Research and Review of Polymethyl-methacrylate Copolymers in the Development of Enhanced Pharmaceutical Delivery Systems

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Acceptance of Senior Honors Thesis

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Abstract

The following thesis shall primarily explore the current research regarding the implementation of modifiable microscopic and nanoscopic polymer particles as novel pharmaceutical delivery systems. Polymethacrylate-based copolymers, such as Eudragit™, are sensitive to alterations in pH levels, becoming increasingly more soluble in response to an increase in pH. The selective solubility and modifiable nature of these polymers allows for greater flexibility of treatment options for patients (Patra et al., 2017). The wide range of functionality of polymethacrylate (PMA) may provide solutions to challenges relating to the current treatment and therapy of certain conditions. Research indicates that PMA based micro- and nanoparticle polymer excipients may preserve oral insulin in the treatment of diabetes, act as superior carriers for the therapy of colonic cancer, and target colonic regions in patients for stable, sustained release of medication in the treatment of IBD and its symptoms (Bettencourt & Almeida, 2015). Additionally, an overview of the research performed at Liberty University's Department of Biology and Chemistry in this field under the direction of Michael Korn, Ph. D will be reviewed. The research focused on the experimental activities relating to the synthesis and analysis of the basic characteristics of Eudragit S100 polymer microspheres. Specifically, the constructed microdroplets were visualized via fluorescent microscopy in an attempt to develop microscopic polymeric particles that could be compared to those being developed in industry.

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Introduction to Polymer-Based Delivery Systems

The development of polymeric excipients has greatly advanced therapeutic options and evolved solid oral pharmaceutical delivery systems. Polymeric materials develop into ideal capsules and delivery systems based on the consistency of their organic chemical structure and their activity. Polymers are long chains of repeating monomers at determined length and architecture dependent on method preparation: solvent, pressure, and amount of material present impact polymer synthesis (Liechty et al., 2010). The variable sizes and structures of the polymer and chemical properties of each monomer allow for incorporation of bioactive agents, using the polymer as a carrier. Polymer chains can be synthesized in various structures, but the gross architecture typically assembles in three forms: linear, branched, and crosslinked as seen in Figure 1 (Brady et al., 2017).

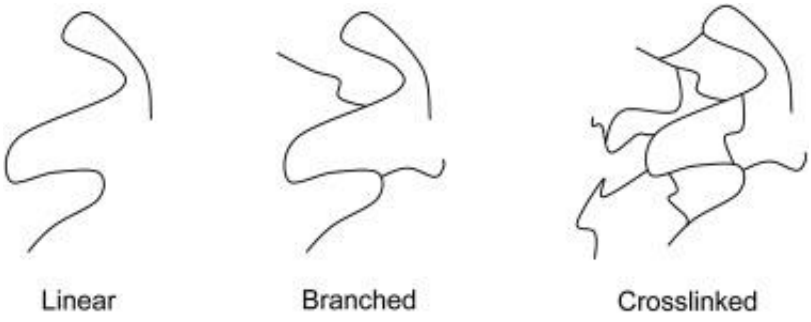

Figure 1: General architectural types of polymer chains (Brady et al., 2017).

Of the three architectural forms, cross-linked polymers chains have been relied on heavily in the development of an excipient due to their ability to form three-dimensional conformations that pack densely (Umar et al., 2015). Dense packing of three-dimensional polymeric systems are optimal carriers for bio-reactive agents, enhancing protection of bio-active agents on the interior

of the polymeric nanoscopic and microscopic microparticle. The polymer can be synthesized with acid-based systems and select acrylic-acid based systems, such as Eudragit. The advantage provided by utilizing Eudragit is due to its nature to swell and condense (Figure 2.) in response to an increase in pH.

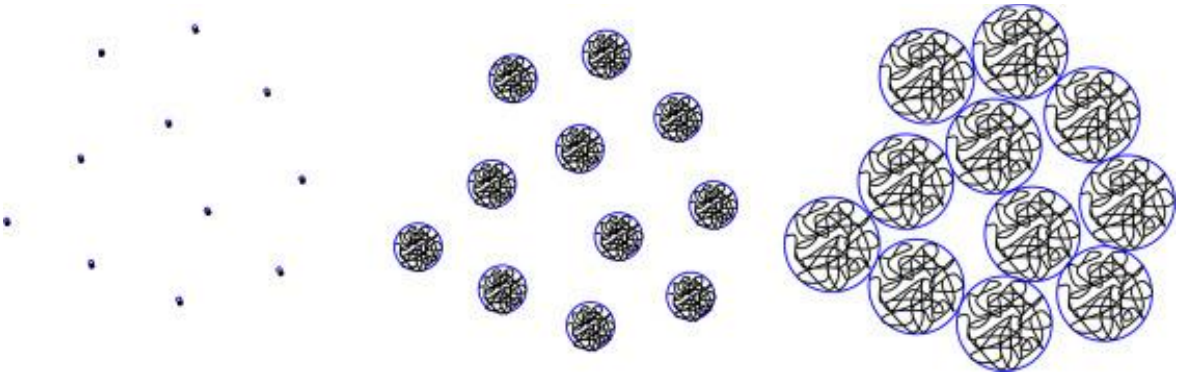

Figure 2: Illustration of progressively reduced condensation at higher pH. The figure demonstrates a progression of how increasing pH from acidic to basic levels impact the physical response of a Eudragit system to a progressively more basic environment. The Eudragit microparticle is densely packed in a low pH environment. As it continues further into a more basic environment, it expands and becomes increasingly more permeable (Brady et al., 2017).

The ability to synthesize polymeric carriers that interact with their environment in a consistently selective manner indicates potential suitably in the delivery of pharmaceutical payloads. Synthesizing polymer systems provide for the incorporation of other therapeutics and superior biodegradation helps to improve reduce excessive accumulation. A new expansion to drug delivery in relation to these polymer systems is that of microscopic or nanoscopic encapsulation, which entails a process by which a particle(s) can be surrounded by polymeric material to form a shell (Ahangaran et al., 2019). This shell can be developed with different morphologies in mind depending on the technique implemented. The ability influences this morphology provides further benefit, giving access to a wider variety of polymer shells that possess their own

characteristics and qualities. Notable morphological structures include mononuclear (basic shell), multiwall, matrix, and polynuclear as seen in Figure 3.

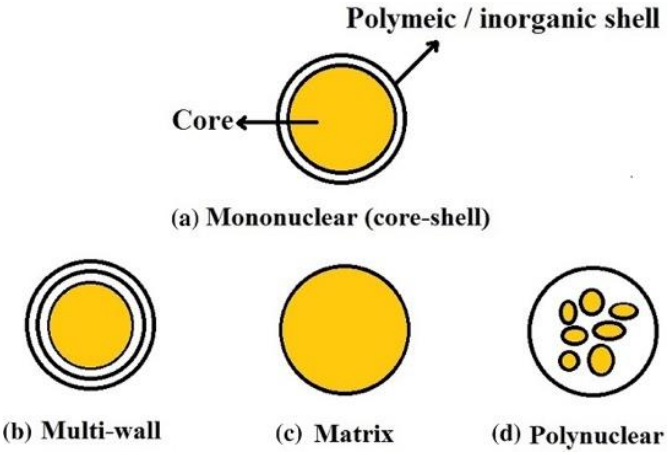

Figure 3: Micro/Nanoparticle general morphology illustrated and classified as four groups: mononuclear, multiwall, matric, and polynuclear. Each particle can be developed into a capsule with implementation of select bioactive agents, which are embedded in the core (yellow area). Each group possesses its own physical characteristics, but the matrix system (c) is of particular interest due to its implementation in industry as being of low-cost and relative ease to which it can be scaled up (Ahangaran et al., 2019).

The purpose of the encapsulation technique is to preserve the bioactive agents form environmental conditions and interfering agents, such as pH, oxygen, and light.

Poly-methacrylate Copolymer

Polymethyl-methacrylate (PMMA) copolymers are of special interest and will be the focus of this review. PMMA has demonstrated its potential to act as a drug carrier based on its biocompatibility, functionality, nontoxicity, and low cost. PMMA polymers are based within a broader family of acrylic polymers, which are typically prepared by free-radical polymerization to develop synthetic, linear copolymers (Figure 4).

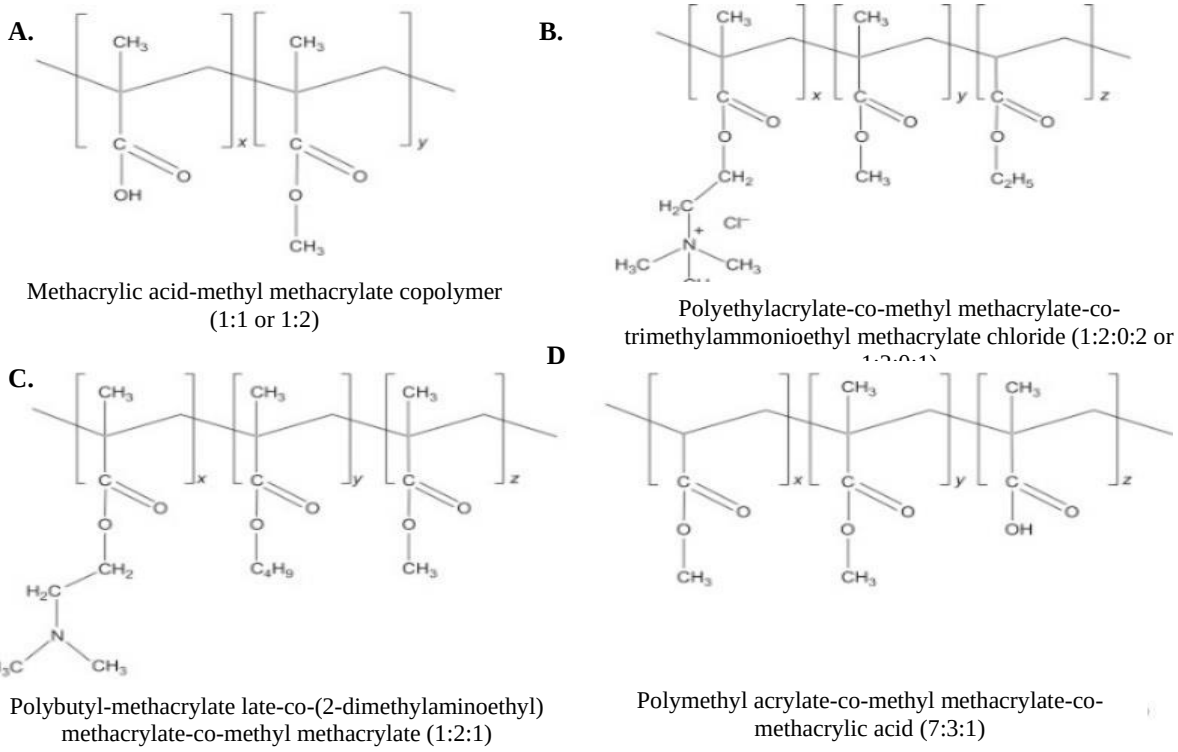

Figure 4: An illustration of generalized acrylic polymers. **A.** Methacrylic acid-methyl methacrylate copolymer (1:1 or 1:2). **B**. Polyethylacrylate-co-methyl methacrylate-co-trimethylammonioethyl methacrylate chloride (1:2:0:2 or 1:2:0:1). **C.** Polybutyl methacrylate late-co-(2-dimethylaminoethyl) methacrylate-co-methyl methacrylate (1:2:1) **D.** Polymethyl acrylate-co-methyl methacrylate-co-methacrylic acid (7:3:1) (Brady et al., 2017).

PMMA polymers can exist in neutral, cationic, and anionic forms depending upon the initial monomer utilized (Umar et al., 2017). The ability to synthesize a polymer with two or more different monomers is referred to as a copolymer. The diversity of various copolymers that can be synthesized and refined to various polymer forms increases the functionality of the polymeric excipients. These various forms contribute to the overall characteristics of the polymer and influence its response to varying environments and interactions with other molecules. The anionic polymer contains acidic functional groups, which dissociate at higher pH and become increasingly more soluble reflective of that increase in pH. At more basic pH levels, acidic groups will lose acidic protons, forming an anionic proelectrolytes which are more soluble than

the uncharged form maintained at low pH. The cationic polymer contains more basic residues, typically swelling and dissociating at low pH values and condensing at higher pH values. At low pH levels, the basic residues can be protonated, ultimately forming a cationic proelectrolytes which is more soluble than the uncharged form maintained at high pH. Neutral polymers do not contain ionic forms and expand in media regardless of pH; however, they do not often dissolve. In efforts to encapsulate materials, a matrix consisting of PMMA is particularly effective due to its thermoplastic properties. The thermoplastic properties of PMMA are characterized by softness and ease of molding at high temperatures, but its hardness and solidity upon cooling (Liechty et al., 2010). The PMMA shell is typically synthesized via suspension and emulsion techniques. Its final structure appears more amorphous due to the presence of the methyl group on its backbone, which inhibits extremely close packing. PMMA polymers possess several other specific qualities that indicate its mechanical suitability as capsule material, including high values of chemical stability, a high Young's modulus, and low elongation at break (Ahangaran et al., 2019).

Emulsion Encapsulation Techniques

Polymer microparticles of PMMA are often constructed with emulsifying techniques. Emulsion polymerization can be used to encapsulate materials with more hydrophobic residues due to association with the polymer. The basic method of encapsulation with emulsion polymerization requires methyl-methacrylate acid (MMA) (Figure 4.A) and hydrophobic molecules/compound to load into the core of the PMMA microparticle. The materials are added with a polar solvent and an emulsifier with high-speed stirring (Shannaq & Farid, 2015). As the MMA begins to polymerize, it will become increasingly more immiscible and separate from the water alongside the hydrophobic material (Figure 5).

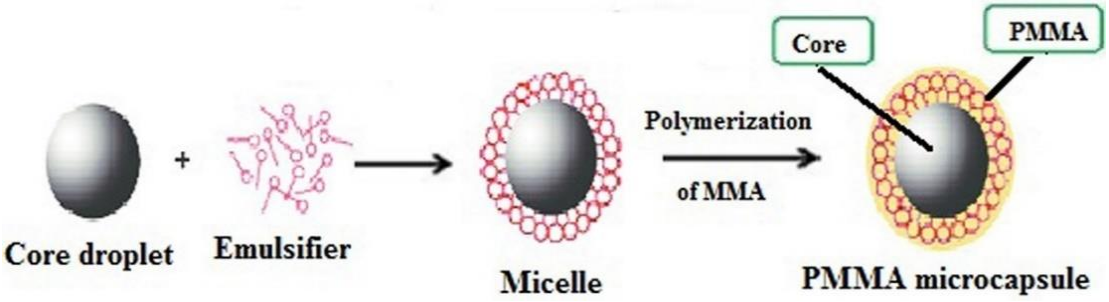

Figure 5: A general scheme of PMMA encapsulation of a material using an emulsion polymerization method. The core droplet contains a hydrophobic material and the MMA. The droplet is added to an aqueous phase that already contains an emulsifier during high-speed stirring, forming a micelle. The MMA begins to polymerize, and the growing polymer becomes increasingly immiscible and begins to separate from the aqueous phase (Ahangaran et al., 2019).

Another prominent technique is solvent evaporation emulsion, which can encapsulate reactive materials, an important aspect of utilizing the polymer microparticles for drug delivery (Koh & Lee, 2007). This solvent evaporation from the emulsion technique was investigated in the research performed at Liberty University to explore the physical characteristics of size, shape, and density of the generated microparticles. The solvent evaporation technique can be performed from an oil-in-water emulsion for incorporation of hydrophobic agents or water-in-oil-in-water emulsion for incorporation of hydrophilic agents (Ju et al., 2002). The solvent evaporation from the standard oil-in-water emulsion entails the dissolution of the polymer and core material of choice (hydrophobic) in an organic phase brought about by volatile solvent with a low boiling point. The organic phase is added to an aqueous phase that contains an emulsifier, creating the conditions for an oil-in-water emulsion as the solvent begins to evaporate. Evaporation can be influenced by altering conditions of temperature and pressure. As the solvent evaporates, the polymer material in the organic phase associates with the organic material and aqueous phase. This process converts the emulsion to suspension as microcapsules are formed as seen in Figure 6 (Shirin et al., 2011).

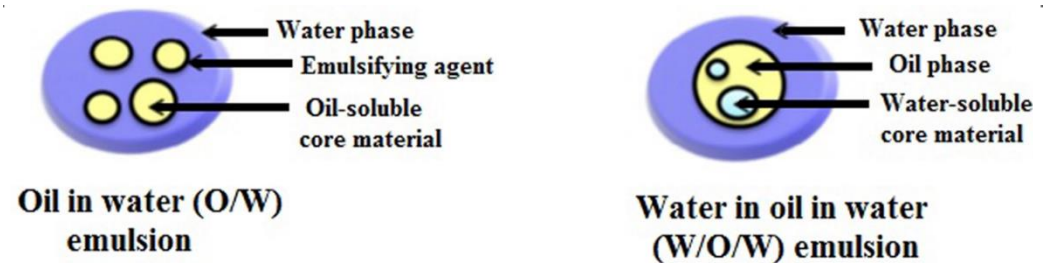

Figure 6: General oil-water (O/W) and water-in-oil-in-water (W/OW) emulsification systems illustrated. This method relies upon solvent evaporation to aid in the formation of micro/nanoparticles: the core material and polymer are dissolved in a volatile solvent to form an organic phase. The organic phase is added to an aqueous phase containing an emulsifier under high stir. As the solvent evaporates and the MMA polymerizes around the core, insoluble micro/nanoparticles are left behind. The W/O/W technique operates similarly, but the hydrophilic agent added is dissolved in an aqueous phase before being added with the polymer in the volatile solvent (Bakry et al., 2016).

Eudragit Polymeric Systems

The PMMA systems described above are industrially recognized under the name of Eudragit™, a registered trademark of Rohm Pharmaceuticals, but currently developed by Evonik Industries. Eudragit includes a wide variety of polymer systems, including copolymers based on methacrylic acid, methacrylic esters, and acrylic esters (Evonik Industries, 2018). These various polymers can be anionic, cationic, or neutral. Eudragit based polymer microparticles provide the necessary flexibility to service a wide variety of biological environments and provide specified, therapeutic drug delivery through simple modification of functional groups (Patra C.N. et al., 2017). Amino-alkyl methacrylate copolymers residue triggers immediate release, methacrylic acid copolymers are implemented for a delayed release, and methacrylic ester copolymers can be time-controlled (Figure 7).

Figure 7: The addition of various functional groups can greatly impact the activity and action of the Eudragit polymer (Evonik Industries, 2018).

Polymer chemistry for the Eudragit excipients is that of a methacrylate base, which is noted in the previously discussed PMMA polymeric micro- and nanoparticles. The mode of delivery can vary depending on need: the polymeric particles can come in an aqueous form, powder form, or organic solution. Eudragit L100 (1:1) and S100 (1:2) (Figure 4. A) type polymers are designed to remain solid and condensed in low pH environments and gradually increase in solubility corresponding with an increase in pH and can be used in tandem with each other (Figure 8).

Figure 8: Eudragit microparcticle dissolution increases with pH. The rate of this dissolution varies based upon the specific Eudragit product used allowing for enhanced selectivity based upon need. (Evonik Industries, 2018).

Due to their pH sensitivity, Eudragit L100 and S100 polymers are being investigated for their ability to target the more basic areas of the body, such as the gastrointestinal and colonic regions (appx. pH of 5-7). Both the gastrointestinal and colonic regions are difficult to treat therapeutically via an oral route due to the degradative environment of the stomach with its low pH (appx. 2) (Júnior, W. F. D. S. et al., 2017). Eudragit L100 and S100 are copolymers and contain slight variances in the ratio of carboxyl to ester groups (1:1 or 1:2, respectively) (Figure 4) Eudragit L100 and S100 copolymer systems dissolve in pH conditions above 5.5 (Figure 8). Due to the acidic nature of the Eudragit polymers, some bioactive agents are notably incompatible as they react with carboxylic acids or esters found within PMMA and its derivatives. Several non-steroidal anti-inflammatory drugs have been known to interact with the microparticle, including flurbiprofen, ibuprofen, diflunisal, and piroxicam. Another interaction chemically based in electrostatic influence on ammonium groups, which can be present in specific types of Eudragit (particularly RS100 and RL100, which contains ammonium groups) (Nikam V.K. et al., 2011). Bioactive agents with low pK_a values due to carboxylic moiety can negatively affect the ability to disperse the core molecule(s) that provide treatment (Singh, S., Neelam, A. S., & Singla, Y et al., 2015) Further investigation is required to consider at what specific levels of acidity does a molecule begin to interact with the polymer matrix and at what point this becomes problematic.

Application of Eudragit

Applying Eudragit to Oral Insulin

Eudragit polymeric systems have been researched as a potential pharmaceutical excipient for the oral delivery of insulin. Research performed by Raghavendra Mundargi, Vidhya

Rangaswamy, and Tejraj Aminabhavi attempted to test this novel method of insulin delivery. Polymer capsules have previously been tested for their ability to preserve the hormone, insulin from highly acidic regions in the digestive tract and denaturation of proteolytic enzymes. The delivery of insulin, however, is inhibited by poor permeability across biological membranes, due primarily to insulin's high molecular weight. In the following experiment, a pH-sensitive oral delivery system was synthesized from Eudragit S100 and L100 copolymers prepared by solvent evaporation from an emulsion. 500mg of Eudragit L100 was dissolved in methanol (5mL) and dichloromethane (15mL). Human insulin (20mg) was dissolved in HCl (0.5mL, 1M) and then added to the Eudragit L100 solution, homogenized, and stirred in an ice bath. The combined solution was added to a sufficient emulsifier and stirred until solvent evaporation. The shape, size, and surface morphology of the particles was analyzed via scanning electron microscopy (SEM) (Figure 9).

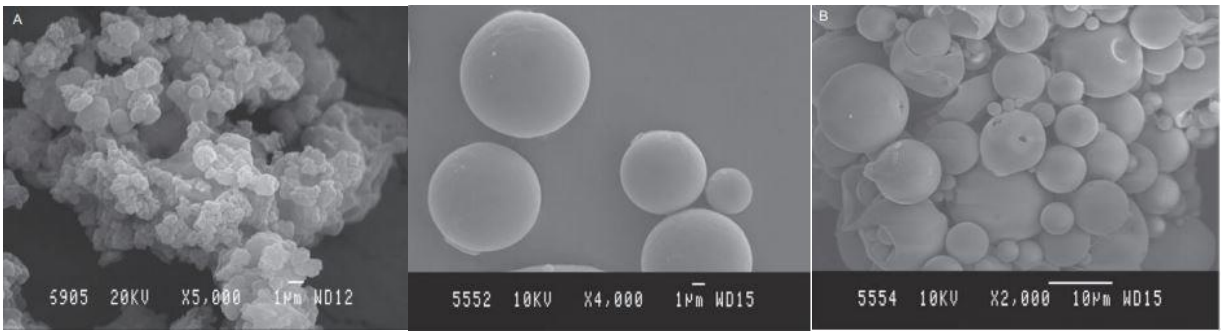

Figure 9: Eudragit microspheres visualized by scanning electron microscopy after synthesis, revealing spherical shapes; smooth surface; and size ranging from 1µm-40µm. (Mundargi R.C., Rangaswamy V., & Aminabhavi T.M., 2011).

Following the physical characterization, the drug loading (%) and encapsulation efficiency (%) was measured by removing 50mg of the microspheres, extracting the insulin with 0.1M HCl (25mL), filtered, and analyzed using high-performance liquid chromatography for content. The

drug loading (%) was calculated by taking the weight of the drug in the microspheres (determined by HPLC) divided by the weight of the microspheres (50mg). The encapsulation efficiency (EE) was determined by taking the drug loading divided by the theoretical loading. Eudragit L100 had an encapsulation efficiency of 52%. The insulin was then loaded into the Eudragit RS100 and L100 (1:1) similar solvent evaporation methods and then tested for encapsulation efficiency, yielding only 26% (EE). The quality of the structural integrity of the released insulin (obtained from extraction to calculate EE) was analyzed by circular dichroism (CD), which is an absorption spectroscopy technique to compare the chirality of one molecule to a native control. In terms of proteins, it is often the secondary structure that is analyzed. The results of the CD can qualitatively and quantitatively indicate whether a protein conformation is close to its native, functional form. In this experiment, the human insulin loaded into the Eudragit L100 microspheres was not substantially different than that of native human insulin (Figure 10).

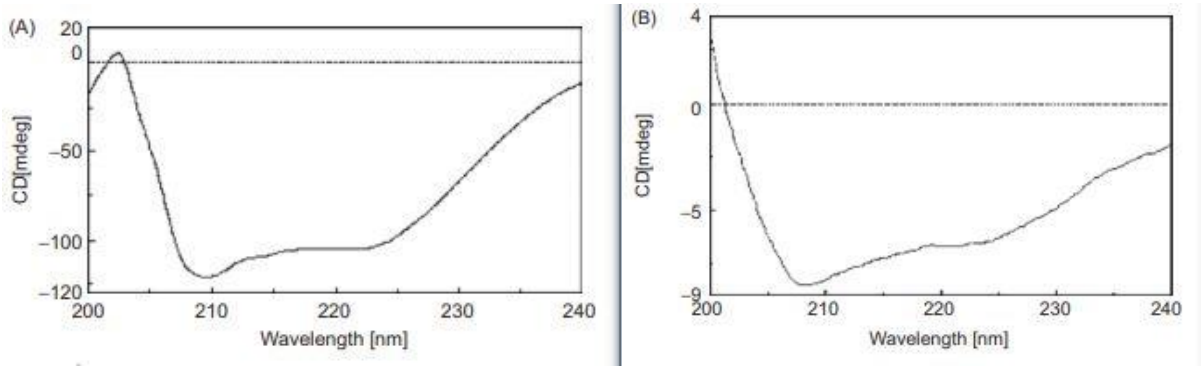

Figure 10: Circular dichroism spectra of native insulin-human (A) and insulin released from Eudragit L100 at pH 7.4 (B). The released insulin does not demonstrate significant differences in the conformation of secondary structure from the native human insulin control (Mundargi R.C., Rangaswamy V., & Aminabhavi T.M., 2011).

The insulin loaded Eudragit L100 and Eudragit L100/RS100 combination microspheres were placed in pH 1.2 and 7.4 (at 37°C) solution buffers. Both sets of microparticles (100mg) were placed in the pH 1.2 buffer before a 2mL aliquot was taken every hour and analyzed for insulin by HPLC. The microparticles were then moved to a 7.4 buffer solution after two hours. Consistent to the hypothesis, Eudragit L100 and the Eudragit L100/RS100 barely released any of the loaded insulin (<9%) in the two hours at pH 1.2. When the buffer solution was changed to 7.4, the microparticles immediately began to release insulin and, within 5 hours, nearly 100% of the insulin was released for both forms of microparticles. The *in vivo* experiment was performed by inducing diabetes on male Wistar rats. Diabetes was brought on by the injection (intravenous) of 150mg/kg of alloxan in saline (0.9%NaCl). After a period of ten days, the rats began to show signs of diabetes with frequent urination, weight loss, and extremely high levels of blood glucose (300mg/dL). The diabetic rats were placed into three groups, each containing six rats. The first group was given placebo polymer microspheres. The second group was given insulin loaded Eudragit L100. The third group was given insulin loaded Eudragit L100/RS100 combination. All groups took the treatment orally (200g). Over a period of 200 minutes, each rodent was subjected to a glucose measurement by providing a drop of blood for a calibrated glucometer. The placebo microspheres of group one did not have any noticeable impact on blood glucose levels. The second group, which was given the Eudragit L100 loaded with insulin, noticed an inhibition level of 86%; the blood glucose level dropped from 455 to 62(mg/dL). The third group was given the combination of Eudragit L100/RS100 and experienced and inhibition of 42%; a drop of 319-258mg/dL (Figure 11).

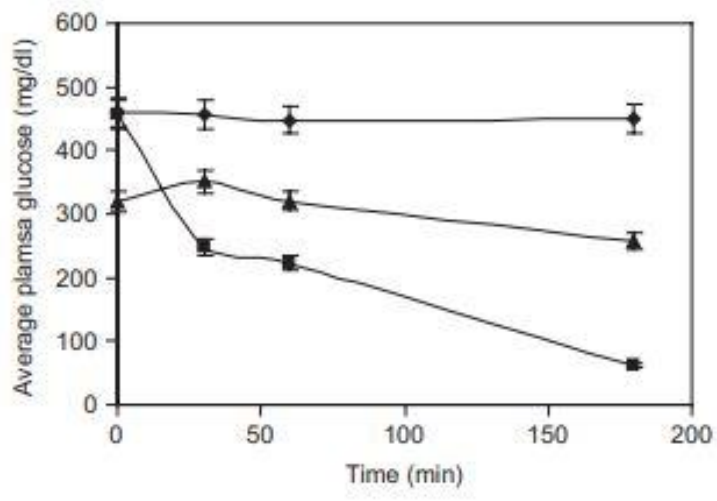

Figure 11: In vivo Results of BGL (blood glucose level) in the Wistar Rats. The (◆) represents the control, (■) represents the Eudragit L100 (20 IU/200g) loaded with insulin, and (▲) represents the Eudragit L100/RS100 (20 IU/200mg) combination (Mundargi R.C., Rangaswamy V., & Aminabhavi T.M., 2011).

Applying Eudragit to Colorectal Cancer

Eudragit polymer microparticles may fill a void in current treatment methods in conventional chemotherapy. The following research was performed by Subudhi, M., et al. Jain, A., Jain, A., Hurkat, P., Shilpi, S., Gulbake, A., and Jain, S (2015). Due to absorption from the intestines and unintended areas of the body, conventional chemotherapy treatments are currently inhibited from reaching the target, the colonic region at reasonable concentrations. The typical bioactive agent used for the treatment of colorectal cancer is 5-fluorouracil (5-FU) (Dangi, R. et al., 2014). Eudragit microparticles make for an ideal delivery system to the colonic region due to their pH sensitivity while traveling the gastrointestinal system. Eudragit S100 (ES) can also be paired with other organic molecules, such as citrus pectin to enhance time-release or target an organ/system more specifically. Citrus pectin belongs to a family of complex polysaccharides and are being studied for their ability to act as biodegradable carriers of pharmaceutical treatment

and can be synthesized into nanoparticle forms similar to that of ES. Citrus pectin was used to target colorectal cancer cells more specifically due to acting as a ligand for galectin-3 receptors, which are overexpressed on colorectal cancer cells (Leclere, L., Cutsem, P. V., & Michiels, C., 2013). The combination of the pH-sensitive Eudragit to survive the gastrointestinal tract and the specificity of the citrus pectin's interaction with colorectal cancer cells is believed to enhance delivery of cancer therapy. In this experiment, citrus pectin nanoparticles (CPNs) were investigated as to their ability to deliver the loaded 5-FU to the colon at high concentrations, avoiding undue absorption prior to reaching the targeted system. The citrus pectin nanoparticles were coated with Eudragit S100 (E-CPNs) to test whether delivery of the loaded 5-FU to the targeted system was enhanced compared to CPNs. Subudhi, M., et al. formulated two basic types of nanospheres: citrus pectin nanoparticles and Eudragit S100 coated citrus pectin nanoparticles; both visualized via transmission electron microscopy (TEM) and scanning electron microscopy (SEM) as seen in Figure 12.

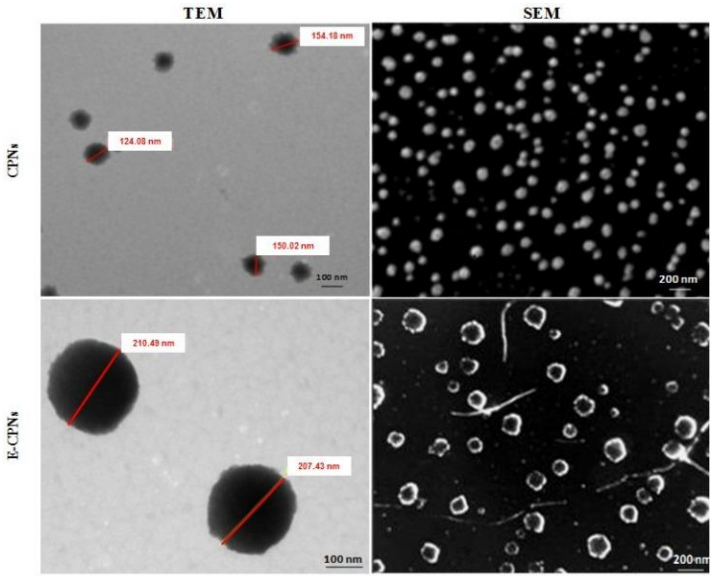

Figure 12: Citrus pectin nanoparticles (CPN) and Eudragit S100 Coated CPN(E-CPN) visualized with TEM and SEM (Subudhi, M. et al., 2015).

The diameter CPN was estimated to be 174.65 ± 5.32nm while the diameter of the E-CPNs was estimated to be 218.12 ± 10.25nm. The E-CPN formulation was prepared with solvent evaporation techniques. Analysis indicated entrapment efficiency to be approximately 35.15% and the drug loading capacity was that of 20.84%. The CPN nanoparticles indicated an entrapment efficiency of 38.75% and drug loading capacity of 21.25%. The GI tract was then mimicked by simulating the fluids and progression of pH and the Eudragit nanoparticles were analyzed to determine responsiveness to the changing pH and monitored for drug release. This *in vitro* process took place over a staggered period of time, allowing the Eudragit nano-polymeric systems to respond in a realistic timeframe. The amount of drug release was evaluated based on UV-spectroscopy, yielding a measurement for anticipated drug release. Over pHs ranging from 1.2-7.0, the Eudragit polymers began to release their payload at approximately the four-hour mark at a pH of 6.8 and continued to release contents of up to 79.24% of its contents at pH of 7.0 and the end of twenty-four period (Figure 13.).

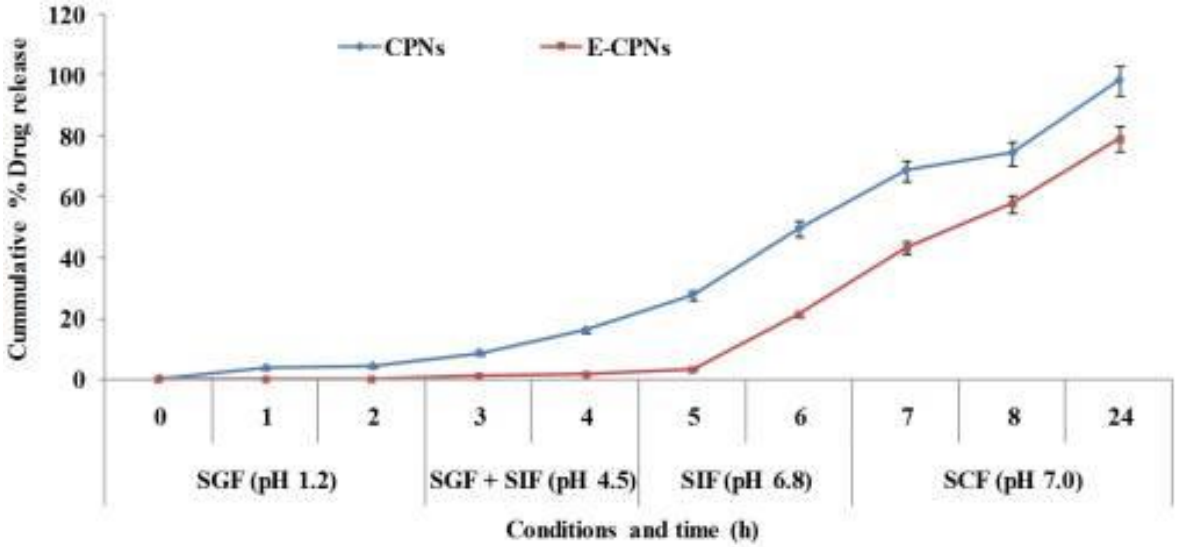

Figure 13: The CPN and E-CPN capsules released 5-FU dependent on pH, simulated environment, and time. GF: Simulated gastric fluid; SIF: Simulated intestinal fluid; SCF: Simulated colonic fluid (Subudhi, M. et al., 2015).

The *in vitro* cytotoxicity was measured as a means of determining the capacity to control growth of cancer cells over increased concentrations of 5-FU. The Eudragit polymers were able to control growth by as much as 90% at a concentration of 5μg/mL to approximately 80% at a concentration of 30μg/mL. Percent growth was determined on a plate-by-plate basis and expressed as a ratio of average absorbance of the test well sample to the average absorbance of a corresponding control well. An *in vivo* study was also performed on Wistar strain rats of both sexes. Four groups of rats were formed; the first group acted as a control and received no drug, the second group received a solution of 5-FU (75mg), group three was given citrus pectin nanoparticles loaded with 5-FU (75mg), and group four received a combination of Eudragit and citrus pectin nanoparticles loaded with 5-FU(75mg). In two-hour increments after administration of the drugs, a rat was sacrificed. Its gastrointestinal tissue was homogenized and treated to be analyzed by HPLC in efforts to record 5-FU recovery at the various time intervals. In both the small intestines and the colonic regions, the Eudragit imbued with citrus pectin nanoparticles had the highest recovery at the eight-hour mark (Figure 14).

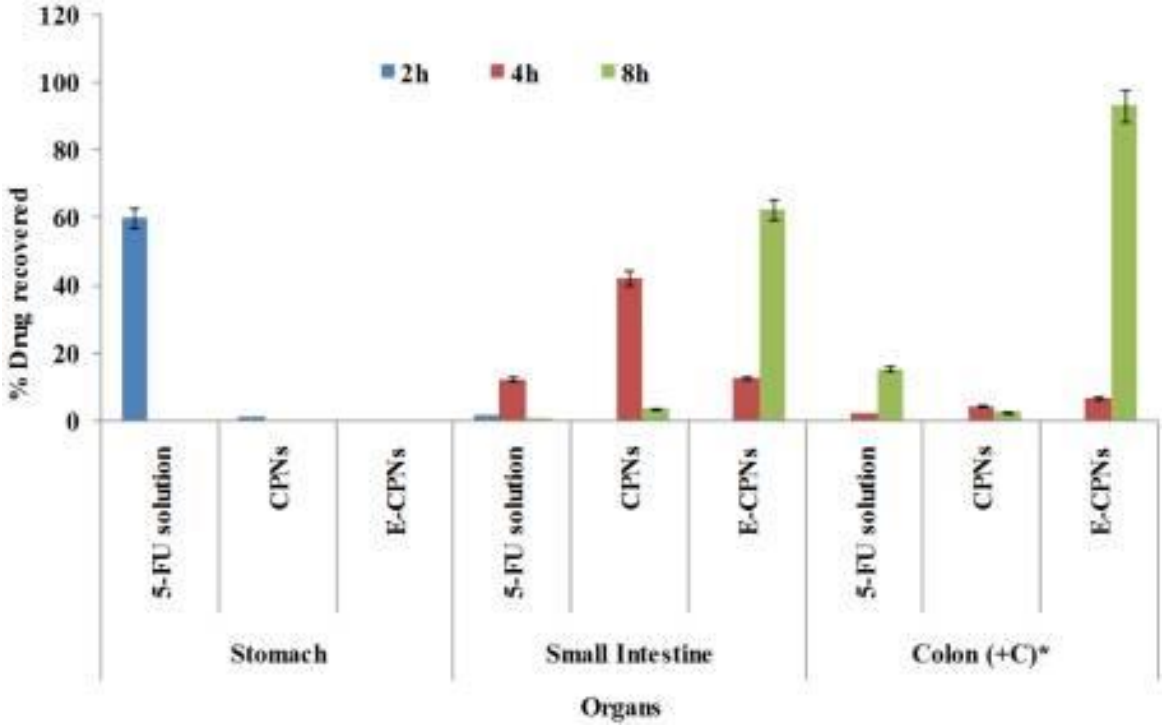

Figure 14: Drug(5-FU) recovery along the gastrointestinal tract in various regions of the gastrointestinal tract is substantially different for each method of delivery. The 5-FU in a 5-FU solution is recovered maximally in the stomach (59.80% ± 4.83%) at the 2-hour mark. The 5-FU loaded in CPNs is recovered maximally in the small intestine (42.10% ± 2.95%) at the 4-hour mark. The 5-FU loaded in E-CPNs was recovered maximally in the colon at the 8-hour mark (Subudhi, M. et al., 2015).

These results indicated that the Eudragit combined with the citrus pectin nanoparticle coating provides an enhanced benefit by maintaining structure until reaching the targeted area, enhancing drug exposure over a longer period of time, making it a potentially viable method for further exploration of cancer treatment.

Applying Eudragit to Colonic Inflammation

Eudragit polymer microparticles may potentially mitigate inflammatory bowel disease (IBD), which is often characterized by ulcerative colitis and Chron's disease Naeem, M., Kim,

W., Cao, J., Jung, Y., & Yoo, J.-W. (2014). studied the potential of Eudragit to provide colonic specific drug delivery. The treatment is based on reducing inflammation to promote mucosal and tissue healing (Naeem M., et al., 2014). IBD provides an added challenge besides the traditional difficulties in providing treatment to specified organs/systems. Due to being subjected to a high frequency of diarrhea, pharmaceutical treatments are eliminated frequently through these constant bouts of diarrhea. To overcome this challenge, not only should the excipient system be able to dissolve in high pH environments, but also in a prolonged manner that can aid despite the frequent bowel movements. Nanoparticles are more easily absorbed by inflamed tissue and mucosa than that of larger systems (Schmidt et al., 2013). Eudragit S100 polymers are sensitive to alterations in pH, dissolving at pH values greater than 7. The ileum, however, promotes an environment that is also above pH values of 7 and may be responsible for premature release and absorption of bioactive agents prior to reaching the targeted colonic region. To enhance targeting, Eudragit S100 was combined with enzyme degradable azo-polyurethane (ES-Azo). The azo-group is reduced by colonic microflora and allows for enhanced targeting when combined with the pH-sensitive Eudragit. This combination was analyzed against ordinary Eudragit S100 (ES) nanoparticles. Coumarin (C-6) was implemented as a hydrophobic model drug given that many of the current treatments for IBD, such as glucocorticoids or immunosuppressants are hydrophobic in nature (Lichtenstein, G.R., et al., 2006). The ES nanoparticles and ES-Azo nanoparticles were developed by an oil in water emulsion/solvent evaporation method. 100mg of ES was dissolved with 2mg of C-6 in 10mL of acetone and ethanol (7:3, v/v). The solution was slowly (0.33 mL/min) injected using a syringe into 40mL of citrate buffer (pH 5.0) that contained 0.1% (w/v) of PVA under constant stir. Upon complete evaporation of the solvent, the nanoparticles were centrifuged at 20,000rpm for half an hour

before being washed with deionized water (3x). The same procedure was performed for the Es-Azo combination, combining 50mg of ES and 50mg of the Azo-polyurethane. The nanoparticle morphology, the structural integrity of the protein, the drug loading, and the entrapment efficiency were measured to determine the validity of the method. The nanoparticles generated were visualized for description of size and morphology via scanning electron microscopy (SEM). (Figure 15).

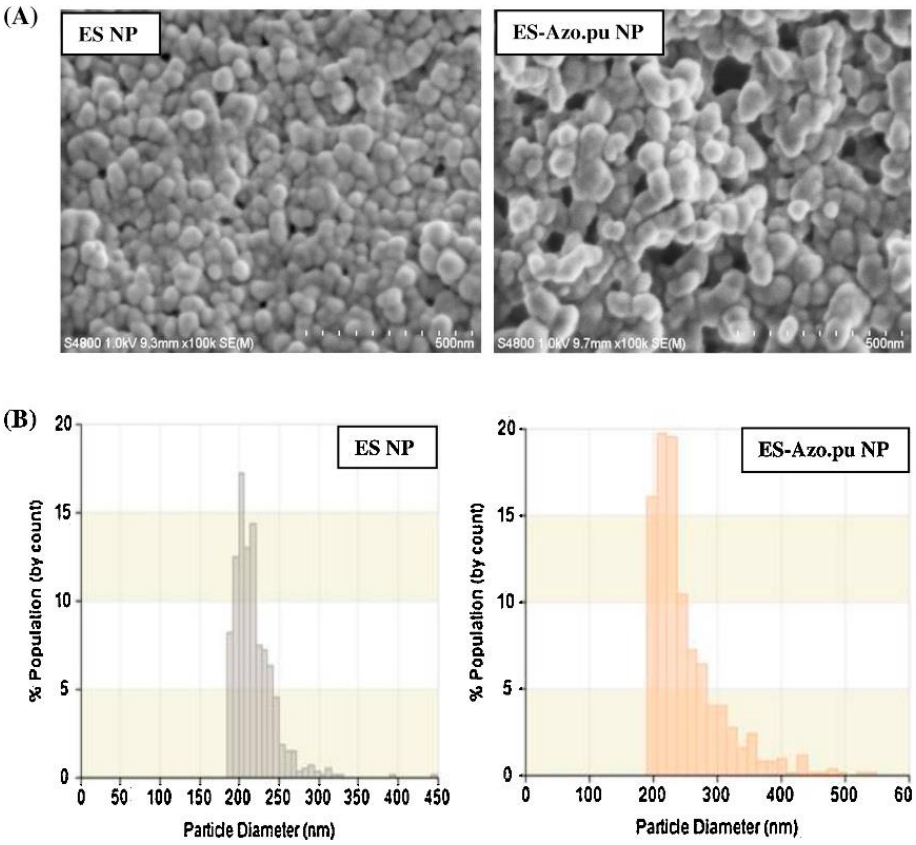

Figure 15: Eudragit S100 nanoparticle (ES-NP) and Eudragit S100 combined with Azo-polyurethane nanoparticles (Es-Azo-NP) characterized by SEM and distributed by size. The ES-NP mean diameter 214 ± 27nm while the mean diameter of the ES-Azo-NP was 244 ± 38nm. (Naeem M., et al., 2014).

Maintaining the structural integrity of bioactive molecules is crucial in developing an excipient system to transport proteins, hormones, and drug molecules to targeted areas. The biomolecule

must reach the intended location without impacting native structure or it may risk reduction of function. The integrity of bioactive structures can be studied via differential scanning calorimetry (DSC), which is a thermoanalytical technique that tests the difference in heat applied and the corresponding changes in the sample's heat capacity. Alterations in heat capacity can signify phase or structural changes, which can reduce or effectively eliminate the functionality of a biomolecule given that function is dependent on structure. The entrapped biomolecule inserted within both forms of nanoparticle (ES and ES-Azo) was able to maintain the native structure and did not experience a phase or structural change when compared to the control, which experienced an alteration of heat capacity at 210°C. The drug loading and entrapment efficiency was determined by using a fluorescence multi-well plate reader. Once C-6 was loaded, an equal mass of both ES and ES-Azo nanoparticles were taken and dissolved in ethanol/dimethyl sulfoxide (1:1). Once dissolved, ES and Es-Azo was transferred to the fluorescence multi-well plate reader and analyzed for C-6 fluorescence intensity emitted at 505nm. The absorbance reflects a concentration that can determine the amount of C-6 available. When comparing the recovered C-6 for the ES and ES-Azo particle to the original mass added, encapsulation efficiency can be determined. The entrapment efficiency for ES and ES-Azo nanoparticles was 52 ± 2.4% and 58 ± 3%, respectively. *In vitro* release for ES nanoparticle and Es-Azo nanoparticle was tested at varying pHs to reflect the change in pH of the gastrointestinal tract and mimic biological exposure time to each environment: pH 1.2 (stomach) for two hours, pH 4 (upper small intestine) for four hours, and pH 7.4 (colon) for at least four hours. SEM was utilized to determine morphological changes to the nanoparticle in response to each pH. The results indicate that no notable morphological changes occur for ES or ES-Azo at pH 1.2 or pH 4.0 (Figure 16), consistent with the pH sensitivity that characterizes Eudragit S100. At pH 7.4,

ES exhibited drastic alteration to its morphology indicative of dissolution and degradation. ES-Azo appeared to swell, but not dissolve or lose its inherent morphological structure at pH of 7.4 (Figure 16). These results indicate that a combination of Eudragit S100 and Azo-polyurethane to be a more suitable nanoparticle than only EudragitS100 in terms of treating colonic inflammation brought about from IBD due to reaching the colon while maintaining particulate form. In treating colonic mucosa, maintaining particulate form has shown to improve bio-adhesion and absorption of treatment, avoiding removal from frequent diarrhea (Lamprecht, A., Schäfer, U. & Lehr, C, 2001).

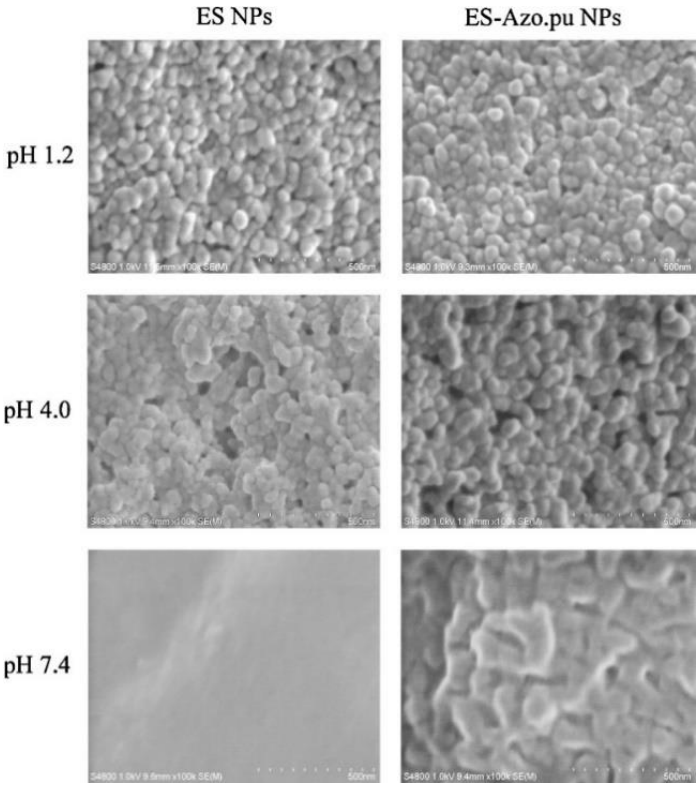

Figure 16: Morphological changes of nanoparticles (ES & ES-Azo) at increasing pH levels: 1.2, 4.0. and 7.4. The alterations of pH environment were introduced to mimic exposure to travel along the gastrointestinal tract. Each SEM was taken after the particles were left in pH 1.2 for two hours, pH 4.0 for four hours and pH 7.4 for at least four hours. (Naeem, et al., 2014).

Aliquots were taken from each pH environment at specified time intervals and analyzed for C-6 concentration via the fluorescent plate reader and compared to the original concentration, resulting in an estimation of C-6 release as pH became increasingly basic and represent travel through the gastrointestinal tract (Figure 17). The ES-Azo-NP released more C-6 early on in the digestive tract. Upon reaching the target pH, the ES-Azo-NPs steadily increased the release of C-6 in a more controlled manner than ES-NPs, which released less C-6 in lower pH environments and nearly all C-6 upon reaching a pH of 7.4. This sudden release of C-6 is indicative of the dissolution of ES-NP at pH 7.4 and its challenge to provide treatment. Given a sudden release, the treatment will not be able to act over a prolonged time period and be quickly voided from the body. ES-Azo-NP demonstrated more controlled release over a longer period of time indicative of a more effective treatment mechanism for colonic inflammation and damage. This experiment was followed by another one, which attempted to determine the impact of colonic microflora on the Es-Azo-NP. Rat cecum(cecal) contents (5%) were added to a PBS solution (pH 5.5) to test whether colonic microflora (also found in the cecum) enhanced the rate of release in ES-Azo-NP compared to the release of ES-Azo-NP release in control PBS solution (pH 5.5) over a period of twenty-four hours (Figure 17). The cecum content solution had significantly more release over the twenty-four hour period than that of the control by approximately double.

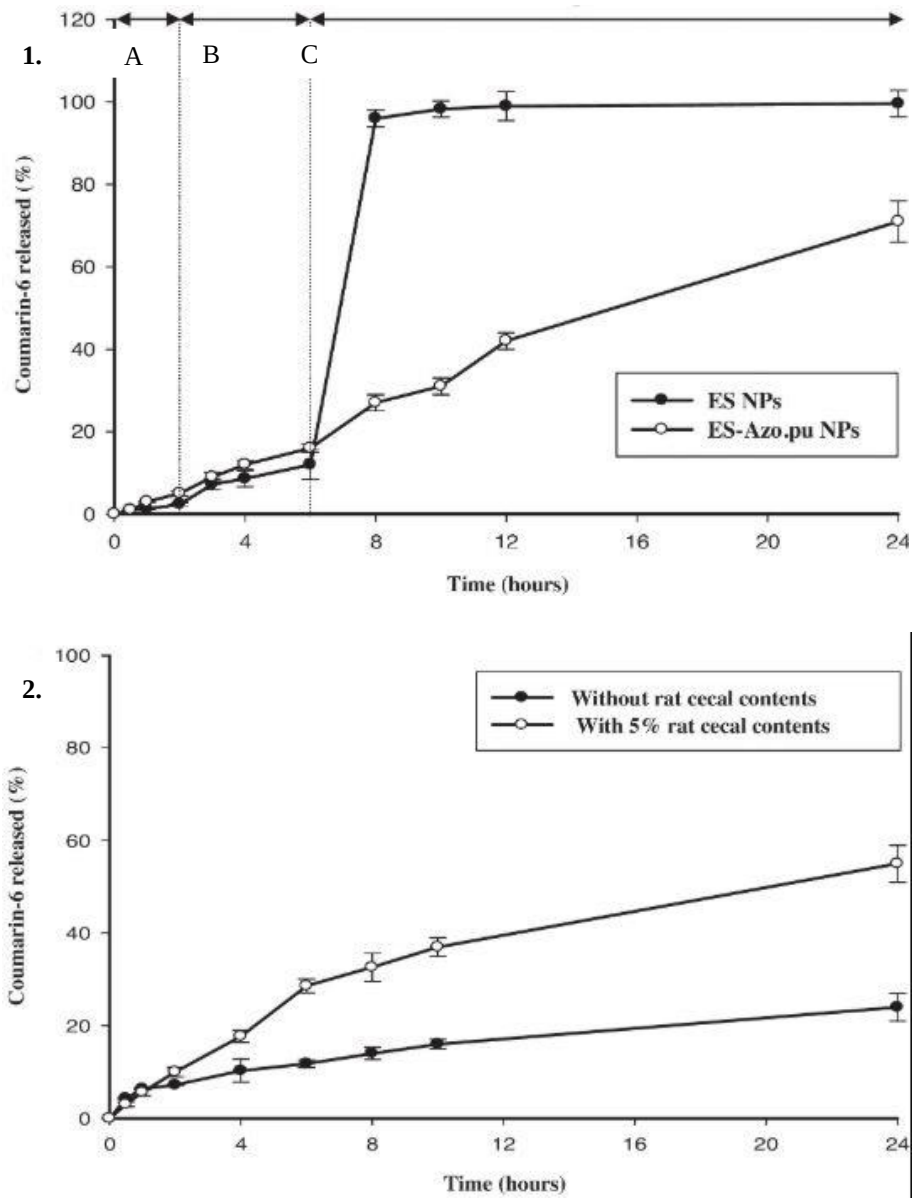

Figure 17: **1**.) ES-NP and Azo-ES-NP coumarin-6 release (%) over a period of twenty-four hours in pH 1.2 (A), pH 4.0 (B), and pH 7.4 (C). **2.**) C-6 release (%) of ES-Azo-NP over a twenty-four hour period at pH 5.5 in presence of rat cecum contents (5%) and without (Naeem M., et al., 2014).

Colitis was introduced to male Sprague-Dawley rats via rectal administration of a hapten solution, 2,4,6-trinitrobenzenesulfonic acid and ethanol (15mg/0.3mL). The *in vivo* localization

of ES and ES-Azo nanoparticles in the gastrointestinal was evaluated utilizing the colitis-induced rats. Three groups were formed; the first group was given orally a C-6 solution (0.15mg/kg C-6/10% ethanol/0.1% Tween-80); the second group was administered orally the C-6 (0.15mg/kg) loaded Eudragit S100 nanoparticles (suspended in PBS, pH 5.0); the third group was given orally the C-6 (0.15mg/kg) loaded ES-Azo combination (suspended in PBS, pH 5.0). After eight hours, the rats were euthanized and the gastrointestinal region and divided into four regions: colon, cecum, small intestine, and stomach. Each region was homogenized and extracted with ethanol/dimethyl sulfoxide (1:1) to determine potential drug recovery via a fluorescent plate reader assay. The C-6 solution was almost entirely absorbed in the gastrointestinal tract, primarily the stomach. Only 20% of the total dose of the total dose remained after the 8-hour period. The Eudragit S100 was also largely absorbed before reaching the targeted colonic region, with a remaining total dose of 32%. Minimal amounts of the C-6 remained for the Eudragit S100 at the ileum and only very small quantities made it to the cecum and colon, 7% and 4% respectively. These minimal amounts suggest the pH-sensitive polymer is not itself suitable for the treatment of IBD and its associated symptoms. The ES-Azo nanoparticle demonstrated a significantly lower absorption than that of the Eudragit S100 with 17% of the amount of C-6 available and unabsorbed in the cecum and 42% being found in the colon after 8-hours, implying a more effective controlled release of treatment rather than premature removal (Figure 18). This data suggests that Eudragit S100 can be successfully modified with azo-polyurethane to enhance of delivery of treatment distant colonic regions in the body.

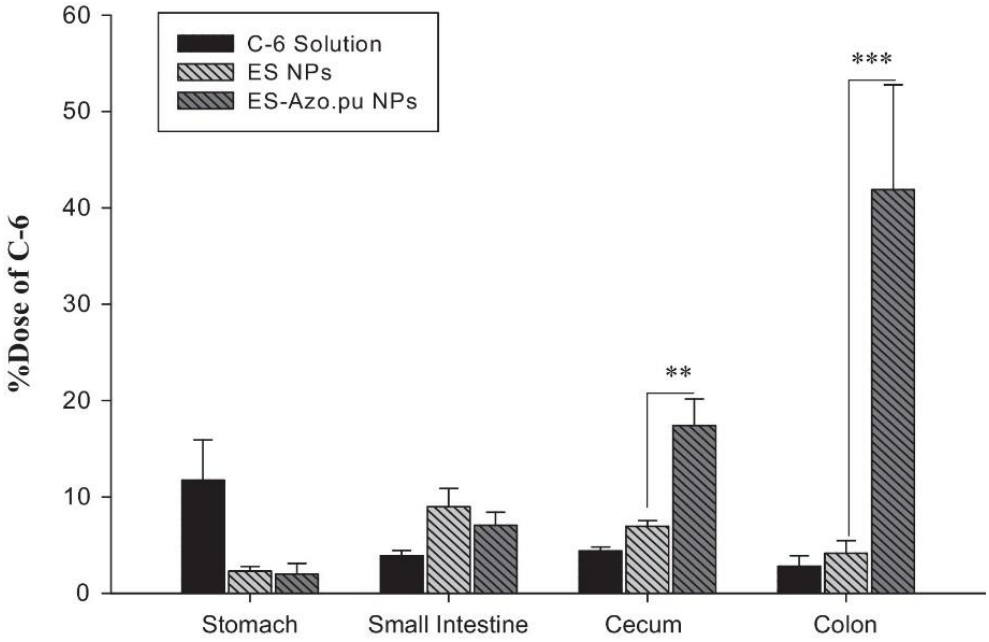

Figure 18: %Dose of C-6 remaining (not absorbed) for C-6 solution, ES-NPs, and ES-Azo-NPs in specific gastrointestinal after eight hours. (Naeem, et al., 2014).

Report on Research Developing Polymeric Microparticles

The purpose of the research performed at Liberty University focused on the development of polymeric microparticles. These microparticles were constructed utilizing similar techniques to industrial synthesis and allowed for analysis of the polymer matrix physical characteristics (Steiert, E. et al., 2018). A primary goal of the research was to develop and adjust methods of synthesizing a tight polymer weave in the form of polymer microspheres that maintain structure in highly acidic environments and dissolve in basic environments. The Eudragit S100 utilized is a polymer based in polymethyl-methacrylate chemical structure (Figure 19). This Eudragit S100 polymer is specifically designed to maintain structure in low pH and dissolve in higher pH as a result of protonation/de-protonation of the chemical structure.

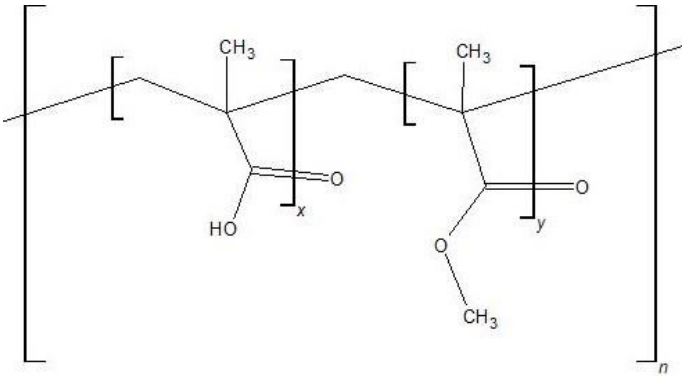

Figure 19: The illustrated molecular structure of Eudragit S100, an anionic copolymer based on methacrylic acid and methyl-methacrylate (1:2) (Drawn via ChemSketch).

Experimental Section

Three types of Eudragit S100 microspheres were generated and named JAD1, JAD2, and JAD3 to indicate the order in which they were synthesized and the initials of the individual responsible for their synthesis. The Eudragit S100 microspheres were developed with a single emulsion solvent evaporation technique. Eudragit S100 (400 mg) was added to 20mL of an immiscible organic solvent and allowed to stir at 1000rpm until the Eudragit S100 completely dissolved. Twenty drops of diH_2O were added to aid in dissolution. diH_2O (40mL) was placed in a separate beaker and mixed with 1mg of fluorescein (for visualization) and 380mg of Tween80, the emulsifying agent, which prevents separation of newly formed droplets from recoalescing. This mixture was added drop-wise to the dissolved Eudragit S100 solution. Another 80mg of Tween80 was added to the combined solution then stirred at 1000rpm for 30 minutes before the final addition of 0.4mL of 1M acetic acid. An aliquot was removed and visualized under both microscope (Figure 20) and the ZOE Fluorescent Cell Imager. JAD1 was left open for a week in order to allow for solvent evaporation. JAD2 and JAD3 implemented a vacuum to aid in removal of the solvent. JAD3 did not include the addition of the 1M acetic acid, was stirred at reduced

speeds of 800rpm, and was heated (35°C) to expedite solvent evaporation. The JAD3 solution was spread out in a water bath to avoid aggregation of the polymer. Previously made particles (CLS1-polymeric particles made by Carson L. Smith the previous year), were cut open via cryostat and visualized via ZOE Fluorescent Cell Imager to examine the physical characteristics of the core of the microparticles (Figure 21).

Results and Discussion

Three iterations of polymeric microparticles were developed: JAD1, JAD2, and JAD3. JAD1 and JAD2 utilized acetic acid, but the results were poor due to extensive loss of product as the polymer hardened and adhered to the sides of the Erlenmeyer flask during stirring. It is hypothesized that this is due to excessive acid, which may harden the polymer too suddenly. The ideal microscopic particles did not consistently form in JAD1 or JAD2; what did not coat the walls of the Erlenmeyer flask would aggregate and adhere together, forming a large polymer mass in solution rather than individual microspheres. Very few microscopic particles were found in solution with the use of the ZOE Fluorescent Cell Imager and much of the product was not recovered from the large aggregated mass. The minimal amount of microparticles recovered revealed much larger diameters than anticipated and undefined shapes. JAD3 was more successful as it seemingly did not lose much product on the walls of the Erlenmeyer and did not aggregate into a large mass in solution (Figure 21). The results of JAD3 were surprising as it did not include the acetic acid, which aids in the hardening of the microspheres. JAD3 developed as fine particles that were visualized via a microscope and ZOE Fluorescent Cell Imager to reveal microspheres approximately 15-50μm in diameter. The fluorescein introduced to polymeric microspheres appeared to accumulate densely on the surface of the microspheres. The core of the spheres appeared significantly less dense under visualization after cleavage with cryostat

techniques and did not show much incorporation of the fluorescent molecule (Figure 21). The results indicate that if cores are significantly less dense, the polymers could be ideal for carrying pharmaceutical payloads. The accumulation of fluorescein on the surface of the matrix may cause concern if other bioactive molecules act similarly and coat the surface of the microsphere rather than become incorporated into the core reliably. Future research ought to explore entrapment efficiency and determine a method to reliably insert a bioactive agent into the core of the microparticle. If the biomolecule is immobilized on the surface of the microspheres rather than placed within the core, it may be exposed to the acidic environment of the stomach or harsh enzymes causing degradation, inhibiting it from having an effect on the targeted biological system/organ. Future research should also test the incorporation of larger biomolecules such as alkaline phosphatase and researching the dissolution properties of the polymer capsule by introducing the microspheres to a variety of pHs.

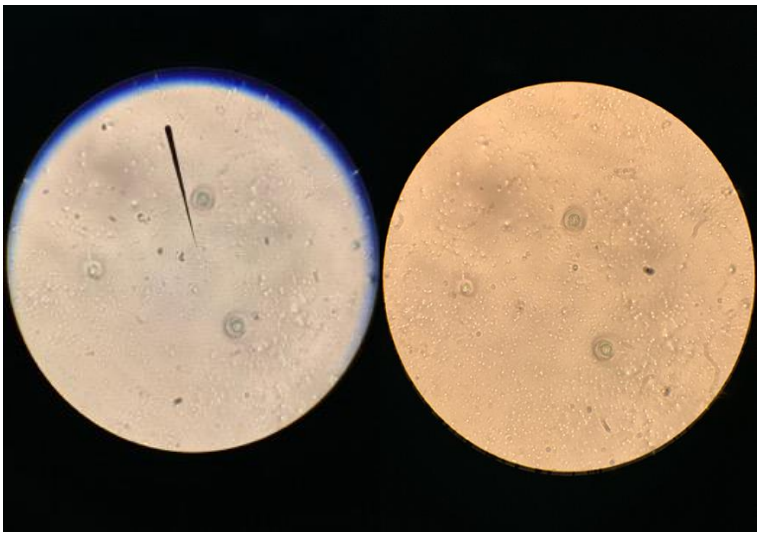

Figure 20: Microscopic visualization of polymer spheres from an aliquot of JAD3 taken after formation (Images taken by J. Dyess).

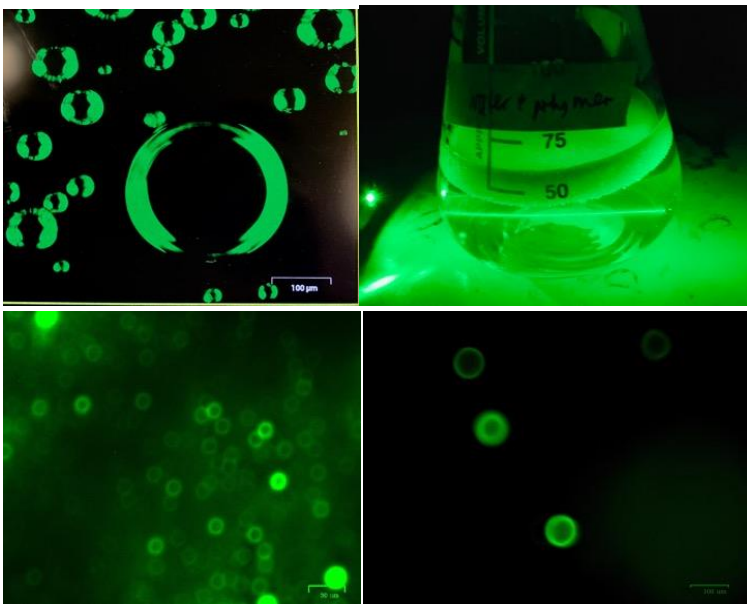

Figure 21: Microspheres Containing Fluorescein at 100µm (upper left) and 30µm (upper right) Magnification: samples were imaged at 488nm using ZOE Fluorescent Cell Imager. Cryostat segment visualized via ZOE (bottom left). Polymer micro-particles scattering light in solution (bottom right) (Images taken by J. Dyess).

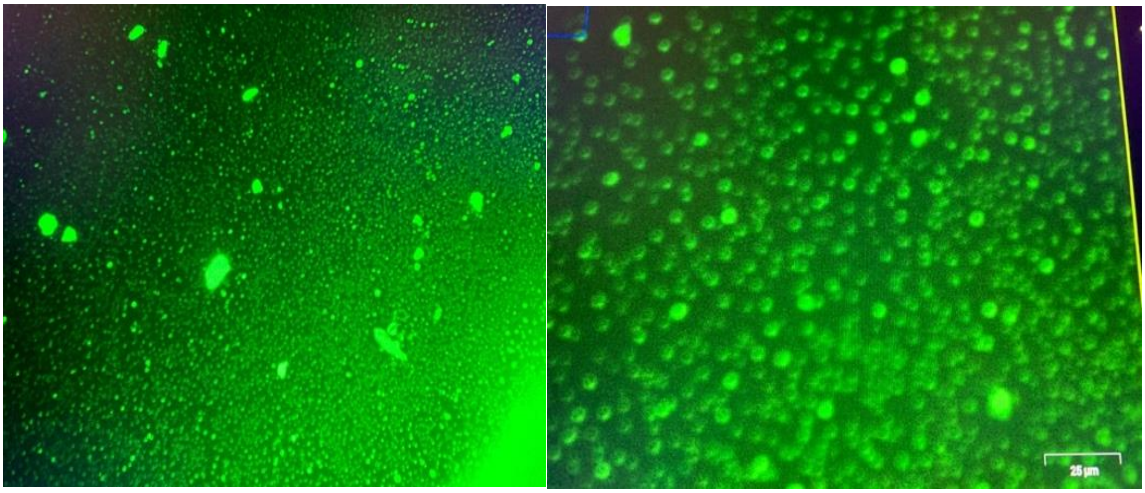

Figure 22: JAD3 microdroplets visualized with ZOE (Images taken by J. Dyess).

Conclusion

Polymeric micro- and nanoparticles are continually advancing medical technology, enhancing prospective benefits for the treatment of patients with an increased capacity to

intelligently target previously challenging biological systems. The flexibility to alter or modify polymer microspheres with the selectivity of monomers allows for intelligent excipients to treat more effectively. Polymethylmethacrylate systems are a prime source of modifiable and effective nano/microspheres for pharmaceutical delivery, most notably under the family of Eudragit polymers. Not only are the techniques required to generate Eudragit microspheres consistently simplistic, but the capacity to modify with functional groups allows for novel and more effective options in treatment for a variety of illnesses such as diabetes, cancer, and irritable bowel syndrome. The research performed at Liberty University explored some of the physical characteristics of Eudragit polymer microspheres and the difference that methods of synthesis have on the characteristics of the microparticle. Further research ought to focus on modifying Eudragit to more easily encapsulate non-steroidal, anti-inflammatory drugs, and other more acidic compounds. Efforts to improve technique and consistency of development of these micro- and nanoparticles is due; the significance of this research may explore novel opportunities in the field of biotechnology.

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