulose isolated from licorice residue: Water sensitivity and mechanical strength. *Industrial Crops and Products*, *117*, 252-259.

Hartzell, A. L., Maldonado-Gómez, M. X., Yang, J., Hutkins, R. W., & Rose, D. J. (2013). In vitro digestion and fermentation of 5-formyl-aminosalicylate-inulin: A potential prodrug of 5-aminosalicylic acid. *Bioactive Carbohydrates and Dietary Fibre, 2*(1), 8-14.

Hong, H.-J., Jeong, H. S., Roh, K.-M., & Kang, I. (2018). Preparation of Mesalazine-Clay Composite Encapsulated Alginate (MCA) Bead for Targeted Drug Delivery: Effect of Composite Content and CaCl 2 Concentration. *Macromolecular Research*, *26*(11), 1019-1025.

Hong, H.-J., Kim, J., Kim, D.-Y., Kang, I., Kang, H. K., & Ryu, B. G. (2019). Synthesis of carboxymethylated nanocellulose fabricated ciprofloxacine–Montmorillonite composite for sustained delivery of antibiotics. *International Journal of Pharmaceutics*, *567*, 118502.

Huang, L., Chen, X., Nguyen, T. X., Tang, H., Zhang, L., & Yang, G. (2013). Nano-cellulose 3D-networks as controlled-release drug carriers. *Journal of Material Chemistry B, 1*(23), 2976-2984.

Hussain, F., Ajjan, R., Moustafa, M., Anderson, J., & Riley, S. (1998). Simple method for the determination of 5-aminosalicylic and N-acetyl-5-aminosalicylic acid in rectal tissue biopsies. *Journal of Chromatography B: Biomedical Sciences and Applications, 716*(1-2), 257-266.

Kojima, H., Yoshihara, K., Sawada, T., Kondo, H., & Sako, K. (2008). Extended release of a large amount of highly water-soluble diltiazem hydrochloride by utilizing counter polymer in polyethylene oxides (PEO)/polyethylene glycol (PEG) matrix tablets. *European Journal of Pharmaceutics and Biopharmaceutics, 70*(2), 556-562.

Larsson, K., Quinn, P., Sato, K., & Tiberg, F. (2006). *Lipids: Structure, Physical Properties and Functionality* (Vol. 19): Oily Press Bridgwater.

Lau, E. T., Giddings, S. J., Mohammed, S. G., Dubois, P., Johnson, S. K., Stanley, Steadman, K. J. (2013). Encapsulation of hydrocortisone and mesalazine in zein microparticles. *Pharmaceutics, 5*(2), 277-293.

Lee, M. C., Tan, C., & Abbaspourrad, A. (2019). Combination of internal structuring and external coating in an oleogel-based delivery system for fish oil stabilization. *Food Chemistry, 277*, 213-221.

Lee, S. S., Lim, C. B., Pai, C. M., Lee, S., Park, I., Seo, M. G., & Park, H. (2002). Composition and pharmaceutical dosage form for colonic drug delivery using polysaccharides. In: Google Patents

Li, Y., Fortner, L., & Kong, F. (2019). Development of a Gastric Simulation Model (GSM) incorporating gastric geometry and peristalsis for food digestion study. *Food Research International, 125*, 108598.

Lin, N., Huang, J., Chang, P. R., Feng, L., & Yu, J. (2011). Effect of polysaccharide nanocrystals on structure, properties, and drug release kinetics of alginate-based microspheres. *Colloids and Surfaces B: Biointerfaces, 85*(2), 270-279.

Maderuelo, C., Zarzuelo, A., & Lanao, J. M. (2011). Critical factors in the release of drugs from sustained release hydrophilic matrices. *Journal of Controlled Release, 154*(1), 2-19.

Marchetti, L., & Andrés, S. C. (2020). Use of nanocellulose in meat products. *Current Opinion in Food Science*.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, Dupont, D. (2014). A standardised static in vitro digestion method suitable for food–an international consensus. *Food & Function, 5*(6), 1113-1124.

Mladenovska, K., Raicki, R., Janevik, E., Ristoski, T., Pavlova, M., Kavrakovski, Goracinova, K. (2007). Colon-specific delivery of 5-aminosalicylic acid from chitosan-Ca-alginate microparticles. *International Journal of Pharmaceutics, 342*(1-2), 124-136.

Murdan, S., Gregoriadis, G., & Florence, A. T. (1999). Inverse toroidal vesicles: precursors of tubules in sorbitan monostearate organogels. *International Journal of Pharmaceutics, 183*(1), 47-49.

Nokhodchi, A., Raja, S., Patel, P., & Asare-Addo, K. (2012). The role of oral controlled release matrix tablets in drug delivery systems. *BioImpacts: BI, 2*(4), 175.

Ojijo, N. K., Neeman, I., Eger, S., & Shimoni, E. (2004). Effects of monoglyceride content, cooling rate and shear on the rheological properties of olive

oil/monoglyceride gel networks. *Journal of the Science of Food and Agriculture, 84*(12), 1585-1593.

Pai, C., Lim, C., Lee, S., Park, I., Park, H., Seomoon, Wilding, I. (2000). *Pharmacoscintigraphic and Pharmacokinetic Evaluation of Colon Specific Delivery System in Healthy Volunteers.* Paper presented at the Proceedings of the International Symposium on Controlled Release Bioactive Materials.

Patel, K., Patel, C., Panigrahi, B., Parikh, A., & Patel, H. (2010). Development and validation of spectrophotometric methods for the estimation of mesalamine in tablet dosage forms. *Journal of Young Pharmacists, 2*(3), 284-288.

Peng, B. L., Dhar, N., Liu, H., & Tam, K. (2011). Chemistry and applications of nanocrystalline cellulose and its derivatives: a nanotechnology perspective. *The Canadian Journal of Chemical Engineering, 89*(5), 1191-1206.

Pérez-Monterroza, E. J., Chaux-Gutiérrez, A. M., & Nicoletti, V. R. (2018). Encapsulation of avocado oil in amylose solution from cassava starch. *Journal of Food Processing and Preservation, 42*(5), e13594.

Perez, J. A. V., Remacho, C. R., Rodriguez, J. R., Pulido, J. M. O., De La Fuente, E. B., & Martinez-Ferez, A. (2019). Optimization of Oleogel Formulation for Curcumin Vehiculization and Lipid Oxidation Stability by Multi-response Surface Methodology. *Chemical Engineering Transactions, 75*, 427-432.

Peskar, B., Dreyling, K., May, B., Schaarschmidt, K., & Goebell, H. (1987). Possible mode of action of 5-aminosalicylic acid. *Digestive Diseases and Sciences, 32*(12), S51-S56.

Qin, J., Di, X., Wang, X., & Liu, Y. (2015). Development and validation of an LC-MS/MS method for the determination of mesalazine in beagle dog plasma and its application to a pharmacokinetic study. *Biomedical Chromatography, 29*(2), 261-267.

Quinteros, D. A., Manzo, R. H., & Allemandi, D. A. (2010). Design of a colonic delivery system based on cationic polymethacrylate (Eudragit E100)-mesalamine complexes. *Drug Delivery, 17*(4), 208-213.

Rasmussen, S., Bondesen, S., Hvidberg, E., Hansen, S. H., Binder, V., Halskov, S., & Flachs, H. (1982). 5-aminosalicylic acid in a slow-release preparation: bioavailability, plasma level, and excretion in humans. *Gastroenterology, 83*(5), 1062-1070.

Rubinstein, A. (1990). Microbially controlled drug delivery to the colon. *Biopharmaceutics & drug disposition, 11*(6), 465-475.

Sagiri, S. S., Singh, V. K., Kulanthaivel, S., Banerjee, I., Basak, P., Battachrya, M., & Pal, K. (2015). Stearate organogel–gelatin hydrogel based bigels: Physicochemical, thermal, mechanical characterizations and in vitro drug delivery applications. *Journal of the Mechanical behavior of Biomedical Materials, 43*, 1-17.

Schroeder, K. (2002). Role of mesalazine in acute and long-term treatment of ulcerative colitis and its complications. *Scandinavian Journal of Gastroenterology, 37*(236), 42-47.

Sharma, N., Sharma, A., Bhatnagar, A., Nishad, D., Karwasra, R., Khanna, K.,

Sharmaa, D., Kumar, N., Jain, G. K. (2019). Novel gum acacia based macroparticles for colon delivery of Mesalazine: Development and gammascintigraphy study. *Journal of Drug Delivery Science and Technology, 54*, 101224.

Sloka, S. N., Gurupadayya, B., & Kumar, C. (2010). Sensitive spectrophotometric method for the determination of mesalazine in bulk and pharmaceutical formulations. *Der. Pharma Chemica, 2*(4), 389-396.

Soloway, S., & Wilen, S. H. (1952). Improved ferric chloride test for phenols. *Analytical Chemistry*, *24*(6), 979-983.

Soradech, S., Petchtubtim, I., Thongdon-A, J., & Muangman, T. (2016). Development of Wax-Incorporated Emulsion Gel Beads for the Encapsulation and Intragastric Floating Delivery of the Active Antioxidant from Tamarindus indica L. *Molecules*, *21*(3), 380.

Stortz, T. A., Zetzl, A. K., Barbut, S., Cattaruzza, A., & Marangoni, A. G. (2012). Edible oleogels in food products to help maximize health benefits and improve nutritional profiles. *Lipid Technology*, *24*(7), 151-154.

Takaya, T., Ikeda, C., Imagawa, N., Niwa, K., & Takada, K. (1995). Development of a colon delivery capsule and the pharmacological activity of recombinant human granulocyte colony-stimulating factor (rhG-CSF) in beagle dogs. *Journal of Pharmacy and Pharmacology*, *47*(6), 474-478.

Tavares Junior, A. G., de Araújo, J. T. C., Meneguin, A. B., & Chorilli, M. (2020). Characteristics, Properties and Analytical/Bioanalytical Methods of 5-Aminosalicylic Acid: A Review. *Critical Reviews in Analytical Chemistry*, 1-15.

Vishwakarma, N., Ganeshpurkar, A., Pandey, V., Dubey, N., & Bansal, D. (2015). Mesalazine–probiotics beads for acetic acid experimental colitis: formulation and characterization of a promising new therapeutic strategy for ulcerative colitis. *Drug delivery*, *22*(1), 94-99.

Watanabe, S., Kawai, H., Katsuma, M., & Fukui, M. (2002). Colon-specific drug release system. In: Google Patents.

Wright, N. D., Kong, F., Williams, B. S., & Fortner, L. (2016). A human duodenum model (HDM) to study transport and digestion of intestinal contents. *Journal of Food Engineering*, *171*, 129-136.

Zheng, H., Mao, L., Cui, M., Liu, J., & Gao, Y. (2020). Development of food-grade bigels based on κ-carrageenan hydrogel and monoglyceride oleogels as carriers for β-carotene: Roles of oleogel fraction. *Food Hydrocolloids*, *105*, 105855.

Figures

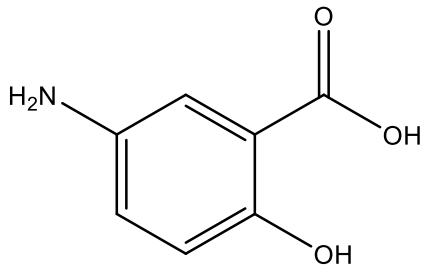

Figure 2.1 The chemical structure of 5-ASA

CHAPTER 3

DEVELOPMENT OF NANOCELLULOSE INCORPORATED OLEOGEL FOR THE ENCAPSULATION AND COLON-TARGETED DELIVERY OF THE 5-AMINOSALICYLIC ACID[1]

[1] Qin, Z. and Kong, F., to be submitted to *Food Hydrocolloids.*

Abstract

5-Aminosalicylic acid (5-ASA) is a drug used to treat ulcerative colitis (UC), a disease characterized by inflammations of the colon. However, during the gastrointestinal (GI) digestion, orally administrated 5ASA could be degraded before reaching the colon. Sorbitan ester-based oleogel, as a new nutra-pharmaceuticals encapsulation delivery method, shows a promising effect on improving the bioaccessibility of active compounds. In this work, 5-ASA was successfully encapsulated into sorbitan tristearate (STS)-based oleogel with nanocellulose incorporated to enhance the gel properties, with encapsulation efficiency up to 99.4%. The results of texture and leaching tests suggested that the addition of nanocellulose to oleogel improved its mechanical strength and thermal stability. A static *in vitro* digestion model was used to evaluate the release of 5-ASA. The results showed that oleogel successfully delayed the release of 5-ASA in the GI tract, with less than 4% released in the stomach and 28% in the small intestine. The addition of cellulose nanocrystal (CNC) at different concentrations affected the release of 5-ASA, indicating that CNC could be used to modulate the bioaccessibility and bioavailability of 5-ASA. The nanocellulose incorporated oleogel encapsulation system developed in this study could be used for the delivery and controlled release of nutrients and bioactive compounds in the human GI tract.

Keywords: 5-Aminosalicylic acid; Oleogel; Nanocellulose, Encapsulation

Introduction

5-Aminosalicylic acid (5-ASA), also known as mesalamine, is a type of aminosalicylate compound used to treat ulcerative colitis (UC), a disease characterized by inflammation of the large intestine (Cohen & Weisshof, 2020). Currently, two forms of 5-ASA are frequently used to treat ulcerative colitis: suppository or enema for the rectal administration and pill for oral administration. The orally administrated 5-ASA has been frequently used for the treatment due to its advantage of the extensive treatment area (Ham & Moss, 2012), increased patient compliance, and simple administration method (Nokhodchi, Raja, Patel, & Asare-Addo, 2012). However, during the digestion process in the stomach and small intestine, 5-ASA could be degraded due to the acidic gastric environment and the enzymatic hydrolysis, diminishing the amount of 5-ASA reaching the ulcerative point in the colon and further causing the unnecessarily increased drug intake amount and potential of adverse effects. So, encapsulation was employed to deliver the active agent to the colon. The transit and colonic absorption of orally administrated 5-ASA depends on the gastrointestinal (GI) motility and pH in the stomach and small intestine as well as the coating material (wall material) of the tablet (Ham & Moss, 2012). The wall material has been widely studied. For example, zein (Lau et al., 2013), ethylcellulose (Rasmussen et al., 1982), chitosan (Mladenovska et al., 2007; Quinteros, Manzo, & Allemandi, 2010), clay (Hong, Jeong, Roh, & Kang, 2018), gum acacia (Sharma et al., 2019) were used to coat the tablets and have shown effectiveness in delaying the release of 5-ASA in the GI tract.

Oleogel, as a type of organogel, has a gel-like, viscoelastic structure consisting of liquid oil and gelator. It is initially proposed to replace solid fat and enhance unsaturated

fat content in the food product. Stortz et al. (2012) proposed the use of oleogel in the controlled delivery of nutraceuticals and pharmaceuticals. It was used to effectively deliver bioactive agents to the desired site in the GI tract like beta-carotene (Chloe, Davidovich-Pinhas, Wright, Barbut, & Marangoni, 2017), fish oil (M. C. Lee, Tan, & Abbaspourrad, 2019; Perez et al., 2019), lutein and neoxanthin (Pérez-Monterroza, Chaux-Gutiérrez, & Nicoletti, 2018). The esters of sorbitol have been widely used as effective gelators for oleogelation. For example, sorbitan monostearate (Span 60) has been proved to be a good oleogelator (Murdan et al., 1999; Swe & Asavapichayont, 2018) and has been used for the encapsulation of bioactive compounds like aspirin, paracetamol, ibuprofen, and hydrocortisone in the GI tract (Jibry, Sarwar, & Murdan, 2006). Sorbitan tristearate (STS), however, has received little attention. Few studies have been conducted to explore the use of STS as an oleogelator for the encapsulation for the controlled release of bioactive agents in the GI tract.

Nanocellulose is an emerging renewable polymeric nanomaterial that holds potential in many different applications (Peng et al., 2011). Nanocellulose mainly includes cellulose nanofibril (CNF) and cellulose nanocrystal (CNC). CNF, with a high aspect ratio, is produced via mechanical homogenization. CNC is produced by chemical hydrolysis. Compared with CNF, CNC has a lower aspect ratio because hydrolysis usually causes cleavage of cellulose fibers transversely. Nanocellulose was used as a wall material for different encapsulation systems and showed a promising effect on the controlled release of drugs because of the 3D network formed between the nanocellulose itself (Lin Huang et al., 2013) and between nanocellulose and other wall materials (Kolakovic, Laaksonen, Peltonen, Laukkanen, & Hirvonen, 2012; N. Lin et al., 2011).

Herein, we report a novel oleogel-based encapsulation system for the controlled release of colon-targeted nutraceuticals and pharmaceuticals. 5-ASA was used as an indicator. STS was chosen as the oleogelator, and cellulose nanocrystal (CNC) or cellulose nanofibers (CNF) was added into the oleogel system to examine the impact of nanocellulose on the gel structure and the release of 5-ASA. The structure of the oleogel was characterized by Fourier-transform infrared (FT-IR) spectroscopy and polarized optical microscopy. The texture of the oleogels was measured using a texture analyzer. The leaching of the oil phase was tested to estimate the stability of the gel system. Release studies were carried out using an *in vitro* static digestion model. The results from this study are useful to develop effective oleogel encapsulation delivery systems for bioactives and pharmaceuticals aiming to improved health benefits.

Materials and Methods

Chemicals and Reagents

Coconut oil was purchased from Acros Organics (Fair Lawn, NJ, USA). Sorbitan tristearate was purchased from Spectrum Chemical Manufacturing Corporation (Gardena, CA, USA). CNF (3.0 wt%, dispersed in water) were purchased from the Process Development Center at the University of Maine (Orono, ME). CNC (12.1 wt% in water) was obtained from the USDA's Forest Products Laboratory. Pepsin from porcine gastric mucosa (P7000, 479 U/mg with hemoglobin as substrate), bile extract from porcine (B8631, 1g=2.512 mM bile salt), lipase from porcine pancreas (Type II, L3126, 114U/mg with olive oil as substrate), 5-aminosalicylic acid, ferric chloride were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All the chemicals were used without

further purification. The dialysis bag with flat width 50 mm and molicular weight cut-off 8,000 Da was purchased from Repligen Corporation (Waltham, MA, USA).

Determination of STS concentration for gel preparation

To choose the proper STS concentrations for the oleogel formation, STS was added to coconut oil at various concentrations (20% - 80% w/w to the final oleogel weight), and the slip melting point of oleogel gelated by STS was determined using the AOCS Official Method Cc 3-25 (American Oil Chemists' Society, 2011). The oleogels with slip melting point higher than 37 ℃ were selected for the encapsulation since these matrixes could still maintain the solid shape in the GI tract, which has a temperature of 37℃.

Encapsulation of 5-ASA in oleogel

To prepare the oleogel, STS with various concentrations (30% - 80% w/w to the final oleogel weight) were chosen for the oleogelation. The reason for selecting this concentration range was that all of the corresponding oleogel formed had a melting point higher than 37℃. STS was completely dissolved in coconut oil at 80 ℃ under magnetic stirring. 5-ASA (10% w/w to the final product weight) was rapidly dispersed in the above formulation with homogenization for 2 min. Oleogel was formed by cooling the hot solution to room temperature. Then, the samples were ground into powders and stored at 4℃ for future use.

Encapsulation of 5-ASA in nanocellulose-integrated oleogel

To prepare the nanocellulose integrated oleogel, STS (60% w/w to the final oleogel weight) was completely dissolved in coconut oil at 80 ℃ under magnetic stirring. CNC or CNF (0.6% - 1.5% w/w) was added to the above formulation with

homogenization for 2 min, respectively. 5-ASA powder (10% to the weight of final product) was rapidly dispersed in the above formulation under homogenization for 2 min using a homogenizer (PRO Scientific Inc, Oxford, CT, USA) at a setting of 4. The matrix was cooled to room temperature to allow the formation of the network, followed by freezing drying to remove water. Then, the sample was ground into powders and stored at 4℃ for future use.

Analysis of mechanical property

The texture of the drug-loaded matrix was evaluated using a TA-XT2i texture analyzer (Stable Microsystems, Surrey, England) coupled with a 3 mm (DI) cylindrical plunger. About 2.5 g (dry basis) 5-ASA encapsulated matrix was formed in a 6 mL cylindrical glass beaker (inner diameter = 13 mm and height = 45 mm) at 4 ℃, and the penetration test was conducted after 24-hour storage. The matrix was compressed to a distance of 8.0 mm at a speed of 1.0 mm/s. Hardness (the highest peak force expressed in gram measured during penetration) was used to characterize the textural properties of samples. The 5-ASA encapsulated oleogel sample without nanocellulose was tested as the control group.

Quantification of 5-ASA

To determine 5-ASA in the microcapsule surface and digestion samples, a new method was developed in this study. A $FeCl_3$ solution with a concentration of 2g/L in 300 mM acetate buffer (pH 3.6) was prepared and used to react with 5-ASA for color formation (Soloway & Wilen, 1952). As a salicylic acid derivative, 5-ASA could conjugate with ferric ion to form intense color at low concentration with a distinct absorption peak at visible light spectrum, which could be used as a convenient

spectrophotometric method for quantifying 5-ASA (Tavares Junior et al., 2020). Due to the complexity of digestion phases, proteins and salts could potentially interfere with the formation of 5-ASA-ferric ion conjugate and the detection of its absorbance. Thus, methanol and acidic medium were required for accurate quantification of 5-ASA. For the analysis of 5-ASA in the intestinal phase, samples were mixed with the aforementioned $FeCl_3$ solution, methanol, and 1M HCl at a ratio of 1:5:9:1. For the determination of encapsulation efficiency and analysis of 5-ASA in the gastric phases, samples were mixed with the $FeCl_3$ solution and methanol at a ratio of 1:5:9 (v/v), respectively, and reacted for 5 min. All samples were subjected to centrifugation at 8,000 rpm (AccuSpin Micro 17, Thermo Fisher Scientific, Germany) for the removal of precipitated proteins and salts. The violet color of the formed conjugate was detected by a UV-Vis Spectrophotometer (Thermo Scientific Evolution, MA, USA) at 510nm, 547 nm, and 500nm, respectively, for the samples taken for the encapsulation efficiency test, and gastric and intestinal release test. Deionized (DI) water, gastric, and intestinal juices without the addition of 5-ASA were used as blank, respectively. 5-ASA dissolved in DI water, gastric, and intestinal juices at different concentrations were used for developing standard curves for analyses of encapsulation efficiency, and the content of 5-ASA in gastric and intestinal phases.

Determination of encapsulation efficiency

To determine the encapsulation efficiency, 5 g oleogel (containing 60% STS) and CNC and CNF incorporated oleogel (containing 60% STS) were washed with 300 mL DI water to remove the surface and unencapsulated 5-ASA. The washing solution was collected and used to react with the $FeCl_3$ in acetate solution according to the method

described above. The amount of 5-ASA was determined by measuring the color by a UV-Vis Spectrophotometer. The encapsulation efficiency (EE) was calculated according to the following equation:

$$EE\ (\%) = \left(1 - \frac{\text{Weight of washed-off 5-ASA}}{\text{Weight of 5-ASA before wash}}\right) \times 100\% \quad (3.1)$$

Oil leaching study

The leaching of the oil phase was studied to determine the stability of the oleogel microcapsules. The higher the amount of leaching, the lower the stability of the microcapsule, as leaching of the oil phase to outside weakens the oleogel network structure. 0.5 g sample (W_0) was placed on a clean filter paper, which was then placed in an oven at 60 °C, and leakage was visually monitored for 10 min. The unleached sample was removed from the filter paper and weighed (W_1). The weight difference was recorded. The leaching values were calculated according to the following equations:

$$\text{Leaching}\ (\%) = \frac{W_1 - W_0}{W_0} \times 100\% \quad (3.2)$$

FT-IR and microscopy analysis

The FT-IR spectra of oleogel samples were collected using a Thermo Nicolet Nexus FT-IR 1100 spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a ZnSe attenuated total reflection attachment (ν_{max} is reported in cm^{-1}). 0.1 g sample was placed onto the ZnSe crystal. Spectral data were collected from 650 to 4,000 cm^{-1} with a resolution of 4 cm^{-1} and 32 scans.

Polarized light microscopy was used to characterize the 5-ASA encapsulated oleogels with/without nanocellulose. An Olympus BX40 microscope (Olympus America, Center Valley, PA, USA) was used to observe the morphology of samples at magnifications of 400×. The slides were prepared by placing a drop of heated samples

(80 °C) between a stationary and moving glass plate, and the prepared samples were stored at 4 °C overnight for oleogelation. 5-ASA incorporated oleogel without nanocellulose were also tested as the control group.

In vitro drug-release studies

The preparation of simulated gastric juice (SGF) and simulated intestinal fluid (SIF) were conducted following the literature (Minekus et al., 2014), and in *vitro* digestion was conducted based on the United State Pharmacopeia (USP General chapter 2003) method and standardized static *in vitro* digestion method (Alminger et al., 2014) with minor modifications. 0.5g matrix was placed in a dialysis bagand 10 ml distilled water was added to simulate the oral administration of the 5-ASA.

Then, 10 ml SGF and pepsin were added, and the pH value was adjusted to 3 to trigger gastric digestion. The release study was carried out for two hours in a shaking water bath at 37 °C and 50 rpm. 0.5 ml gastric buffer was taken hourly, and the volume loss was made up by fresh SGF with pepsin during the digestion. After the gastric digestion, 20 ml SIF, 0.22g lipase, and 0.16g bile were added to the mixture, and the pH of the solution was adjusted to 7 to trigger intestinal digestion. The digestion was conducted in the shaking water bath for two hours. 0.5 ml of the medium was withdrawn at 2 min, 6 min, 10 min, 20 min, 30 min, 40 min, 80 min, and 120 min, respectively, and the same amount of fresh solution was replaced. The withdrawn samples were analyzed for 5-ASA content as described above. The cumulative amount of 5-ASA released from the samples were calculated using the following equation:

$$\text{Cumulative 5-ASA released (\%)} = \frac{M_t}{M_{total}} \times 100\% \qquad (3.3)$$

M_t is the amount of 5-ASA released from the sample at time t, and M_{total} is the total amount of 5-ASA loaded in the sample.

Statistical analysis

Statistical analysis of the experimental results was performed using JMP® software (version 13.2.0, SAS Institute, Inc., Cary, NC, USA) and SPSS Statistics software (version 25, IBM Corp., Armonk, NY, USA). The results were expressed as mean values ± standard deviation (SD). To evaluate the effects of the variables on leaching percentage and hardness, the leaching percentage values (%) and hardness (g-force) were analyzed by one-way analysis of variance (ANOVA). To evaluate the effects of different encapsulation matrix on 5-ASA release at various times, the cumulative 5-ASA released (%) were analyzed by analysis of variance (ANOVA) with two-way interactions. The results were expressed as least-squares means (LS mean) ± the standard error of mean (SEM). Significant differences were determined using Duncan test ($P \leq 0.05$).

Results and Discussion

Melting point of oleogel

The melting point of bulk oleogel is shown in Table 3.1. The slip melting point is the temperature at which the solid-like lipid begins to melt to a liquid-like phase and move upward from a capillary tube due to buoyancy in the aqueous medium. There was an increasing trend of slip melting point when the STS concentration was increased. According to Table 3.1, the slip melting point increased from 35.4 ± 0.2 ℃ to 53.4 ± 0.1℃ with the STS concentration increased from 20% to 80%. This was consistent with

the findings reported by Naeli et al. (2020), who used ethyl cellulose-hydroxypropyl methylcellulose biopolymer as the oleogelator.

The increasing concentration of STS led to a rising slip melting point of the oleogel, which was probably attributed to the increased density of STS crystalline network (Yang et al., 2017). This network could efficiently trap the coconut oil and decrease the surface tension of the mixture. So, the mixture with low STS concentration (loose 3D network and high surface tension) tended to go higher along the capillary tube at a higher temperature. The oleogel with STS concentration higher than 20% (slip melting point higher than 37 ℃) were selected for the encapsulation due to their potential high thermostability in the GI tract where the temperature is around 37℃ (Koziolek et al., 2015).

Texture analysis

The hardness of the matrix expressed in gram-force (g) is shown in Table 3.2. The CNC and CNF integrated oleogel (5-ASA contained) showed harder textures compared to the control group (0.6% to the weight of oleogel). For example, the hardness of the oleogel without nanocellulose was 2812 g, while after the addition of 0.6% CNC and 1.5% CNF, the hardness increased to 3489 and 4029 g, respectively. The results indicated that the addition of CNC and CNF in the oleogel as part of encapsulation wall material could improve the mechanical strength of the oleogel. Previous research showed that the growth of ice crystals in a colloidal suspension could be used to drive the self-assembly of anisotropic building blocks (Romeo et al., 2012), which might help to increase the mechanical strength of the matrix after the removal of moisture. Similarly, Peng et al. (2013) had studied the influence of the freeze-drying process on the structure and found

that the interaction between nanocellulose was improved after the freeze-drying process, which could possibly contribute to the improved mechanical properties of nanocellulose. In this case, during the freeze-drying, the nanocellulose in oleogel was confined in the space between ice crystals and the oleogel network (Svagan, Samir, & Berglund, 2008). Thus the concentration of localized nanocellulose was increased, which led to the increased interaction between nanocellulose (Svagan et al., 2008). After freeze-drying, a low-agglomerate and porous structure could be formed by the nanocellulose in oleogel, and this robust particle network could increase matrix's mechanical performance (Munier, Gordeyeva, Bergström, & Fall, 2016). To better understand the internal structure of nanocellulose incorporated oleogel, a scanning electron microscopy (SEM) analysis could be conducted in future research.

Quantification of 5-ASA

The relationships between the concentration of 5-ASA in the reference samples and the absorbance were linear for 5-ASA in the DI water, gastric digesta, and intestinal digesta in specific 5-ASA concentration ranges. The corresponding standard curves are shown in Figure 3.1. Coefficients of determination (R^2) were 0.9943, 0.9972, 0.9994 for the absorbance and the concentration of 5-ASA in the DI water, gastric phase, and intestinal phase. The corresponding high R^2 values indicated a high correlation of the results. Linear trends were obtained in the concentration range of 0.01–0.5 mg/mL, 0.1–1.0 mg/mL, 0.01–1.5 mg/mL for 5-ASA in the media of DI water, gastric juice, and intestinal juice respectively. The low limit of detection (LOD) of 5-ASA in DI, gastric phase, and intestinal phase was 0.01 mg/ml, 0.1 mg/ml, and 0.01 mg/ml, respectively.

The relatively low LOD values (high sensitivity of the method) could be explained by the violet-colored complex formed by the 5-ASA and Fe^{3+}. Compared with the liquid chromatography method, this spectrophotometry method uses more samples with a higher LOD value (LOD of HPLC method could be as low as 50 ng/mL (Qin, Di, Wang, & Liu, 2015)). However, the method has the advantage of low cost since it avoids using expensive chemicals and a large amount of mobile phase solvent. Additionally, it is easy and quick to conduct since the sample preparation and detection could finish with common laboratory instruments in 5 min. Compared with the traditional UV-VIS spectrometric method, where 5-ASA concentration is directly determined at the wavelength where 5-ASA has the maximum absorption (Mladenovska et al., 2007), this method tests the colored complex formed by 5-ASA and Fe^{3+} and showed high sensitivity. Compared with other UV-VIS spectrometric methods involving the formation of the color complex between 5-ASA and reagents, the reagent ($FeCl_3$) used in this method is cheaper. Therefore, this new spectrometric method based on the formation of colored complex originated from 5-ASA and Fe^{3+} could be used for studies to quantify 5-ASA in the bioanalytical sample with low cost.

Encapsulation efficiency

The 5-ASA concentration in the 300 mL DI water washing off from 5g matrix of oleogel containing 60% STS, and CNC and CNF incorporated oleogel (containing 500 mg 5-ASA theoretically) were under the LOD, which was 0.01 mg/mL. According to Equation 3.1, the encapsulation efficiency (EE) for the 5-ASA in oleogel, CNC and CNF incorporated oleogel was higher than 99.4%. The EE values were much higher than most of the similar studies reported in the literature. For example, Mladenovska et al. (2007)

loaded 5-ASA into chitosan–Ca–alginate microparticles by spray drying, and the EE value was reported as 55 ± 19%; Bahadori et al. (2019) loaded 5-ASA into sodium alginate cores coated with quaternized inulin, with EE of 90%; Lau et al., (2013) encapsulated 5-ASA into zein microparticles, and the highest EE value reached 61.69 ± 9.61%. The high EE values in this study could be explained by the dense network structure of STS assembled oleogel and nanocellulose. The network structure could be responsible for inhibiting the movement or dissolving of 5-ASA into outside media when the matrix was washed with DI water. The high EE value indicated that oleogel and nanocellulose incorporated oleogel could be a promising carrier for the storage and GI delivery of 5-ASA.

Leaching studies

The oil leached (%) from the matrix is shown in Figure 3.2. With the incorporation of either CNC or CNF, the leaching of the oil phase significantly decreased. After the addition of 1.5% CNC and CNF, the leaching value (%) decreased from 80.76 ± 3.2 (control group without nanocellulose incorporated) to 36.23 ± 1.7 and 26.31 ± 4.8, respectively. The result could probably be due to the high thermostability of nanocellulose (Khakalo, Mäkelä, Johansson, Orelma, & Tammelin, 2020) and the network structure formed in oleogel by nanocellulose after the freeze-drying process (Munier et al., 2016). Nanocellulose has a high decomposition temperature which is approximately 200–300 °C (Gan, Sam, Abdullah, & Omar, 2020), and this is much higher than the slip melting point of oleogel gelated by STS. Additionally, research had also shown that hydrogen bonding could occur among the hydroxyl groups of the cellulose chains in the non-aqueous environment (Tanaka & Fukui, 2004), and this

hydrogen bonding could drive nanocellulose to aggregate forming crystalline structures (Peng et al., 2013). The formed crystalline structure could even be enhanced after the freeze-drying due to the rearrangement of the nanocellulose (Peng et al., 2013). Similarly, in this work, the decreased leaching values in the nanocellulose incorporated oleogel could be explained by the strengthened crystalline network formed after freeze-drying, and the 3D network trapped the melted oil as temperature increased which led to a decreased leaching value of oleogel incorporated with nanocellulose.

FT-IR and microscopy analyses

The FT-IR spectra of pure 5-ASA, coconut oil, STS and oleogel, CNC, CNF, CNC-incorporated oleogel, and the CNF incorporated oleogel are shown in Figure 3.3 and Figure 3.4. Compared with the CNC spectra, there was a redshift in the CNC incorporated oleogel (1053 cm^{-1} to 1061 cm^{-1}), implying a possible interaction between CNC and oleogel. Similarly, redshifts were also observed by comparing CNF incorporated oleogel (1061 cm^{-1}) with CNF (1050 cm^{-1}); oleogel (1739 cm^{-1}) with coconut oil (1736 cm^{-1}) and STS (1735 cm^{-1}); 5-ASA (807 cm^{-1};1191 cm^{-1};1145 cm^{-1}) to oleogel encapsulating 5-ASA (808 cm^{-1};1192 cm^{-1};1155 cm^{-1}). These shifts implied that possible interaction between 5-ASA and oleogel, as well as the interaction between nanocellulose (both CNC and CNF) and oleogel, and the interactions could be caused by the hydrogen bonding or the van der Waals' forces.

The microscopy images of 5-ASA contained oleogel, CNC and 5-ASA incorporated oleogel as well as CNF and 5-ASA incorporated oleogel were shown in Figure 3.5. From the photo of bulk oleogel and 5-ASA encapsulated oleogel (60% STS), the network was formed during the oleogelation process, which was caused by the self-

assembling of the STS during the cooling process (Sagiri et al., 2016). The growing crystals were interconnected to form a network structure that could entrap the coconut oil and reduce the leaching of 5-ASA in the digestive media. After adding CNC and CNF into the oleogel, this network structure could still be observed. Moreover, the positions where 5-ASA were located were overlapped with some fine networks, which could probably be the nanocellulose fiber.

In vitro drug-release studies

The release of 5-ASA from oleogels made with different STS concentrations is shown in Figure 3.6, and the effect of incorporation of CNC and CNF into the oleogel (containing 60% STS) were shown in Figure 3.7 and Figure 3.8, respectively. As for the effect of STS, the formation of STS assembled oleogel caused a significant reduction in the accumulative 5-ASA released (%) compared to the control group (pure 5-ASA without encapsulation) and the group of 5-ASA mixed with coconut oil during both gastric and intestinal digestion. The reduced release could be attributed to the minor role stomach played in the lipid digestion and the properties of the oleogel itself. The gastric digestion, which last 2-5 hours, contributed very little to the lipid hydrolysis compared to intestinal digestion. Therefore, the structure of the oleogel-based matrix remained relatively intact after gastric digestion. Moreover, STS, similar to sorbitan monostearate, could not be fully digested in the human small intestine (Anneken et al., 2000; Wick & Joseph, 1953), which could explain the relatively low amount of released 5-ASA in the small intestine when STS was used to form the oleogel. The relatively high slip melting point of the oleogel (more than 37°C) and the dense network could effectively arrest the movement of the coconut oil contained 5-ASA to the digestion media, so the diffusion of

5-ASA into the media was reduced during the small intestinal digestion. The LS mean of the accumulative release of 5-ASA-coconut oil samples was higher than that of pure 5-ASA, which indicated that coconut oil could not impede the release of 5-ASA. In contrast, the movement of coconut oil to the outside media could accelerate the release of the 5-ASA from the matrix.

The low STS concentration groups (30%, 40%, and 50%) showed a small but significantly lower accumulative release (%) compared to the high STS concentration groups (60%, 70%, and 80%). This could be explained by the potentially increased brittleness of the oleogel with high gelator concentration, and the brittleness was usually associated with the breakage of the gelation network under stress (i.e., the hydrodynamic forces generated by the motility of the GI tract simulated by the shaking water bath) (Sagiri et al., 2016). Another possible reason is related to the surface area in different oleogel samples. It was observed the oleogel particles with higher STS concentrations were less likely to agglomerate together, which led to a larger total surface area as compared with the samples with lower STS content, eventually causing a faster release of the encapsulated ingredient.

As for the effect of CNF, by compared with the pure 5-ASA samples and coconut samples, all CNF groups showed a significantly lower amount of 5-ASA released calculated by the least square mean (LS mean), which indicated that the oleogel structure was still effective to immobilize the encapsulated 5-ASA after the addition of CNF. In addition, at the same STS concentration level, the addition of CNF did not cause significant changes to the cumulative amount of 5-ASA released. Although the hydrophilicity caused by hydroxyl groups (Tyagi, Lucia, Hubbe, & Pal, 2019) in CNF

could probably increase the trend of sample to break down and disperse into the aqueous digestive juice, which could lead to increased release of 5-ASA, the low concentration of CNF added in this study did not cause a significant effect. As for the effect of CNC, when compared with the pure 5-ASA samples and coconut samples, similar to CNF, all CNC groups showed a significantly lower amount of 5-ASA released. Additionally, at the same STS concentration level, the addition of 0.6%, 1.2%, and 1.5% CNC did not cause significant changes in the cumulative 5-ASA released. However, a small but significant increase in the accumulative 5-ASA release (%) was observed with the addition of 0.9% CNC, which has not been observed in the CNF added samples. The increased accumulative release after the addition of CNC could be attributed to hydrophilicity caused by hydroxyl groups (Tyagi et al., 2019) and the high brittleness (Nair, Zhu, Deng, & Ragauskas, 2014) of CNC than CNF. Thus, the oleogel with CNC incorporated could probably be more prone to fracture during the motility of the GI tract simulated by the shaking water bath, and the fine particles with increased hydrophilicity (compared with the oleogel without CNC added) could be more prone to disperse in water. This could cause an increased contact area between oleogel particles and digestive juice, which led to the increased release of 5-ASA. Additionally, it was observed that CNC incorporated oleogel particles were less likely to adhere to each other compared to the samples without the addition of nanocellulose, leading to a larger surface area during the digestion compared to the groups without added CNC, which may have contributed to the release of 5-ASA. Further studies may be conducted to confirm this, in which a larger solid matrix with a fixed shape instead of powder may be applied for the digestion study to determine whether this difference in 5-ASA release was caused by the surface area.

Additionally, there was also a possibility that the addition of a certain amount of CNC could slightly interrupt the network structure, which was not discernible enough to be observed under the polarized light microscope. To better understand this, a SEM microscopy test could be helpful. The increased 5-ASA release caused by the addition of CNC could be useful for the targeted/local treatment of UC at the end of the ileum and the proximal colon, which need relatively higher intraluminal 5-ASA concentration and earlier release of 5-ASA in the corresponding position, compared with the UC in the distal colon.

Conclusion

A novel method for encapsulation of 5-ASA was established to improve its bioaccessibility in the colon. The oleogel based encapsulation system effectively reduced the release of 5-ASA in the upper GI tract. The addition of CNF and CNC improved the mechanical strength and reduced the leaching of oil, which indicated that the addition of nanocellulose into oleogel could improve the stability and reduce the quality loss of the product during storage and transportation. Moreover, the addition of CNC at different concentrations to the oleogel affected the release of 5-ASA during the digestion, which indicated that the controlled release property of the oleogels could be modulated by adjusting the amount of CNC added. Additionally, a new rapid spectrophotometric method was developed to determine the concentration of 5-ASA with relatively high sensitivity and low cost, which could be used as an alternative method for quantification of 5-ASA in food and biological systems.

References

Alminger, M., Aura, A. M., Bohn, T., Dufour, C., El, S., Gomes, A.,Requena, T. (2014). In vitro models for studying secondary plant metabolite digestion and bioaccessibility. *Comprehensive Reviews in Food Science and Food Safety, 13*(4), 413-436.

American Oil Chemists' Society. (2011). In F. David (Ed.), Official methods and recommended practices of the AOCS (6th ed.). Champaign, IL: AOCS Press

Anneken, D. J., Both, S., Christoph, R., Fieg, G., Steinberner, U., & Westfechtel, A. (2000). Fatty acids. *Ullmann's Encyclopedia of Industrial Chemistry*.

Bahadori, F., Akinan, B. S., Akyil, S., & Eroglu, M. S. (2019). Synthesis and engineering of sodium alginate/inulin core-shell nano-hydrogels for controlled-release oral delivery of 5-ASA.Chloe, M., Davidovich-Pinhas, M., Wright, A. J., Barbut, S., & Marangoni, A. G. (2017). Ethylcellulose oleogels for lipophilic bioactive delivery–effect of oleogelation on in vitro bioaccessibility and stability of beta-carotene. *Food & function, 8*(4), 1438-1451.

Cohen, R. D., & Weisshof, R. (2020). A comprehensive review of topical therapies for distal ulcerative colitis. *Gastroenterol Hepatol (NY), 16*, 21-27.

Domingues, R. M., Gomes, M. E., & Reis, R. L. (2014). The potential of cellulose nanocrystals in tissue engineering strategies. *Biomacromolecules*, *15*(7), 2327-2346.

Dufresne, A. (2013). Nanocellulose: a new ageless bionanomaterial. *Materials Today, 16*(6), 220-227.

Gan, P., Sam, S., Abdullah, M. F. b., & Omar, M. F. (2020). Thermal properties of nanocellulose-reinforced composites: A review. *Journal of Applied Polymer Science, 137*(11), 48544.

Ham, M., & Moss, A. C. (2012). Mesalamine in the treatment and maintenance of remission of ulcerative colitis. *Expert review of clinical pharmacology, 5*(2), 113-123.

Han, Y., Yu, M., & Wang, L. (2018). Soy protein isolate nanocomposites reinforced with nanocellulose isolated from licorice residue: Water sensitivity and mechanical strength. *Industrial Crops and Products, 117*, 252-259.

Hong, H.-J., Jeong, H. S., Roh, K.-M., & Kang, I. (2018). Preparation of Mesalazine-Clay Composite Encapsulated Alginate (MCA) Bead for Targeted Drug Delivery: Effect of Composite Content and CaCl 2 Concentration. *Macromolecular Research, 26*(11), 1019-1025.

Huang, L., Chen, X., Nguyen, T. X., Tang, H., Zhang, L., & Yang, G. (2013). Nano-cellulose 3D networks as controlled-release drug carriers. *Journal of Materials Chemistry B, 1*(23), 2976-2984.

Jibry, N., Sarwar, T., & Murdan, S. (2006). Amphiphilogels as drug carriers: effects of drug incorporation on the gel and on the active drug. *Journal of Pharmacy and Pharmacology, 58*(2), 187-194.

Khakalo, A., Mäkelä, T., Johansson, L.-S., Orelma, H., & Tammelin, T. (2020). High-Throughput Tailoring of Nanocellulose Films: From Complex Bio-Based Materials to Defined Multifunctional Architectures. *ACS applied Biomaterials, 3*(11), 7428-7438.

Kolakovic, R., Laaksonen, T., Peltonen, L., Laukkanen, A., & Hirvonen, J. (2012). Spray-dried nanofibrillar cellulose microparticles for sustained drug release. *International Journal of Pharmaceutics, 430*(1-2), 47-55.

Koziolek, M., Grimm, M., Becker, D., Iordanov, V., Zou, H., Shimizu, J., Wanke, C., Garbacz, G., Weitschies, W. (2015). Investigation of pH and temperature profiles in the GI tract of fasted human subjects using the Intellicap® system. *Journal of Pharmaceutical Sciences, 104*(9), 2855-2863.

Lau, E. T., Giddings, S. J., Mohammed, S. G., Dubois, P., Johnson, S. K., Stanley, R. A., Halley, P. J., Steadman, K. J. (2013). Encapsulation of hydrocortisone and mesalazine in zein microparticles. *Pharmaceutics, 5*(2), 277-293.

Li, Z., & Xia, W. (2020). Coarse-grained modeling of nanocellulose network towards understanding the mechanical performance. *Extreme Mechanics Letters, 40*, 100942.

Lee, M. C., Tan, C., & Abbaspourrad, A. (2019). Combination of internal structuring and external coating in an oleogel-based delivery system for fish oil stabilization. *Food Chemistry, 277*, 213-221.

Lin, N., Huang, J., Chang, P. R., Feng, L., & Yu, J. (2011). Effect of polysaccharide nanocrystals on structure, properties, and drug release kinetics of alginate-based microspheres. *Colloids and Surfaces B: Biointerfaces, 85*(2), 270-279.

Marchetti, L., & Andrés, S. C. (2020). Use of nanocellulose in meat products. *Current Opinion in Food Science*.

Mladenovska, K., Raicki, R., Janevik, E., Ristoski, T., Pavlova, M., Kavrakovski, Z., Dodov, MG.,Goracinova, K. (2007). Colon-specific delivery of 5-aminosalicylic

acid from chitosan-Ca-alginate microparticles. *International Journal of Pharmaceutics, 342*(1-2), 124-136.

Murdan, S., Gregoriadis, G., & Florence, A. T. (1999). Inverse toroidal vesicles: precursors of tubules in sorbitan monostearate organogels. *International Journal of Pharmaceutics, 183*(1), 47-49.

Naeli, M. H., Milani, J. M., Farmani, J., & Zargaraan, A. (2020). Development of innovative ethyl cellulose-hydroxypropyl methylcellulose biopolymer oleogels as low saturation fat replacers: Physical, rheological and microstructural characteristics. *International Journal of Biological Macromolecules, 156*, 792-804.

Nair, S. S., Zhu, J., Deng, Y., & Ragauskas, A. J. (2014). High performance green barriers based on nanocellulose. *Sustainable Chemical Processes, 2*(1), 1-7.

Nokhodchi, A., Raja, S., Patel, P., & Asare-Addo, K. (2012). The role of oral controlled release matrix tablets in drug delivery systems. *BioImpacts: BI, 2*(4), 175.

Patel, K., Patel, C., Panigrahi, B., Parikh, A., & Patel, H. (2010). Development and validation of spectrophotometric methods for the estimation of mesalamine in tablet dosage forms. *Journal of Young Pharmacists, 2*(3), 284-288.

Peng, Y., Gardner, D. J., Han, Y., Kiziltas, A., Cai, Z., & Tshabalala, M. A. (2013). Influence of drying method on the material properties of nanocellulose I: thermostability and crystallinity. *Cellulose, 20*(5), 2379-2392.

Peng, B. L., Dhar, N., Liu, H., & Tam, K. (2011). Chemistry and applications of nanocrystalline cellulose and its derivatives: a nanotechnology perspective. *The Canadian Journal of Chemical Engineering, 89*(5), 1191-1206.

Pérez-Monterroza, E. J., Chaux-Gutiérrez, A. M., & Nicoletti, V. R. (2018). Encapsulation of avocado oil in amylose solution from cassava starch. *Journal of Food Processing and Preservation, 42*(5), e13594.

Qin, J., Di, X., Wang, X., & Liu, Y. (2015). Development and validation of an LC-MS/MS method for the determination of mesalazine in beagle dog plasma and its application to a pharmacokinetic study. *Biomedical Chromatography, 29*(2), 261-267.

Quinteros, D. A., Manzo, R. H., & Allemandi, D. A. (2010). Design of a colonic delivery system based on cationic polymethacrylate (Eudragit E100)-mesalamine complexes. *Drug delivery, 17*(4), 208-213.

Rasmussen, S., Bondesen, S., Hvidberg, E., Hansen, S. H., Binder, V., Halskov, S., & Flachs, H. (1982). 5-aminosalicylic acid in a slow-release preparation: bioavailability, plasma level, and excretion in humans. *Gastroenterology, 83*(5), 1062-1070.

Romeo, H. E., Hoppe, C. E., Lopez-Quintela, M. A., Williams, R. J., Minaberry, Y., & Jobbágy, M. (2012). Directional freezing of liquid crystalline systems: from silver nanowire/PVA aqueous dispersions to highly ordered and electrically conductive macroporous scaffolds. *Journal of Materials Chemistry, 22*(18), 9195-9201.

Sagiri, S. S., Kasiviswanathan, U., Shaw, G. S., Singh, M., Anis, A., & Pal, K. (2016). Effect of sorbitan monostearate concentration on the thermal, mechanical and drug release properties of oleogels. *Korean Journal of Chemical Engineering, 33*(5), 1720-1727.

Sharma, N., Sharma, A., Bhatnagar, A., Nishad, D., Karwasra, R., Khanna, K., Sharmaa, D., Kumar, N., Jain, G. K. (2019). Novel gum acacia based macroparticles for colon delivery of Mesalazine: Development and gammascintigraphy study. *Journal of Drug Delivery Science and Technology, 54*, 101224.

Soloway, S., & Wilen, S. H. (1952). Improved ferric chloride test for phenols. *Analytical Chemistry*, *24*(6), 979-983.

Stortz, T. A., Zetzl, A. K., Barbut, S., Cattaruzza, A., & Marangoni, A. G. (2012). Edible oleogels in food products to help maximize health benefits and improve nutritional profiles. *Lipid Technology*, *24*(7), 151-154.

Svagan, A. J., Samir, M. A. A., & Berglund, L. A. (2008). Biomimetic foams of high mechanical performance based on nanostructured cell walls reinforced by native cellulose nanofibrils. *Advanced Materials, 20*(7), 1263-1269.

Swe, M. T. H., & Asavapichayont, P. (2018). Effect of silicone oil on the microstructure, gelation and rheological properties of sorbitan monostearate–sesame oil oleogels. *Asian Journal of Pharmaceutical Sciences, 13*(5), 485-497.

Tavares Junior, A. G., de Araújo, J. T. C., Meneguin, A. B., & Chorilli, M. (2020). Characteristics, Properties and Analytical/Bioanalytical Methods of 5-Aminosalicylic Acid: A Review. *Critical Reviews in Analytical Chemistry*, 1-15.

Tanaka, F., & Fukui, N. (2004). The behavior of cellulose molecules in aqueous environments. *Cellulose, 11*(1), 33-38.

Tyagi, P., Lucia, L. A., Hubbe, M. A., & Pal, L. (2019). Nanocellulose-based multilayer barrier coatings for gas, oil, and grease resistance. *Carbohydrate Polymers, 206*, 281-288.

The United States Pharmacopeial Convention, Inc,Rockville, MD(2003), p.*2303*

Wick, A. N., & Joseph, L. (1953). Sorbitan Monostearate Metabolism, Lack of Deposition upon Chronic Feeding. *Journal of Agricultural and Food Chemistry, 1*(5), 398-399.

Yang, S., Li, G., Saleh, A. S., Yang, H., Wang, N., Wang, P., Yue, X., Xiao, Z. (2017). Functional characteristics of oleogel prepared from sunflower oil with β-sitosterol and stearic acid. *Journal of the American Oil Chemists' Society, 94*(9), 1153-1164.

Tables

Table 3.1 Melting point of oleogel with different concentrations of STS

STS Concentration (w/w) (%)	Melting point (°C)
20	35.4 ± 0.2
30	41.9 ± 0.1
40	44.0 ± 0.2
50	49.5 ± 0.6
60	50.7 ± 0.2
70	52.5 ± 0.7
80	53.4 ± 0.1

All values are expressed as mean ± standard deviation, *n*=3

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Table 3.2 Hardness of the oleogel (60% STS) and nanocellulose integrated oleogels (60% STS) encapsulating 5-ASA.

Sample	nanocellulose concentration (w/w%)	Hardness (gram-force)
Oleogel	0	2994±169 [a]
CNC integrated oleogel encapsulating 5-ASA	0.6	3612±372 [abcd]
	0.9	3169±136 [ab]
	1.2	3798±351 [bcd]
	1.5	3396±405 [abc]
CNF integrated oleogel encapsulating5-ASA	0.6	4122±72 [d]
	0.9	4118±538 [d]
	1.2	3609±266 [abcd]
	1.5	4015±19 [cd]

All values are expressed as mean ± standard deviation, *n*=3

Different letters in the same column indicated values are significantly different at p-value ≤ 0.05 using one-way analysis of variance (ANOVA)

Figures

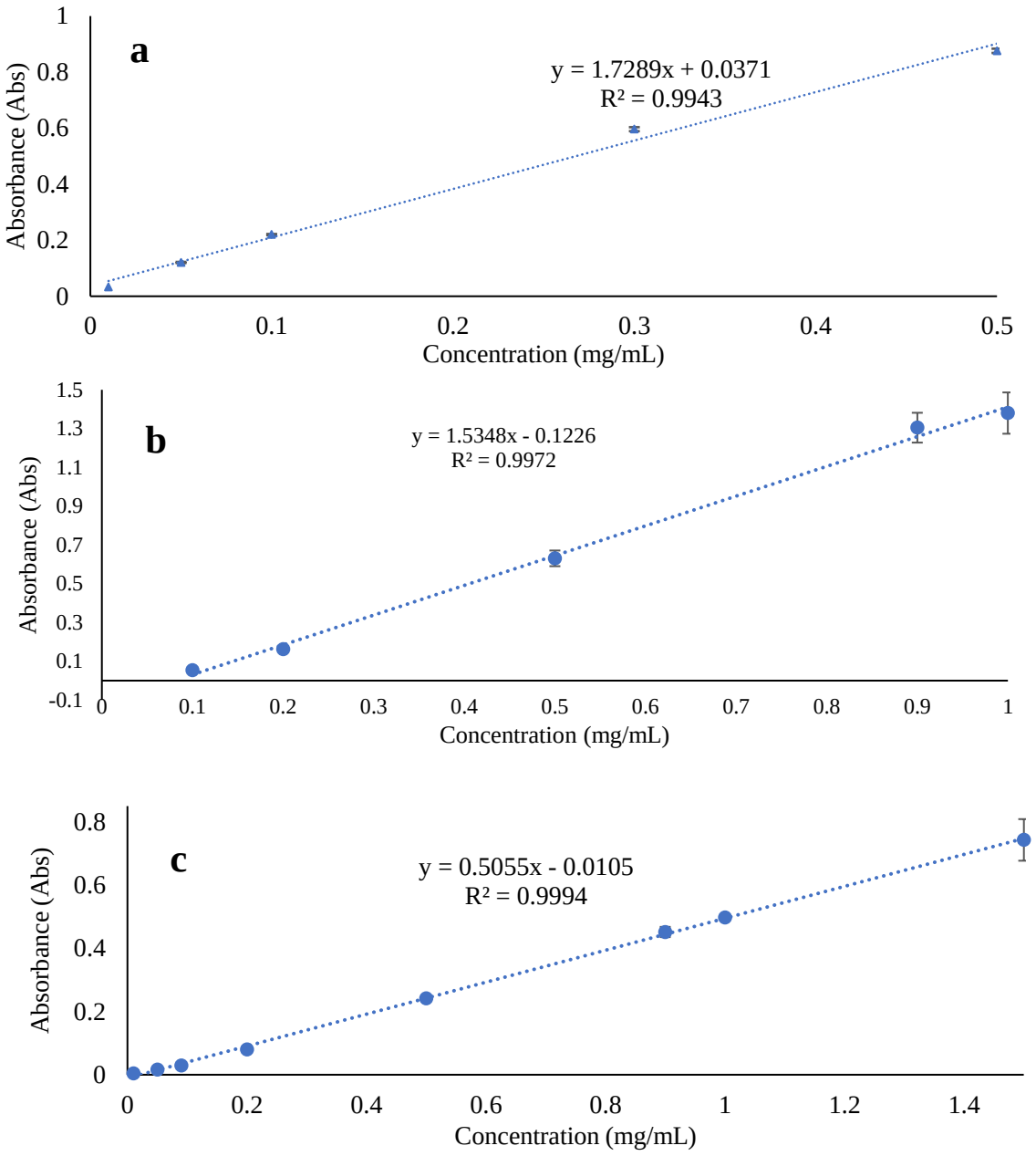

Figure 3.1 Calibration curves for the 5-ASA (a) in the DI water; (b) in the gastric phase; (c) in the intestinal phase. Values are expressed as mean or mean ± standard deviation (error bar), *n*=3

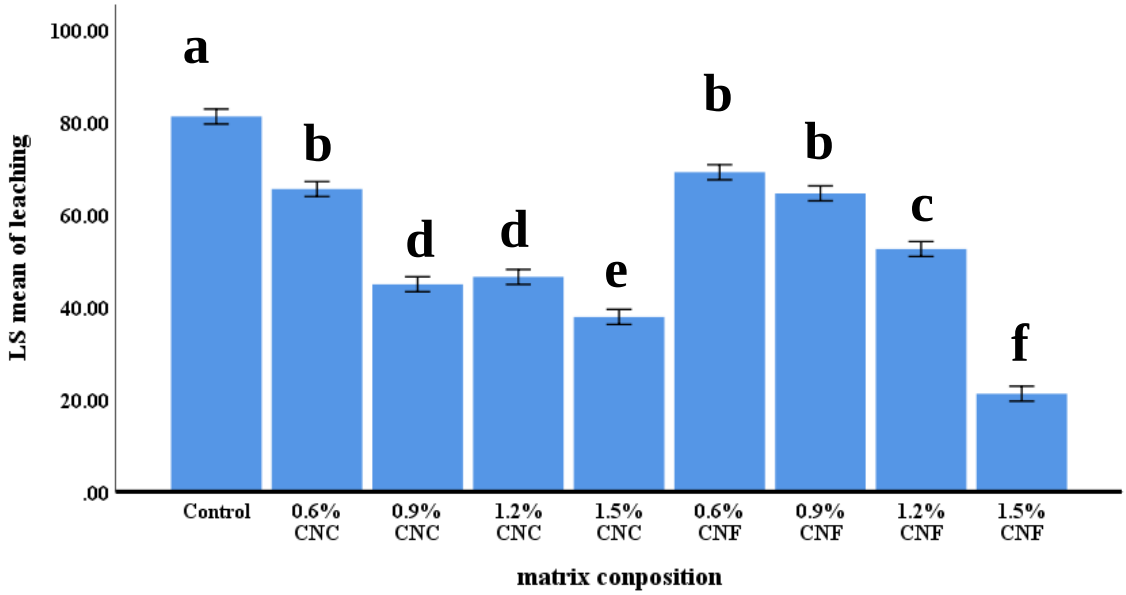

Figure 3.2 The percent oil leaching of 5-ASA encapsulated oleogel (coconut oil and STS), and CNC and CNF incorporated oleogel encapsulating 5-ASA. The data were analyzed using 2-way ANOVA with interactions and expressed as LS means ± standard error of mean (SEM), *n*=3

Different letters indicate significant statistical difference at p-value ≤ 0.05 in Duncan tests

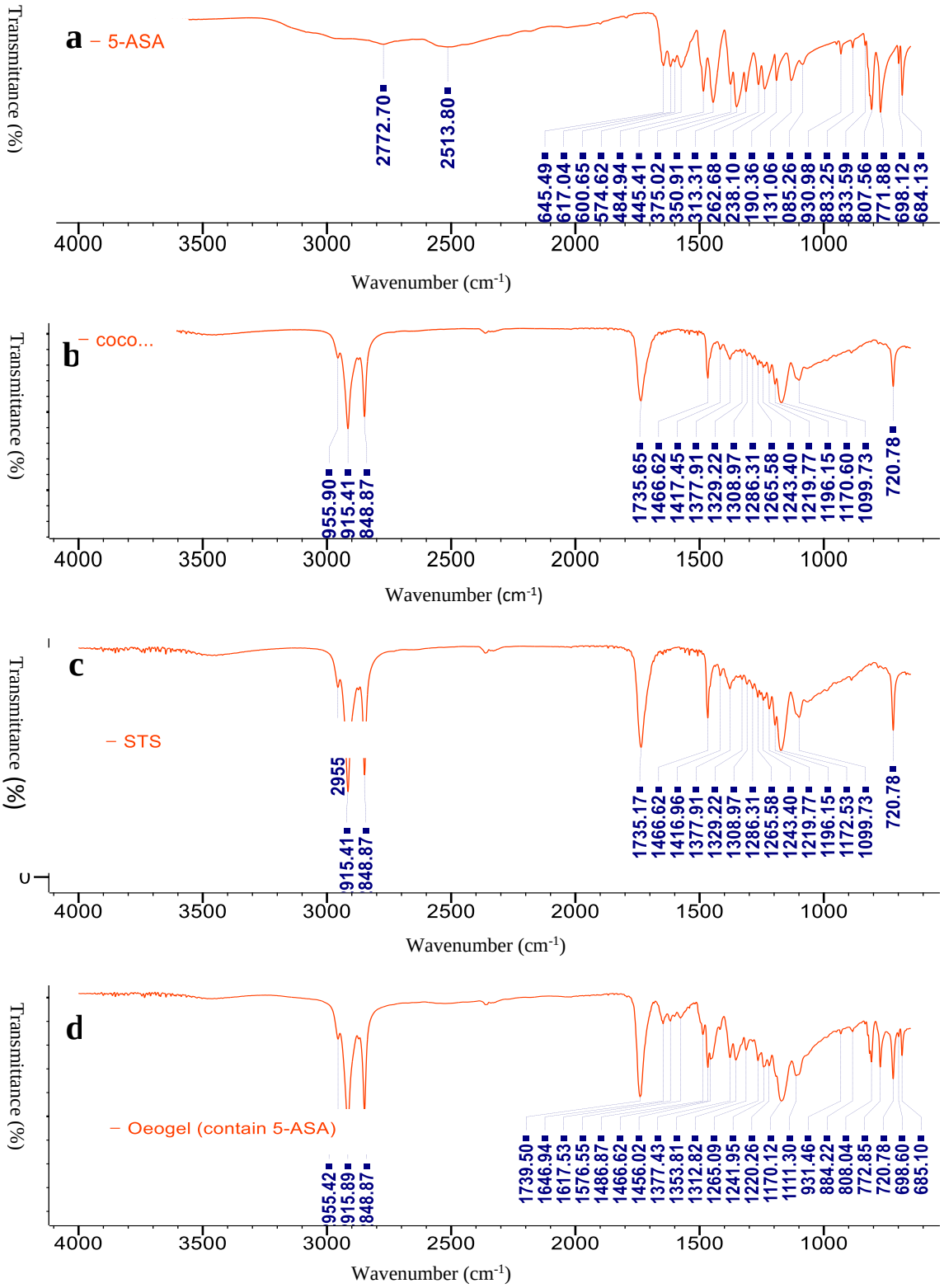

Figure 3.3 FT-IR spectra for a) 5-ASA , b) coconut oil, c) STS and d) oleogel encapsulating 5-ASA

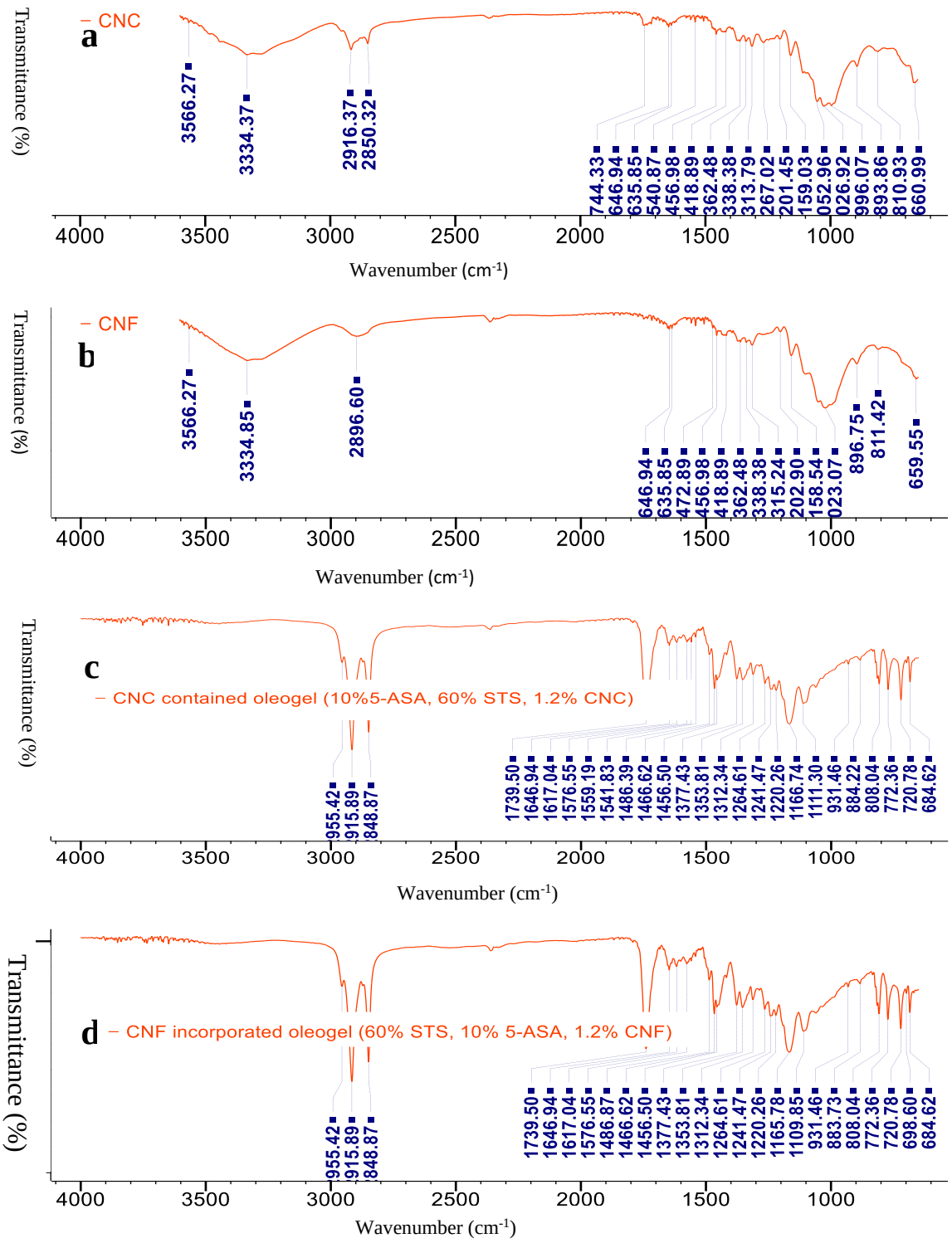

Figure 3.4 FT-IR spectra of 5-ASA encapsulated oleogel containing a) CNC, b) CNF, c) 1.2% CNC and d) 1.2% CNF

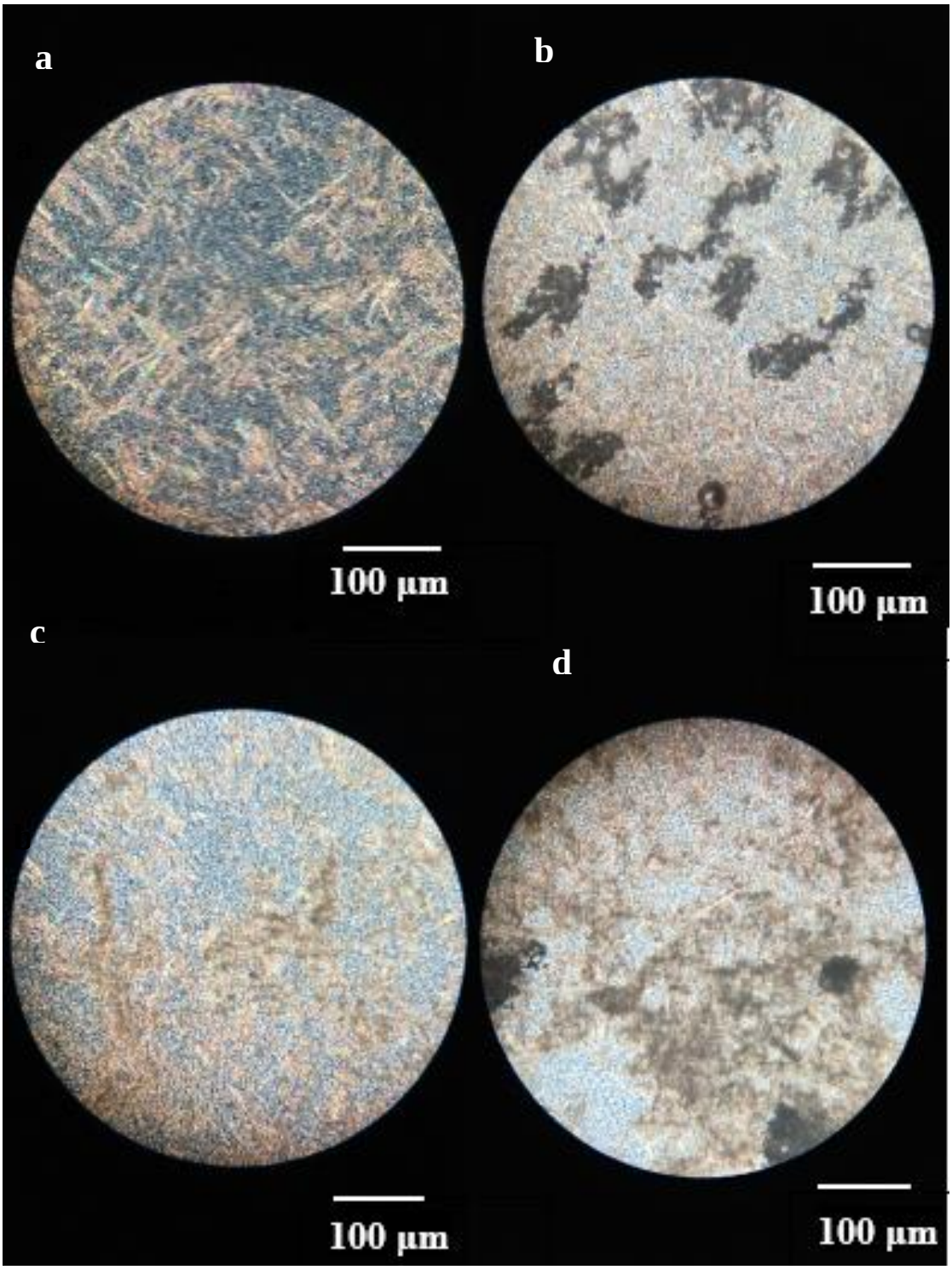

Figure 3.5 Polarized light micrographs of a) bulk oleogel, b) 5-ASA encapsulated oleogel, c) 0.9% CNC incorporated oleogel encapsulating 5-ASA, and d) 0.9% CNF incorporated oleogel encapsulating 5-ASA. Magnification at 400×

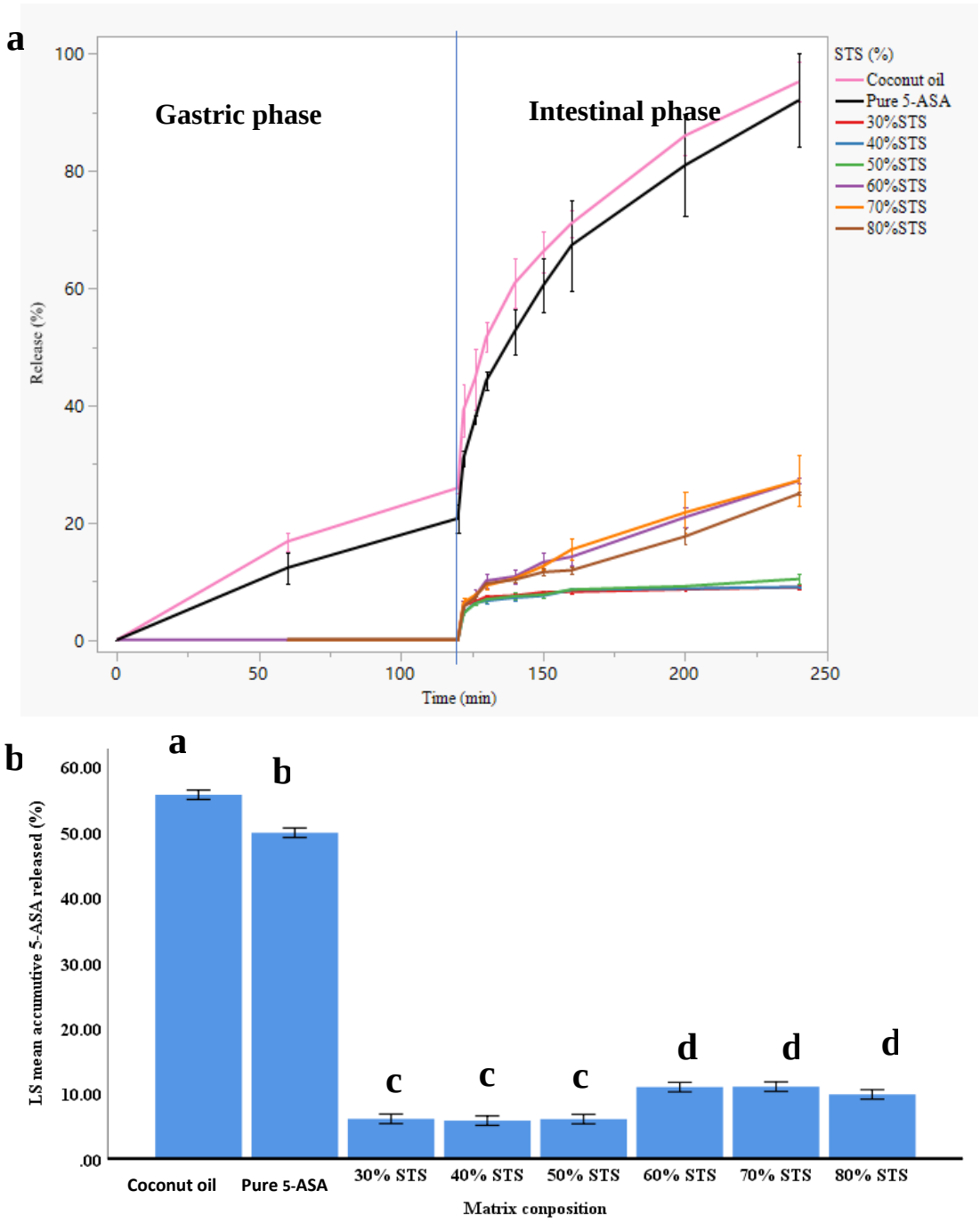

Figure 3.6 Release of 5-ASA during *in vitro* digestion from oleogel with various STS concentrations, coconut oil and control group (unencapsulated) (a) in the gastric and intestinal phase; (b) LS mean accumulative released (%)

All values are expressed as mean ± standard deviation (error bar), *n*=3; different letters indicate significant statistical difference at p-value ≤ 0.05 in Duncan tests

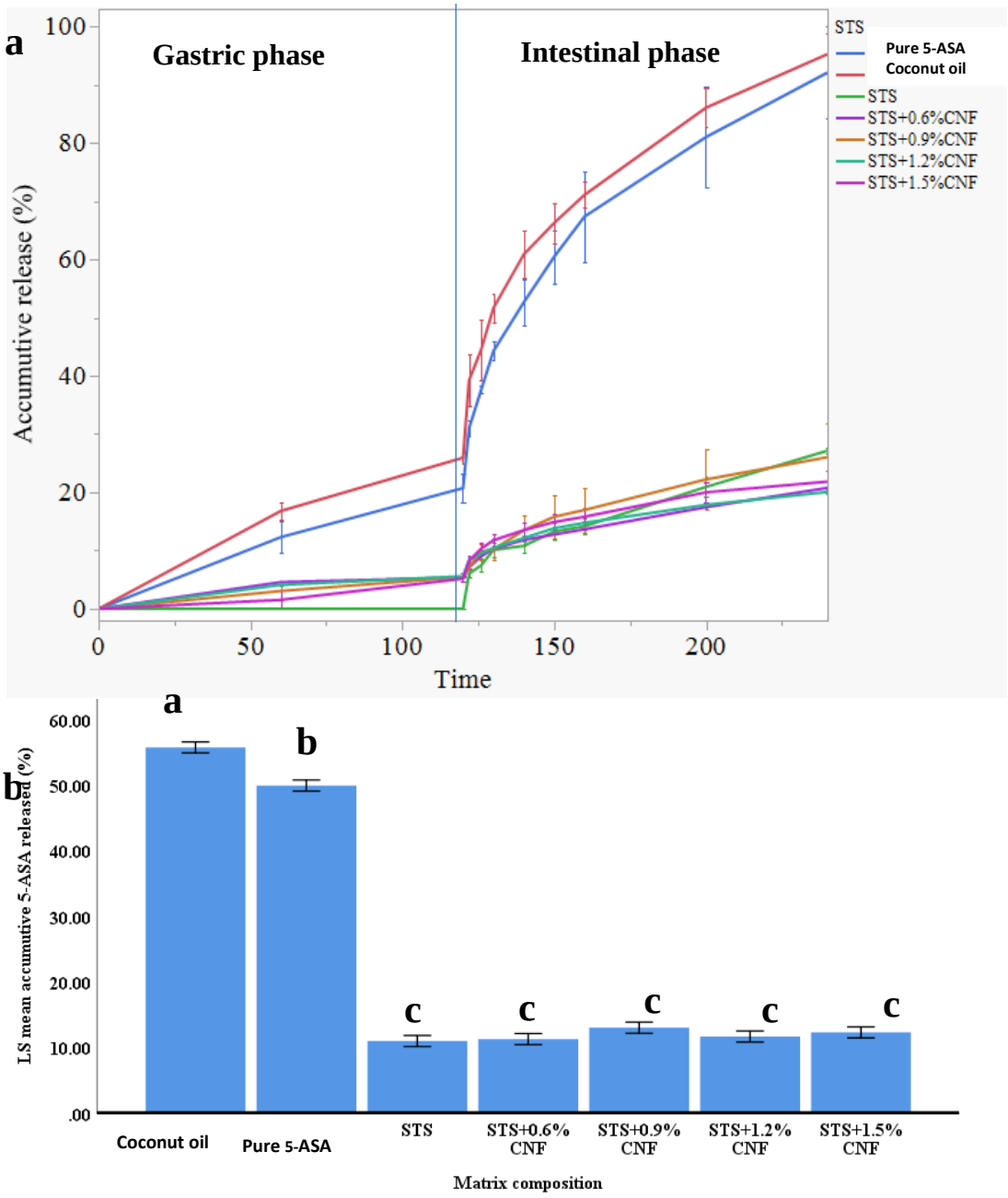

Figure 3.7 Release of 5-ASA from CNF incorporated oleogel, coconut oil and control group (unencapsulated) (a) in the gastric and intestinal phase; (b) LS mean accumulative released (%)

All values are expressed as mean ± standard deviation (error bar), *n*=3; different letters indicate significant statistical difference at p-value ≤ 0.05 in Duncan tests

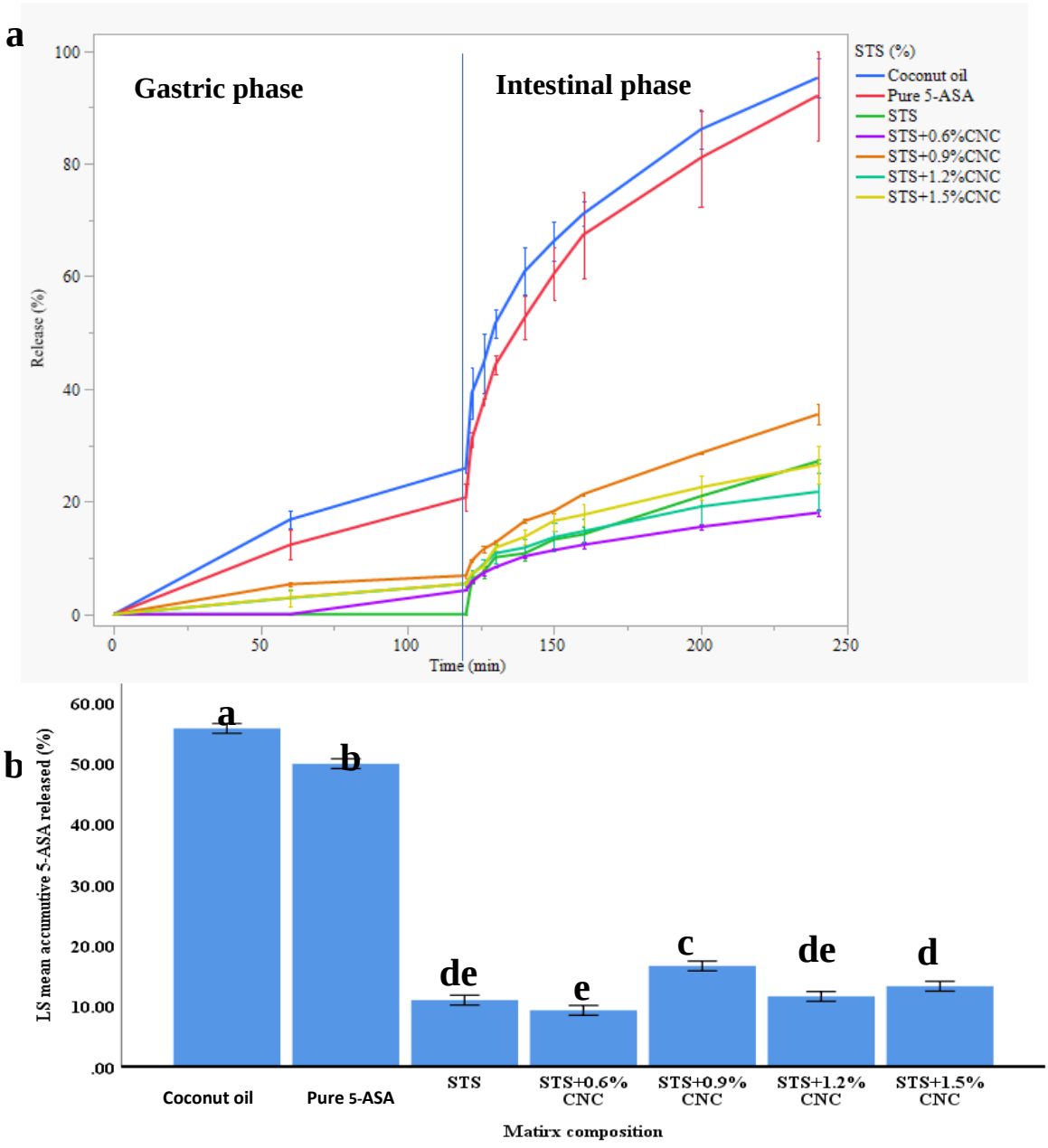

Figure 3.8 Release of 5-ASA from CNC incorporated oleogel, coconut oil, and control group (unencapsulated) (a) in the gastric and intestinal phase; (b) LS mean accumulative released (%)

All values are expressed as mean ± standard deviation (error bar), *n*=3; different letters indicate significant statistical difference at p-value ≤ 0.05 in Duncan tests

CHAPTER 4

CONCLUSIONS

In this study, a novel method for encapsulation of 5-ASA was established with improved colon-targeted release property. The oleogel based method showed high encapsulation efficiency up to 99.4%, and effectively delayed the release of 5-ASA in the gastrointestinal (GI) tract, with less than 30% released in the upper GI tract for most samples. The addition of CNC and CNF as additive materials increased the mechanical strength and reduced the leaching of oil, improving the stability of the product. Additionally, adding CNC at different concentrations to the oleogel significantly affected the release of 5-ASA during the digestion, which indicated that, by adjusting the CNC to oleogel ratio, the release site and rate could be modified. In contrast, the addition of CNF had not changed the 5-ASA release profile significantly due to small testing amount which could probably cover the effect of CNF. Additionally, a new spectroscopy-based method was developed in this study for the quantification of 5-ASA. The method is quick and inexpensive and could be used as an alternative way for 5-ASA measurement.

There are still some limitations existing in this study. First, the concentration range (0.6% -1.5%) of nanocellulose tested was relatively small, which may have limited the influence of nanocellulose in the oleogel, especially for the CNF incorporated oleogel. Second, the static digestion model used in this study oversimplified the conditions and motility of the GI tract, which may have caused an over-or under-estimated 5-ASA release. Third, the results showed that the proposed encapsulation method delayed the 5-

ASA released in the stomach and small intestine and allowed most of the 5-ASA to enter the colon. It is helpful to further examine the release of 5-ASA in the colon to confirm that the 5-ASA could be released from the matrix under the mechanical mixing of colonic lumen and the structural decomposition caused by colonic bacteria. Therefore, future research can include the following topics:

1. Increase the concentration range of nanocellulose added into the oleogel to better understand the effect of nanocellulose on the release of bioactive encapsulated oleogels.
2. Conduct the digestion test with dynamic stomach and small intestine models to obtain more comprehensive information about the bioaccessibility of oleogel encapsulated bioactives.
3. Study the release of the encapsulated 5-ASA in colon using in vivo and/or in vitro methods.

APPENDIX A

List of Abbreviations:

5-ASA:	5-Aminosalicylic Acid
CNC:	Cellulose Nanocrystals
CNF:	Cellulose Fibrils
EE:	Encapsulation efficiency
GI:	Gastrointestinal
IBS:	Irritable Bowel Syndrome
SMP:	Slip Melting Point
STS:	Sorbitan Tristearate
UC:	Ulcerative Colitis