"DEVELOPMENT AND OPTIMIZATION OF LIGAND APPENDED NANO-STRUCTURED LIPID CARRIER SYSTEMS FOR THE TREATMENT OF INFLAMMATORY BOWEL DISEASE"

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IN

PHARMACEUTICAL TECHNOLOGY &

BIOPHARMACEUTICS

BY

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CERTIFICATE

This is to certify that the dissertation work entitled "Development and Optimization of Ligand appended Nano-structured lipid carrier systems for the treatment of Inflammatory Bowel Disease" submitted by Ms. Gurpreet Kaur Sinhmar with Regn. No. (14MPH106) in partial fulfillment for the award of Master of Pharmacy in "Pharmaceutical Technology and Biopharmaceutics" is a bonafide research work carried out by the candidate at the Department of Pharmaceutics, Institute of Pharmacy, Nirma University under our guidance. This work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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DECLARATION

I hereby declare that the dissertation entitled "Development and Optimization of Ligand appended Nano-structured lipid carrier systems for the treatment of Inflammatory Bowel Disease", is based on the original work carried out by me under the guidance of Dr. Mayur M. Patel, Associate Professor, Department of Pharmaceutics, Institute of Pharmacy, Nirma University and Dr.Bhoomika M. Patel, Assistant Professor, Department of Pharmacology. I also affirm that this work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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TABLE OF CONTENTS

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DEDICATED TO
MY
GRANDPARENTS
PARENTS AND
RONAK

"Development and Optimization of Ligand appended Nanostructured lipid carrier systems for the Treatment of Inflammatory bowel disease"

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Inflammatory bowel disease is a cascade of inflammatory conditions marked by aberrations in small intestine and colon. It is a long lasting disease and the treatment includes long term doses that causes severe adverse effects as a result of systemic absorption. Hence a local targeted treatment that aids delivering the drug at the site of inflammation serves as a safe therapy.

Budesonide is a glucocorticoid that inhibits various inflammatory cells causing induction in remission of active disease. The present study deals with formulation of a targeted preparation of budesonide loaded **nano-structured lipid carriers (NLCs)** that helps local delivery of drug without getting degraded in body. The NLCs were designed by using combination of a solid lipid Compritol ATO 888 and a liquid lipid Labrafac WL 1349, a medium chain triglyceride (MCT). The main advantage of using a MCT was that it itself serves an inhibitory effect in intestinal inflammation by inhibition of TNF-α and Interleukins and thereby enhancing the healing activity of the formulation.

NLCs were prepared by using hot homogenization technique. Various preliminary trials were performed for optimization of the NLCs in which process as well as formulation parameters were studied. Based on preliminary trials, 3 factors viz. concentration of drug, concentration of surfactant and concentration of co-surfactant were optimized using Box-Behnken Design. The lyophilized NLCs were converted to pellets by using simple extruder-spheronizer and were enteric coated in order to protect the pellets from degradation in upper part of gastro-intestinal tract. Characterization of the formulation and in-vivo studies were performed on colitis-model to ensure the efficacy of the prepared formulation and parameters such as myeloperoxidase activity, determination of inflammatory markers and histopathology was studied.

Inflammatory bowel disease (IBD) is a series of chronic inflammatory disorders of gastrointestinal that tend to relapse. The two major features of IBD are Ulcerative colitis and Cohrn's disease that differ from each other in terms of affected area of small intestine and colon.[1] The GIT is marked by inflamed lesions which are located specifically in colon in Cohrn's disease and is spread throughout in Ulcerative colitis. The inflammatory process is facilitated by defects in both the barrier function of the intestinal epithelium and mucosal immune systems. The treatment comprises of oral administration of anti-inflammatory agents, corticosteroids, and/or antibiotics. The oral administration of corticosteroids has been effective in patients with active Crohn's disease and ulcerative colitis.

Among the various currently available nano-particular systems, solid lipid nanoparticles (SLN) have been proved to be effective vectors for the treatment of inflammatory bowel disease[2]. SLNs have been proved to be stable in GIT and has the ability to modulate immune system. A better approach is to develop nanostructured lipid carriers because of its ability to encapsulate higher amount of drug due to its imperfections in lipid molecules.[3]

Budesonide, a non-halogenated corticosteroid, is highly effective in the treatment of IBD due to its greater topical anti-inflammatory activity than many other glucocorticoids. Therefore, a dosage form capable of delivering budesonide to the colon rather than upper GIT can be envisaged to result in high local concentration, thus enhancing the effectiveness of therapy. It exhibits low oral bioavailability and extensive first pass metabolism.[4] These characteristic properties give it better adverse effect profile that makes it first choice for the induction of remission of disease. Available formulations of Budesonide includes Budenofalk and Entocort. It helps inhibits the inflammatory cytokines and thus help heal the inflammation. Long term treatment using Budesonide causes long term side effects related to glucocorticoid.

The aim of the present study was to develop colon targeted drug delivery system comprising of nanostructured lipid carriers **(**NLCs) loaded with Budesonide for the treatment of inflammatory bowel disease. Targeted delivery of drug leads to avoidance of first pass metabolism and ligand appended drug loaded nanostructured lipid carriers provide a promising drug delivery system for local treatment of inflammatory bowel disease.

Folate receptors comprise of a family of glycosyl phosphatidylinositol–anchored, which are high-affinity receptors for folic acid (FA) and are the products of at least 4 different genes: FRα, FRβ, FRγ, and FRδ. Folate receptor α (FRα) has been exploited widely as a target for therapy and imaging in oncology but newer targeting therapies have focused on FRβ as a therapeutic target on macrophages in inflammatory diseases. It has been reported that elevated levels of functional glycosylated FRβ are expressed only on activated macrophages involved in inflammatory responses, but not on quiescent resident macrophage.[5]

During inflammatory conditions, Mannose receptors are highly expressed by activated macrophages and thus can be exploited for targeted delivery of drug. The macrophages are highly concentrated on the inflamed tissue and hence the over-expressed mannose receptors [6] can be chosen as a target that would lead to the attachment of ligand appended NLCs to the receptors and hence deliver the drug near/ close to the site of inflammation.

The transferrin receptor (TfR) is overexpressed in inflamed colon tissue, basically in elevated expression in both the basolateral and apical membranes of enterocytes and thus can be used as a target to deliver the formulation to the site of inflammation.[6] The increased levels of this receptor was noted in the colon biopsies from IBD patients as well as in excised tissues from colitis induced rat models. The level of TfR are elevates in activated immune cells like lymphocytes and macrophages also.

Thus targeting colon can be achieved by selectively binding our formulation to the receptors which are over-expressed on the macrophages or intestinal cell surface during the inflammatory stage.

2.1 Introduction to colon targeted drug delivery systems

Colon targeted drug delivery systems (CoDDS) has been a ground-breaking approach for site specific treatment of disease. This gained importance to CoDDS is due to the recently recognized importance of this region of the gastrointestinal tract (GIT), essentially for local as well as systemic delivery.[7] Topical (local) treatment of colonic disorders and oral delivery of drugs and proteins destroyed in the upper GIT are areas where such an approach is beneficial. It serves as an alternative for the treatment of serious local diseases such as inflammatory bowel disease, colorectal cancer, irritable bowel syndrome, infectious disease, etc. Now-a-days great focus is given to colon as a potential target for the absorption of peptides, proteins, and vaccines following oral administration because of the high residential time and the low proteolytic activity. Specific systemic absorption in the colonic region presents emaculated possibilities for the treatment of diseases susceptible to the diurnal rhythm, such as angina pectoris, nocturnal asthma, rheumatoid arthritis, etc. The classes of drugs suitable for CoDDS include anti-inflammatory, anti-amoebic, anticancer, antihypertensive, anti-asthmatic and others. Limitation of conventional drug delivery systems for treatment of colonic disorders is they do not reach the site of action in sufficient concentration. Hence an effective and a safe therapy for these disorders, using site-specific drug targeting possess a difficult and challenging task to the formulation department. The therapeutic benefits of targeted drug delivery to the colon include:

i. Intact delivery of drug in.

ii. Dose reduction and patient's compliance

iii. Reduced side effects (due to dose reduction).

iv. Increased residence time which improves absorption of drugs.

v. Advantageous for administering peptides, because the hydrolytic enzyme activity is lower in the colon than in the small intestine.

Oral or rectal route can aid the colon targeted delivery. Rectal dosage forms such as suppositories and enemas does not always give a therapeutic effectiveness due to high variability in the distribution of these forms. Suppositories are only effective in the rectum because of the confined distribution, and enema solutions can only offer topical treatment to the sigmoid and descending colon. Therefore, oral administration can be taken into

consideration for achieving site specific delivery. Conventional oral formulations degrades in the stomach or intestine and are absorbed from these regions thereby reducing the availability of therapeutic agent to colon. The active ingredients gets absorbed and degraded in the upper part of gastrointestinal tract and hence pose a major hurdle with the delivery of drugs by the oral route which has to be mitigated in order to successfully deliver the drug to colon.

Several approaches have been explored by researchers for formulation of CoDDS which include: pH dependent, Time dependent, Microflora activated, Prodrug based and Pressure controlled based systems.[8] A number of CoDDS have being developed taking advantage of the luminal pH in the ileum and the microbial enzymes in the colon, such as pectinase, amylase, dextrase, glycosidase, and azoreductase.

2.1.1 Anatomical and physiological characteristics of the colon

To clearly understand CoDDS, an insight of anatomy and physiology characteristics of colon should be necessarily understood. The colon consists of caecum, ascending colon, transverse colon, descending colon and sigmoid colon. The transverse colon is the largest and most versatile region with the overall length of colon of 1.5 m long. The colon histologically consists of four layers, the serosa, muscularis externa, submucosa and mucosa. Plicae semilunares are found on the surface, which are crescentic folds, which helps increase the surface of the colon to approximately 1300 cm^2.

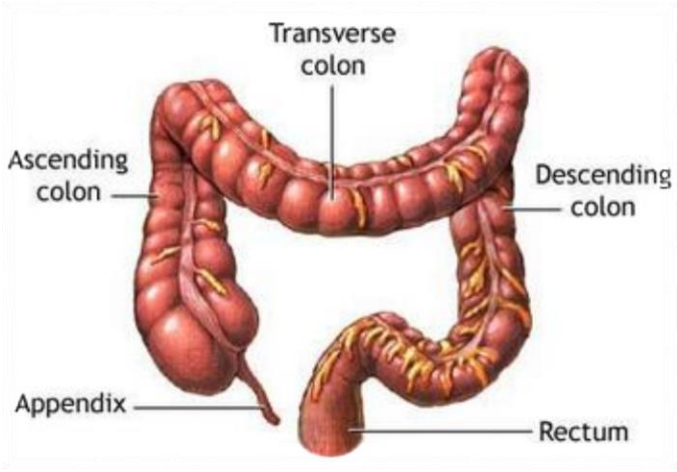


Figure 2.1 Ascending colon, transverse colon and descending colon

-The normal GI tract has a number of defense mechanisms that prevent infections. The stomach is sterile due to its high acidic pH. The latter part of the GI tract has normal bacterial flora which inhibit the growth of other organisms. Some species of normal flora produce short-chain fatty acids or antibiotics such as 'clostin' which prevent growth of pathogens.

2.1.2 Factors affecting performance of the oral CoDDS[9,10]

Various factors influences the performance of the oral CoDDS. Intact drug delivery without degrading in upper GI organs is necessity for targeted drug delivery. The major factors governing the colon drug delivery are the gastrointestinal pH, gastrointestinal transit time, gastric emptying, colonic microflora and colonic absorption, which are briefed below.

2.1.2.1 Gastrointestinal pH

The pH of entire GIT plays a vital role in the performance of the oral CoDDS. The pH of GI tract varies with inter and intra subject. Food habits, medical history and severity of disease influences the pH of the gastrointestinal fluid.

2.1.2.2 Gastrointestinal transit time[11]

Drug delivery to the colon through oral route depends on the gastric emptying and small bowel transit time. The gastric emptying is highly dependent on food. Usually doses taken before food passes out the stomach within a span of 1 hour which can stay for long till 10 hours if taken after food. In small intestine, the transit time is more consistent as compared to stomach, ranging between 3 to 4 h, regardless of conditions such as physical state, size of dosage form, and presence of food in the stomach.

2.1.2.3 Gastric emptying

The rate of gastric emptying significantly influence the arrival of an oral dosage form at the colon. Feeding state and particle size strongly affect the residence time of particles in the stomach. The rate of gastric emptying primarily depends on the caloric contents of the ingested

meal. An increase in acidity, osmolarity and caloric value slows down gastric emptying. Depression slows down gastric emptying rate, whereas stress increases it.

2.1.2.4 Colonic micro flora

The slow movement through the colon allows the drug delivery system to come in contact with a wide range of microbial population thriving there. They are degraded by the action of polysaccharidase and glycosidase enzymes and the ultimate products of fermentation are short chain fatty acids, carbon dioxide, hydrogen, methane and hydrogen sulphide. In the proximal region of the colon, carbohydrate fermentation predominates and results in a relatively low pH. In the distal regions, there is little carbohydrate fermentation, resulting in higher pH.

The enzymatic availability of the microbial flora in the colon make it a target for drug delivery. Through the microbial action, the drug that are to be released in the colon can be poorly absorbed from the drug delivery system. Infact, the drugs that disintegrate only by microbial activity can be taken as an opportunity to deliver the formulation at the site of inflammation. Hence this technique is suited for the drugs that are unstable in the upper part of GIT and can be well absorbed from colonic region. The microflora in the colon remains quite consistent but it may be affected by disease state or administration of antibiotics given during infectious and age. Dietary factors do not influence the microflora.

2.1.2.5 Absorption of drugs from the colon

Drugs are absorbed passively by paracellular or transcellular routes. Transcellular absorption involves the passage of drugs through cells and this is the route most lipophillic drugs take. Paracellular absorption involves the transport of drugs through the tight junctions between cells and is the route most hydrophilic drugs take. Studies in the rat have indicated that paracellular absorption is constant throughout the small intestine, but transcellular absorption appears to be confined to the small intestine, with negligible colonic absorption by this route. The poor paracellular absorption of many drugs in the colon is due to the fact that epithelial cell junctions are very tight. The absorption of drugs from colon is slow due to the low surface area also the colon is very large in diameter. It is thus, not ideally suited for absorption. The slow rate of

transit in colon however lets the drug stay in contact with the mucosa for a longer period than in small intestine and this compensates the lower surface area.

2.1.3 Approaches for CODDS[10]

Various formulation approaches that can be exploited for the development of CoDDS are discussed in detail below.

2.1.3.1 pH dependent system

Based on the feature that pH of GIT increases progressively from the various parts of GIT i.e. stomach to small intestine to the distal part of ileum, pH sensitive polymers work. Enteric polymers are those that are insoluble in the acid pH of stomach thereby avoiding dissolution of drug until it reaches small intestine. These polymers are sensitive to pH 5 to 7.5[12] depending on their chemical properties. Dosage form coated with the pH sensitive polymer provides delayed release of drug thereby avoiding the drug to dissolve in the acidic pH of stomach. These are able to maintain its integrity at lower pH values of stomach and proximal small intestine. Therefore, are able to deliver the drug at the ileocecal junction. Enteric coated tablets or similar formulations or gelatin capsules with multiparticulate doses are preferentially used in pharmaceutical dosage form as it has relatively low cost of manufacturing and are easy to scale up. Eudragit polymers are widely used pH sensitive polymers for coating purposes. Various grades available are Eudragit L 100 which dissolves at pH > 6.0, Eudragit S 100 at pH >7.0, Eudragit L30D at around pH of 5.6, Eudragit , Eudragit FS 30D at a pH greater than 7.0 are widely used to develop pH sensitive formulations. They can either be used alone or in combination depending on the properties to be achieved.

2.1.3.2 Time dependent system

This technique refers to delaying the drug release until it reaches the colon. Gastric emoting time widely varies but the transit time of small intestine happens to be relatively constant with little or no variations. This technique to design a time released delivery aims at resisting the acidic environment of stomach and get released at the predicted time point further to which drug delivery to colon is ensured. Time required to transit from the oral cavity to the colon is the lag time here.

2.1.3.3 Microflora activated system

Naturally occurring polysaccharides from plant origin (eg. pectin, guar gum, inulin), animals (eg. chitosan, chondroitin sulfate), algal (eg. alginates), or microbial (eg. dextran) origin can be used to formulate colon targeted drug delivery system. Simple saccharides are formed by the breakdown of such polysaccharides. This technique deals with protecting the drug from the hostile conditions of upper GIT. Hydrolysis of the glyosidic linkages on entering colon triggers the release of the entrapped active ingredient. The saccharolytic species responsible for this biodegradation are Bacteroides and Bifidobacterium.

2.1.3.4 Prodrug approach

The increasing number of potent and selective peptide and non-peptide drugs being investigated in the past few years has influenced the demand for development of targeted drug delivery. Designing a prodrug is an area of drug research that helps in overcoming pharmaceutical and pharmacokinetic problems associated with the parent drug molecule. This approach involves the formation of a covalent linkage between the drug and the carrier. It is designed in such a way that upon oral administration it remains intact in the stomach and small intestine. In general, a prodrug is a pharmacologically inactive component of a parent molecule that requires on the spot or enzymatic transformation within the body in order to release the active component, having improved delivery over parent compounds. The problem of the degradation of drugs due to the adverse environment of the upper GIT can also be overcome by the formation of a prodrug that has improved stability.

2.2. Introduction to Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is the wide taxonomy for a series of chronic relapsing gastrointestinal (GI) diseases that has two principle features namely ulcerative colitis (UC) and Crohn's disease (CD). While UC and CD are considered different from each other, they share many clinical features which are characterized by cycles of relapsing and remitting mucosal inflammation. In case of UC, the inflammation is limited to the colon which extends proximally from the rectum and is continuous, in some cases involving the entire colon (pancolitis).[13] Crohn's disease may include inflammation in any region of the GI tract, with the terminal ileum and the colon most affected. The inflammation occurs in a discontinuous in manner. Although the etiology of disease is undefined, certain parameters that tend to play role involve genetics, microbiome, environmental stress and immune dysfunction. No cure for IBD is available but there are many therapeutic strategies that plays promising role to attain and maintain remission from inflammatory episodes.

For the treatment of both the diseases, steroids are commonly prescribed but in acute conditions. During chronic conditions, its long term doses may cause severe undesirable systemic adverse effects. Alternate treatment for IBD includes drugs like amino-salicylates, antibiotics, and immuno-suppressive agents. These therapies are able to induce and maintain remission for short term, while 70% of patients suffering from IBD has to undergo at least one surgical intervention in their lifetime. A systematic review by Talley et al highlighted the variable performance of current IBD therapies across IBD phenotype, location, stage and severity of disease. Other conventional oral dosage forms have a limited ability to treat IBD as they are aimed to achieve systemic delivery of therapeutics, which causes side effects and toxicity after their distribution in body. Local oral preparations pose to be better drug delivery design for IBD. It is an improved therapy as it aims at delivering the drug to the site of inflammation and doesn't get absorbed systemically. Hence the currently available formulations are either delayed or controlled release dosage forms. Physiological conditions in the GI tract, in particular the colon are taken advantage of through this technique. For example, prodrugs of 5-aminosalicylic acid (5-ASA), such as sulfasalazine or olsalazine, depends on the enzymatic activity of colonic bacteria for their cleaving into active moieties. COLAL-PRED® (prednisolone sodium metasulfobenzoate) system, non-starch polysaccharide coatings and matrix formulations depend on enzymatic degradation that is specific to colonic bacteria. pH sensitive coatings is another technique used to deliver drug based on the pH of the system. The

limitation of this approach is their inconsistent efficacy and inter-patient variability. One reason for varied efficacy of these conventional colon targeted delivery approaches maybe because of the difference in physiological conditions which may either be signified by pH, the gastrointestinal transit time or the colonic microbes.

Constant attempts to overcome these issues have focused on improved understanding of the physiology of the GI tract during active IBD and following GI tract resection, as well as rational design of oral formulations. These considerations not only improve bio-distribution of therapeutics to the colon, but also confer specific accumulation and cellular uptake within diseased tissue. Recent pharmaceutical advances have applied nanotechnology to oral dosage form design in an effort to overcome the limitations of conventional formulations. General physiological considerations for colonic drug delivery Drug delivery to the colon relies on a number of physiological factors to ensure optimal efficacy following oral administration. Considerations should be made during formulation design to the residence time of the formulation in the GI tract, how the GI environment affects the delivery of the formulation and dissolution of the drug at the site of action, the intestinal fluid volume, and the propensity of the formulation or drug to be metabolized in the GI tract through enzymatic or microbial degradation. For instance, consideration of the formulation transit time through the GI tract is critical to ensuring delivery of the drug to the site of action. Small intestinal transit time is generally accepted as 4 hours, with individual variability ranging from 2 to 6 hours. In contrast, colonic transit times can vary significantly, with ranges from 6 to 70 hours reported. Additional confounders influencing GI transit time include gender, with females having significantly longer colonic transit times, and the time of dosing with respect to an individual's bowel movements. Differences in pH along the GI tract have been exploited for the purposes of delayed release therapies. The highly acid stomach environment rises rapidly to pH 6 in the duodenum and increases along the small intestine to pH 7.4 at the terminal ileum. Ceacal pH drops below pH 6 and again rises in the colon reaching pH 6.7 at the rectum. However, pH ranges can exhibit variability between individuals, with factors such as water and food intake as well as microbial metabolism being major determinants.

In addition to influencing pH, fluid matter ratios may also affect the colonic delivery of drugs. For instance, free fluid volumes, bile salts and digestive enzyme levels in the GI tract are significantly altered following food intake. Intestinal fluid secretion also affects the viscosity of the mucous-gel layer, which may influence the ability of drugs to be taken up by cells at the

site of action. Finally, we are now appreciating the importance of the intestinal microbiome in GI physiology. The GI tract plays host to over 500 distinct bacterial species, with many estimating the number of species to be close to 2,000. These bacteria play pivotal roles in both digestion and intestinal health, including digestion and metabolism of fatty acids, proteins and carbohydrates. The majority of the intestinal microbiome resides in the anaerobic colon and fermentation of carbohydrates is the main source of nutrition for this population. The relatively exclusive fermentation of non-starch polysaccharides by the colonic microbiome is exploited in formulations that use non-starch polysaccharide coatings. While there appears to be considerable variation in the composition of the microbiome between individuals, which is influenced by both genetic and environmental factors, the dominant Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria species appear to be consistent and represent the majority of the colonic flora. Despite this, the microbiome is exposed to temporal disruption (dysbiosis) by disease and medications (e.g. antibiotics), and is influenced by factors such as diet, lifestyle and geographical distinctions. It is therefore clear that within healthy individuals there is variability in the physiology of the GI tract. Adding to this complexity are the changes in physiology associated with GI disease, which will further affect the efficacy of orally administered formulations. These physiological factors are dynamic, inter-related and remain an important challenge in dosage form design. Depending on disease severity, gastrointestinal pathologies can affect some or all of the physiological variables for oral drug delivery. Many acute GI infections will cause dysbiosis and drive increased intestinal fluid secretion, and may increase or decrease bowel motility, while more chronic conditions, such as IBD, can drastically and permanently alter the physiology of the GI tract.

2.2.1 Changes in the physiology of the GI tract during active IBD

An often overlooked confounder when considering oral delivery strategies in IBD is the change in the physiological condition of the GI tract associated with chronic inflammation. Mucosal inflammation in IBD causes pathophysiological changes, such as (i) a disrupted intestinal barrier due to the presence of mucosal surface alterations, crypt distortions and ulcers, (ii) increased mucus production and (iii) the infiltration of immune cells (e.g. neutrophils, macrophages, lymphocytes and dendritic cells). During relapse of IBD, patients suffering from severe mucosal inflammation may exhibit altered GI motility and diarrhea, which in turn affects intestinal volume, pH and mucosal integrity. The inflammatory response at the mucosa, along with severe diarrhea, will also disrupt the resident microbiome, which can alter microbial

metabolism in the GI tract. Thus active inflammation significantly alters the physiology of the GI tract, which can affect the efficacy of conventional approaches to colon targeted drug delivery.

2.2.2 Transit time and microbial considerations

Alterations to GI physiology in states of disease are often dynamic and inter-related, and therefore difficult to examine in isolation. For instance, orocecal transit time (OCTT), the time taken for the meal to reach the cecum, has been shown to be delayed in both CD and UC patients compared to healthy controls. However, significantly faster OCTTs have been observed in IBD patients with the dysbiotic condition—small intestinal bacterial overgrowth (SIBO). These observations have been confirmed experimentally in humanized mice, following dietary manipulation of the gut microflora. Changes in the composition of the microbiome (dysbiosis) are common in GI disease, with alterations in physiology, inflammatory state, or as a result of treatment regimens. While it is generally accepted that the bacterial load is relatively static in IBD, the diversity of the microbiome is reduced with increases in major species such as Bacteroides, Eubacteria and Peptostreptococcus acting to the detriment of other populations. It is not known what precipitates the initial dysbiosis, and whether dysbiosis precedes or is a symptom of disease. However, there is some evidence to suggest that physiological factors such as dysmotility and increased luminal fluid (diarrhea) may play a role. Studies in animal models have shown that prolonged water secretion into the bowel, leading to decreased colonic transit times, can alter the colonic microbiome. Therefore, not only can the microbiome affect intestinal transit times, but conversely, transit times may also alter the intestinal microbiome.

In contrast to OCTT, colonic transit is significantly faster in IBD patients, likely due to the diarrhea that is a hallmark of the disease. UC patients may exhibit transit times twice as rapid as a healthy individual, leading to difficulties in targeting specific regions of the colon with conventional formulations. Studies using conventional delayed release formulations have shown asymmetric bio-distribution in the colon, with higher retention of drug in the proximal colon and significantly lower drug concentrations in the distal colon. Thus transit time in itself may not be a reliable approach to colon-targeted drug delivery in IBD.[14]

2.2.3. Changes in colonic pH

There is little evidence to suggest major alterations to small intestinal pH in IBD patients, however colonic pH is significantly lower in both UC and CD patients. In the colon, intestinal pH is influenced by microbial fermentation processes, bile acid metabolism of fatty acids, bicarbonate and lactate secretions, and intestinal volume and transit times. As all of these factors may be disrupted during active IBD, changes in luminal pH in the colon are not surprising. While normal colonic pH ranges from 6.8 in the proximal colon and rises to 7.2 in the distal colon, this can significantly vary in active UC patients from pH 5.5 to as low as 2.3. Similarly, reported colonic pH values for CD patients are approximately 5.3, irrespective of disease activity. These pH changes are likely to affect the composition of the colonic microbiome and thus colonic transit times, which can influence the release of drug from formulations requiring bacterial fermentation or enzymatic activity. Likewise, pH changes can affect the release of compounds from pH-dependent release coatings.

2.2.4 Intestinal volume

The composition of the intestinal biomass is altered in disease and is directly related to changes in microbial metabolism, intestinal transit time and luminal pH. In particular, increased fluid secretion and decreased reabsorption can dilute the digestive enzymes that control intestinal transit to allow nutrient absorption. This in turn may influence the intestinal microbiome, which can alter carbohydrate and polysaccharide digestion as well as contribute to changes in intestinal transit times. These changes in intestinal fluid volumes may alter the way conventional formulations are processed in the GI tract and the subsequent local delivery of drugs to the colon.

2.2.5 Mucosal integrity

The epithelial barrier selectively regulates transport from the lumen to the underlying tissue compartments, restricting transport of smaller molecules across the epithelium, while virtually abolishing macromolecule transport. This selectivity is determined by apical transmembrane protein complexes known as tight junctions (TJ). These multi-protein complexes interact directly with underlying epithelium actomycin rings, influencing physiological and pathophysiological stimuli, such as ion transport, luminal glucose transport, water secretion and the transport of cytokines and leukocytes. While these properties make TJ an attractive pharmacological target for enhancing drug absorption, dysfunctional regulation of TJ complex

formation is associated with a loss of epithelial integrity in intestinal inflammatory diseases, such as IBD. Active inflammation not only alters intestinal mucosal integrity, but also significantly alters mucosal metabolism as the tissue attempts to limit further damage and repair. For instance, in an attempt to compensate for the loss of intestinal epithelial integrity accompanying inflammation and tissue ischemia, a number of endogenous protective pathways are activated subtly altering the physiology of the mucosa. In order to augment intestinal barrier function, and perhaps compensate for the reduction in mucous-gel integrity with fluid secretion, the oxygen-sensing transcription factor, hypoxia-inducible factor (HIF), mediates increased expression of intestinal mucins and trefoil factors. The viscosity of the mucous-gel layer is likely to affect the permeability of lipophilic drugs and muco-adhesive formulations. In addition, HIF transcriptionally regulates multi-drug resistance gene 1 (MDR1), which codes for the xenobiotic drug efflux pump, P-glycoprotein (P-gp), that is involved in actively transporting substrate compounds back into the lumen.[15] For example, glucocorticoids are substrates for P-gp and have been shown to stimulate the expression of MDR1, potentially contributing to steroid resistance in IBD. Interestingly, nano-delivery systems have been shown to target both drug and biological mechanisms to overcome multidrug resistance via P-gp inhibition and ATP depletion.

2.2.6 Intestinal resection in IBD patients

Resection of bowel tissue is common among IBD sufferers, with over 70% of IBD patients undergoing at least one surgery in their lifetime. Removal of bowel tissue results in a shortening of the intestine and reduced transit distance through the GI tract, which potentially affects the way conventional oral formulations are processed. Beyond this, resection profoundly changes the physiology of the intestinal tract by altering pH, nutrient absorption, digestion and transit. In particular, resection of the terminal ileum alters water absorption and dilutes residual bile acids in the colon, therefore reducing net colonic fatty acid concentrations. This may profoundly alter microbial metabolism of fatty acids by hydroxylation to produce ricinoleic acid analogues that can drive diarrhea. Diarrhea significantly affects the therapeutic efficacy of conventional oral formulations. The reduction in fatty acids also reduces the "ileal brake"—a nutrient feedback mechanism which slows transit times to allow nutrient absorption. As fatty acids are the most potent stimulant of the ileal brake, a loss of both fatty acid receptors (from resected tissue) and fatty acids from digestion leads to a loss of the ileal brake and a subsequent decrease in intestinal transit time. As a large proportion of IBD patients have undergone

resection of the bowel, these physiological changes should be considered when devising targeted delivery strategies in the GI tract.

2.3. INTRODUCTION TO LIPID NANOPARTICLES[16]

Lipid nanoparticles with a solid matrix are generally two types: solid lipid nanoparticle (SLN) and nanostructured lipid carrier (NLC). Solid Lipid Nanoparticle SLNs are prepared from lipids which are solid at room temperature as well as at body temperature. Different solid lipids are used to produce SLNs, such as, tripalmitin/ Dynasan® 116 , cetyl alcohol , cetyl palmitate , Compritol® 888 ATO , Glyceryl monostearate , Precirol® ATO5, trimyristin/Dynasan® 114, tristearin/Dynasan® 118 , stearic acid , Imwitor® 900. There are several advantages of SLN formulations , such as: degradation in external environment (during storage) and in the gut (following oral administration) of a photosensitive, moisture sensitive, and chemically labile drug molecules can be protected (b) improved bioavailability of highly lipophilic molecules can be achieved, (c) biodegradable and physiological lipids are used to prepare SLNs, (d) scaling up of the formulation technique to industrial production level is feasible at low cost and in a relatively simple way, (e) use of organic solvents can be avoided to produce SLNs.

In contrary, several disadvantages are also associated with SLNs, such as:

(a) SLN dispersions contain high amount of water,

(b) drug-loading capacity of SLNs are limited due to crystalline structure of solid lipid,

(c) expulsion of encapsulated drug may take place during storage due to formation of a perfect crystalline lattice especially when SLNs are prepared from one highly purified lipid,

(d) drug release profile may change with storage time,

(e) polymorphic transitions are possible,

(f) particle growth is possible during storage, and

(g) gelation of the dispersion may take place during storage.

In nanostructured Lipid Carrier drugs are incorporated between the fatty acid chains or in between lipid layers or in amorphous clusters in crystal imperfections within SLN matrix. However, SLNs prepared from one highly purified lipid can crystallize in a perfect crystalline lattice that allows very small space for the incorporation of drugs. In high energetic lipid modifications, α and β′, immediately after preparation of SLN, lipid gets crystallized. However, the lipid molecules undergo a time-dependent restructuring process leading to formation of the low-energetic modifications, βi and β, during storage. Formation of this perfect lipid crystalline structure leads to expulsion of drug. Therefore, despite SLNs being interesting delivery systems, relatively low drug-loading capacity and potential expulsion of the drug during storage led to the discovery of another lipid carrier NLCs. In case of NLCs, spatially very

different lipid molecules are mixed to create a lipid particle matrix as imperfect as possible. Generally, solid and liquid (oil) lipids are mixed to produce NLCs that are still solid at room temperature as well as at body temperature. Due to many imperfections in NLCs, drug-loading capacity is enhanced and drug expulsion during storage is minimized.

NLCs have several advantages, such as:
(a) NLC dispersions with higher solid content can be produced,
(b) drug-loading capacity is better than SLNs,
(c) drug release profile can be easily modulated,
(d) drug leakage during storage is lower than SLNs, and
(e) production of final dosage forms (e.g., tablets, capsules) is feasible.

2.3.1 FORMULATION TECHNIQUES[17]

Various formulation techniques exist for the production of SLNs and NLCs. Among them, high-pressure homogenization (HPH) and microemulsion techniques have been discovered to have a strong potential for scaling up to industrial production scale. The following sections describe different existing size reduction techniques for the development of SLN and NLC formulations. However, in some instances combination of different methods has been utilized to prepare the nanoparticles.

2.3.1.1. High-Pressure Homogenization

HPH is a reliable and suitable technique for and the drug is dissolved or homogeneously dispersed in the melted lipid(s). Then a hot aqueous surfactant solution (preheated at the same temperature) is added to the drug–lipid melt and homogeneously dispersed (pre-emulsion) by a high shear mixing device. Subsequently, this hot pre-emulsion is subjected to a high-pressure homogenizer at the same temperature. This homogenization process is repeated till the nanoemulsion of desired average particle size is obtained. The obtained nanoemulsion is then cooled down to room temperature. During this cooling down, lipid droplets of the nanoemulsion re-crystallize and form lipid nanoparticles with solid matrix. Cold high-pressure homogenization is another technique wherein the lipid(s) is/are melted at 5–10°C above its/their melting points and the drug is dissolved or homogeneously dispersed in the melted lipid(s) in the cold HPH technique. Then the drug-lipid melt is rapidly cooled down and subsequently milled to microparticles. These microparticles are suspended in a cold aqueous

surfactant solution and then homogenized at or below room temperature forming lipid nanoparticles. This cold HPH technique is suitable for hydrophilic or thermo-labile drugs as this method is expected to avoid temperature-induced drug degradation and drug distribution into aqueous phase during homogenization. However, complete avoidance of drug exposure to high temperature is impossible as the drug needs to dissolve or disperse in the molten lipid and some heat is generated during the homogenization process. Generally, scaling up of a process encounters several problems. Nevertheless, usage of the larger scale machines during HPH leads to an even better quality of the product with regard to a smaller particle size and its homogeneity. Additionally, HPH technique is widely used and well-established technique in pharmaceutical and food industry. SLN prepared by HPH can also be produced in non-aqueous dispersion media as long as the dispersion medium does not dissolve the lipid, e.g., liquid polyethylene glycol or oils (e.g., mineral oil) the preparation of lipid nanoparticles. There are two types of HPH, hot HPH and cold HPH.

2.3.1.2. Hot high-pressure homogenization.

In this technique, first the lipid(s) is/are melted at 5–10°C above its/their melting point(s) and the drug is dissolved or homogeneously dispersed in the melted lipid(s). Then a hot aqueous surfactant solution (preheated at the same temperature) is added to the drug–lipid melt and homogeneously dispersed (pre-emulsion) by a high shear mixing device. Subsequently, this hot pre-emulsion is subjected to a high-pressure homogenizer at the same temperature. This homogenization process is repeated till the nanoemulsion of desired average particle size is obtained. The obtained nanoemulsion is then cooled down to room temperature. During this cooling down, lipid droplets of the nanoemulsion re-crystallize and form lipid nanoparticles with solid matrix.

2.3.1.3. Cold high-pressure homogenization.

Similar to hot HPH, the lipid(s) is/are melted at 5–10°C above its/their melting points and the drug is dissolved or homogeneously dispersed in the melted lipid(s) in the cold HPH technique. Then the drug-lipid melt is rapidly cooled down by means of liquid nitrogen or dry ice and subsequently milled to microparticles by means of a ball mill or mortar. These microparticles are suspended in a cold aqueous surfactant solution and then homogenized at or below room temperature forming lipid nanoparticles. This cold HPH technique is suitable for hydrophilic or thermo-labile drugs as this method is expected to avoid temperature-induced drug

degradation and drug distribution into aqueous phase during homogenization. However, complete avoidance of drug exposure to high temperature is impossible as the drug needs to dissolve or disperse in the molten lipid and some heat is generated during the homogenization process. Generally, scaling up of a process encounters several problems. Nevertheless, usage of the larger scale machines during HPH leads to an even better quality of the product with regard to a smaller particle size and its homogeneity. Additionally, HPH technique is widely used and well-established technique in pharmaceutical and food industry. SLN prepared by HPH can also be produced in non-aqueous dispersion media as long as the dispersion medium does not dissolve the lipid, e.g., liquid polyethylene glycol or oils (e.g., mineral oil)

2.3.1.4. Emulsification-Sonication

Emulsification technique consists of similar steps like in HPH. Briefly, the lipid(s) is/are melted at a temperature of 5–10°C above its/their melting point(s) and the drug is dissolved/dispersed in the melted lipid(s). Then a hot aqueous surfactant solution (preheated at the same temperature) is added to the drug-lipid melt and homogeneously dispersed by a high shear mixing device. Coarse hot oil-in-water emulsion obtained is ultra-sonicated using probe sonicator till the desired sized nanoemulsion is formed. Finally, lipid nanoparticles are obtained by allowing hot nanoemulsion to cool to room temperature. However, metallic contamination of the product may happen during sonication by probe sonicator.

2.3.1.5. Microemulsion

Gasco et al[18] developed a Microemulsion method for the preparation of SLNs which has been exploited widely. In this method, first the solid lipid(s) is/are melted and the drug is dissolved/dispersed in the molten lipid(s). Thereafter, non-aqueous dispersion media as long as the dispersion medium does not dissolve the lipid, e.g., liquid polyethylene glycol or oils (e.g., mineral oil) that, aqueous surfactant–cosurfactant solution (pre-heated above the melting temperature of solid lipid) is added to the lipid melt with mild agitation to obtain transparent microemulsion. Subsequently, the microemulsion is dispersed in cold water (2–10°C) with mild agitation, where the microemulsion breaks into ultrafine nano-emulsion droplets which immediately crystallize to form SLNs. Strong dilution of the particle suspension due to usage of large volume of water (ratio of hot microemulsion to cold water=1:25–1:50) is the main criteria of this technique. Thus, the excess water needs to remove either by ultra- filtration or by lyophilization to obtain a concentrated dispersion. Another disadvantage of this method is

the necessity of high concentrations of surfactants and co-surfactants, which is not desirable. Industrial scale production of lipid nanoparticles by the microemulsion technique is possible. In the large-scale production, a large temperature controlled tank is used to prepare the microemulsion. Subsequently, the microemulsion is pumped into a cold water tank for the precipitation step. The temperature of the microemulsion and water, temperature flow in the aqueous medium, and hydrodynamics of mixing are the critical process parameters in the large-scale production. Solvent Emulsification-Evaporation In this technique, first the lipid(s) is/are dissolved in a water-immiscible organic solvent (e.g., cyclohexane, chloroform) and then emulsified in an aqueous phase containing surfactants under continuous stirring. The organic solvent evaporates during emulsification, which results in lipid precipitation. As the whole formulation procedure can be conducted in room temperature, this technique is highly suitable for thermo-labile drugs. However, the major concern is the production of very dilute dispersion that needs to be concentrated by means of ultra-filtration or evaporation. Another concern is the use of organic solvent, some of which may remain in the final preparation. Solvent Diffusion In contrary to solvent emulsification–evaporation technique, partially water-miscible organic solvents (e.g., benzyl alcohol, ethyl formate) are used in solvent-diffusion technique. In this case, organic solvents are mutually saturated with water to ensure initial thermodynamic equilibrium of both liquids. The transient oil-in-water emulsion is passed into water under continuous stirring, which leads to solidification of dispersed phase forming lipid nanoparticles due to diffusion of the organic solvent. However, similar to micro emulsion technique, dilute nanoparticle dispersion is produced, which needs to be concentrated by ultra-filtration or lyophilization. Usage of organic solvent is also a concern as some of it may remain in the final preparation. Solvent Injection The basic principle of the solvent injection method is similar to the solvent diffusion method. In case of solvent injection method, lipids are dissolved in a water-miscible solvent (e.g., acetone, isopropanol, and methanol) or water.

The double emulsion (w/o/w) method is based on solvent emulsification–evaporation method wherein the drug and stabilizer are encapsulated in the inner aqueous phase of the w/o/w double emulsion. A stabilizer is necessary to prevent drug partitioning to the outer aqueous phase during solvent evaporation. The method is mainly for the production of lipid nanoparticles loaded with hydrophilic drugs. Formulations so developed are usually named as ‘lipospheres’ due to their comparatively larger particle size than SLNs.

2.3.2 CHARACTERIZATION[19]

Characterization of the lipid nanoparticles is critical owing to its complexity of the system and colloidal size of the particles. Hence to formulate formulation with desired properties, it is essential to characterize these factors to improve the quality, stability, and release kinetics. As a result, accurate and sensitive characterization methods should be employed. There are several important characterization techniques as follows.

2.3.2.1. Particle Size

Molecule size represents the gastrointestinal uptake and their leeway by the reticuloendothelial framework. Henceforth, to decide the molecule size is imperative. Molecule measure under 300 nm are fitting for the intestinal transport. Photon correlation spectroscopy (PCS) and laser diffraction (LD) are the most effective and generally utilized procedures for the molecule size estimation of lipid nanoparticles. PCS is otherwise called dynamic light scattering. The change of the force of the scattered light, brought about by particles development, is measured by this method. PCS is generally exact and delicate strategy. Notwithstanding, just size reach from couple of nanometers to around 3 μl can be measured by PCS. This size extent is sufficient to describe lipid nanoparticles. Then again, LD can gauge greater molecule sizes (>3 μl). LD covers an expansive size extent from the nanometer to the lower millimeter range. This technique depends on the reliance of the diffraction point on the molecule sweep. Littler particles lead to more extraordinary dispersing at high edges than the bigger particles. Nonetheless, it is constantly prescribed to utilize both PCS and LD strategy all the while as both techniques don't specifically gauge molecule sizes, rather molecule sizes are figured from their light scrambling impacts. This is on the grounds that particles are non-circular in numerous cases.

2.3.2.2. Polydispersity Index

Due to the polydispersity nature of SLNs/NLCs, measurement of polydispersity index (PDI) is important know the size distribution of the nanoparticles. The lower the PDI value, the more monodispersed the nanoparticle dispersion is. PDI value less than 0.3 as optimum value. PDI can be measured by PCS.

2.3.2.3. Zeta Potential

The zeta potential (ZP) shows the general charge a molecule obtains in a particular medium. Strength of the nano-dispersion repulsion capacity can be anticipated structure the ZP esteem. The ZP demonstrates the level of shock amongst close and also charged particles in the scattering. High ZP shows profoundly charged particles. For the most part, high ZP (negative or positive) counteracts total of the particles because of electric repugnance and electrically settles the nanoparticle scattering. Then again, in the event of low ZP, fascination surpasses aversion and the scattering coagulates or flocculates. Be that as it may, this supposition is not material for all colloidal scattering, particularly the scattering which contain steric stabilizers. The ZP estimation of −30 mV is sufficient for good adjustment of a nano-dispersion. The ZP of the Nano scatterings can be dictated by PCS.

2.3.2.4. Shape and Morphology

Scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) are extremely helpful methods to decide the shape and morphology of lipid nanoparticles. These methods can likewise decide the molecule size and size dissemination. SEM uses electron transmission from the example surface, though TEM uses electron transmission through the specimen. Rather than PCS and LD, SEM and TEM give direct data on the molecule shape and size. A few SEM and TEM study demonstrated circular state of the lipid nanoparticles. Albeit typical SEM is not extremely sensitive to the nanometer size extent, field emission SEM (FESEM) can identify nanometer size reach. Be that as it may, test readiness (e.g., dissolvable expulsion) may impact the molecule shape. Cryogenic FESEM may be useful for this situation, where fluid scattering is solidified by fluid nitrogen and micrographs are taken at the solidified condition. AFM strategy is likewise picking up ubiquity for nanoparticle portrayal. AFM gives a three-dimensional surface profile dissimilar to electron microscopy which gives two-dimensional picture of an example. AFM straightforwardly gives auxiliary, mechanical, practical, and geological data about surfaces with nanometer-to angstrom-scale determination. In this method, the power acting between a surface and an examining tip results in a spatial determination of up to 0.01 nm for imaging. Direct examination of the initially hydrated, dissolvable containing tests is conceivable as no vacuum is required amid operation and the example does not should be conductive.

2.3.2.5. Crystallinity and Polymorphism

Determination of the crystallinity of the segments of SLN/NLC details is essential as the lipid lattice and in addition the joined medication may experience a polymorphic move prompting a conceivable undesirable medication removal amid capacity. Lipid crystallinity is additionally emphatically corresponded with medication joining and discharge rates. Thermodynamic security and lipid pressing thickness increment, though tranquilize fuse rates diminish in the accompanying request: Super cooled melt, α-change, β′-alteration, and β-adjustment. Be that as it may, lipid crystallization and adjustment changes may be very impeded because of the little size of the particles and the nearness of emulsifiers. Differential checking calorimetry (DSC) and X-Ray diffractometry (XRD) are two generally utilized methods to decide the crystallinity and polymorphic conduct of the parts of the SLNs/NLCs. DSC gives data on the liquefying and crystallization conduct of all strong and fluid constituents of the particles, though XRD can distinguish particular crystalline mixes in view of their precious stone structure. DSC uses the way that diverse lipid changes have distinctive dissolving focuses and softening enthalpies. In XRD, the monochromatic light emission beam is diffracted at points controlled by the separating of the planes in the precious stones and the sort and course of action of the iotas, which is recorded by a finder as an example. The force and position of the diffractions are extraordinary to every kind of crystalline material. XRD example can foresee the way of course of action of lipid atoms, stage conduct, and describe and recognize the structure of lipid and medication particles. Be that as it may, best results are watched when SLN scatterings are explored specifically as dissolvable evacuation may change the alteration. Another two strategies, infrared and Raman spectroscopy are likewise valuable to examine basic properties of lipids. Be that as it may, they have not been broadly used to portray SLNs/NLCs.

2.3.2.6. Assessment of Alternative Colloidal Structures

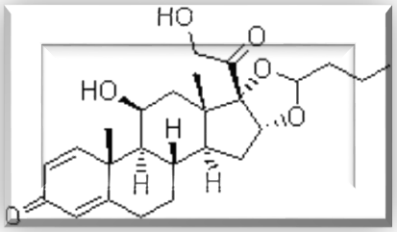
In a few cases, lipid nanoparticles coincide with other colloidal structures (e.g., micelles, liposomes, blended micelles, supercooled melts, and medication nanoparticles). In any case, portrayal and measurement of these colloidal structures are troublesome because of the similitudes in size, low determination of PCS to recognize multimodal appropriations, adjustment of the harmony of the complex colloidal framework amid test planning. Besides, weakening of the first nanoparticle scattering with water may bring about the expulsion of surfactant atoms from the molecule surface and prompt further changes. Henceforth, the

strategies which are touchy to the concurrent identification of various colloidal species and which don't require preliminary strides ought to be utilized. Atomic attractive reverberation (NMR) and electron turn reverberation (ESR) systems are reasonable for this reason. These systems are helpful for exploring dynamic wonders and the qualities of the nano-compartments in colloidal lipid scatterings. Discovery of super-cooled melts because of the low line widths of the lipid protons is conceivable by 1 H-NMR spectroscopy. This strategy depends on the distinctive proton unwinding times in the fluid and semisolid/strong state. NMR likewise can portray fluid nano-compartments in NLCs. ESR requires a paramagnetic twist tests to research SLN scatterings. Immediate, repeatable, and noninvasive portrayal of the conveyance of the twist test between the fluid and the lipid stage can be performed by ESR. In any case, notwithstanding the considerable potential, NMR and ESR have been seldom connected to portray SLNs and NLCs.

2.4. INTRODUCTION TO DRUG:

Budesonide

An epimeric mixture of α and β – propyl forms of 16 α, 17 α-butylidenedioxy -1(β, 2, 1 – dihydroxylpregna- 1, 4 – diene -3, 20-dione.



2.4.1. Molecular formula

$C_{25}H_{34}O_6$

2.4.2. Molecular weight

430.5

2.4.3. Physical Appearance

A white, crystalline powder. Practically insoluble in water: sparingly soluble in alcohol: freely soluble in dichloromethane.

2.4.4. Adverse Effects, Treatment, Withdrawal and Precaution

Inhalation of high doses of budesonide is associated with some adrenal suppression. Systemic absorption may follow nasal use, particularly after high doses or prolonged treatment. The dose of oral budesonide may need to be reduced in hepatic impairment.

When applied topically, particularly to large areas, when the skin is broken, or under occlusive dressings, or when given intranasal, corticosteroids may be absorbed in sufficient amounts to cause systemic effects.

2.4.4.1 Effects on bones

For the suggestion that inhalation once-daily in the morning may have less marked effects on growth and collagen turnover than twice-daily inhalation.

2.4.4.2 Effects on the nervous system

Psychotic behavior has been reported after the use of inhaled budesonide.

2.4.4.3 Hypersensitivity

Contact dermatitis has been reported to topical or intranasal budesonide. An anaphylactic reaction occurred 5 minutes after the first dose of oral budesonide in a patient who had previously reacted in a similar way to mesalamine.

2.4.5 Pharmacokinetics

Budesonide is rapidly and almost completely absorbed after oral administration, but has poor systemic availability (about 10%) due to extensive first pass metabolism in the liver, mainly by the cytochrome P450 isoenzyme CYP3A4.The major metabolites,6- β-hydroxybudesonide and 16- α- hydroxyprednisolone have less than 1% of the glucocorticoids activity of unchanged budesonide. Budesonide is reported to have a terminal half-life of about 2-4 hours

2.4.6 Uses and Administration

2.4.6.1. Budesonide is a corticosteroid with mainly glucocorticoid activity. It is mainly used in asthma, in usual doses of 400 micrograms in daily in 2 divided doses from a metered – dose aerosol; in severe asthma the dosage may be increased up to a total of 1.6mg daily, and guidelines for management of asthma permit 2mg daily. Maintenance dose may be less than 400 micrograms daily but should not be below 200 microgram daily. A doe for children is 50-400 micrograms inhaled twice daily. Budesonide is also available for the management of asthma in the form of a dry powder inhaler; doses are 200-800 micrograms daily, as two divided doses or a single dose daily; up to 800 micrograms twice daily may be given to adults if necessary. Patients for whom budesonide from a pressurized inhaler or dry powder formulation is unsatisfactory may use a nebulized solution. The usual adult dose by this method is 1-2 mg inhaled twice daily. This maybe increased if asthma is serious. Maintenance dose are 0.5 to 1 mg inhaled twice daily .For children between 3 months and 12 years, an initial dose is 0.5 to 1 mg twice daily with a maintenance dose of 0.25 to 0.5 mg twice daily.

Budesonide is also given by inhalation as nebulized solution in the management of childhood croup. The usual dose is 2 mg as a single inhaled dose or two doses of 1 mg, given 30 minutes apart.

2.4.6.2. Budesonide is used topically in the treatment of various skin disorders, as a cream, lotion or ointments containing 0.25%. For recommendations concerning the correct use of corticosteroids on the skin, and the rough guide to the clinical potencies of topical corticosteroids.

2.4.6.3. Budesonide is also used intranasal for prophylaxis and treatment of Rhinitis. In the U.K, two nasal spray preparations are available: one containing 100 micrograms per metered spray and one containing 64 micrograms per metered spray. The initial recommended dose for adults and children over 12 years is either two sprays into each nostrils once daily in the morning or one spray into each nostril twice daily.

This maybe subsequently reduced to one spray into each nostril once daily; treatment maybe continued for up to 3 months. In the USA and some other countries, a nasal spray and a nasal inhaler are available. The intranasal dose maybe expressed in multiples of 32 micrograms, which is the quantity of budesonide delivered from nasal adapter. When given from the nasal inhaler, the recommended initial dose for adults and children over 6 years is 4 sprays into each nostril per morning or two sprays into each nostril twice daily, to give a total daly dose of 256 microgram daily. This is reduced to be lowest dose adequate to control symptoms if no benefit is seen after 3 weeks of treatment, Budesonide must be stopped. When given as an aqueous nasal spray , the recommended initial dose for children and adults over 6 years if one spray each nostril once daily (64 micrograms daily), increasing as necessary up to maximum of 256 micrograms daily for adults and 128 micrograms daily for children aged less than 12 years.

Budesonide is also used as a nasal spray in the management of nasal polyps. In UK, for adults and children over 12 years one spray (containing 64 or 100 micrograms) is given into each nostril twice daily for 3 months.

2.4.6.4. Local formulation of Budesonide are used in management of inflammatory bowel disease.[9] In mild to moderate crohns disease affecting the ileum or ascending colon it is given by mouth as modified release capsules intended for topical effect on GI tract. The recommended dose is 9 mg daily for active disease, as either a single dose before breakfast or in 3 divided doses about 30 minutes before meals, depending on the preparations. Treatment is given for up to 8 weeks, and the doses should be reduced to 2 to 4 weeks before discontinuing the therapy. For recurring episodes of active Crohn's disease, an 8 week course may be repeated. After an 8 week course of active disease, Budesonide 6 mg once daily is

recommended for maintenance of clinical reemission for upto 3 months; thereafter, doses are tapered and therapy is stopped as continued treatment has not shown substantial clinical benefit. There is some absorption of budesonide from the GI tract, and the dose may be reduced in patients with a hepatic impairment especially those with cirrhosis. An enema solution containing 0.22% is also available; it is given in bed times for 4 weeks in the treatment of Ulcerative colitis at the dose of 2 mg.

Local formulations of budesonide are also used in the management of collagenous colitis. It is given by mouth as modified released capsules in dose of 3mg 3 times daily for up to 8 weeks. The doses should be reduced gradually during the last two weeks of therapy.

2.4.6.5. Chronic obstructive pulmonary disease:

For discussion of the value of the inhaled corticosteroids in chronic pulmonary disease, include reference to the use of budesonide. The use of the fixed dose combination of budesonide and formetrol in chronic obstructive pulmonary disease has been reviewed.

2.4.6.6. Collagenous colitis:

Budesonide has been used in a few small controlled studies of the management of the collagenous colitis. Treatment course given orally for 6-8 weeks were found to improve symptoms and histology, and short terms benefits has been confirmed by the meta-analysis, although high rate of relapse after stopping treatment have been reported.

2.4.6.7. Cystic fibrosis: is associated with bronchial hyper responsiveness a small study has suggested that inhalation of budesonide 1.6 mg daily for 6 weeks improves hyper responsiveness slightly and leads to improvement in cough and dyspnea. A large study of budesonide given for two successive 3- months treatment period found improved hyper responsiveness and a tend towards slower decline in the lung function.

2.4.6.8. Inflammatory bowel disease: Budesonide has been given as an enema for the treatment of distal ulcerative colitis, in which context its potency and low systemic availability as a modified release oral dosage form for the management of active crohn’s disease. Systemic review has suggested that it is slightly less effective than conventional corticosteroid therapy, but is associated with fever as adverse effect. It has also been investigated for its potential for delaying relapse in quiescent disease, although any benefit appears short term, and another

systemic review concluded that oral modified release budesonide was not effective in preventing relapse during 12 months of treatment. Similarly. Oral budesonide was ineffective in preventing postoperative recurrence after resection for crohn's disease. Oral budesonide has been tried as an alternative to conventional systemic corticosteroids in ulcerative colitis.

2.5. INTRODUCTION TO LIPIDS

2.5.1. GLYCERYL BEHENATE

2.5.1.1 Nonproprietary Names

BP: Glycerol dibehenate

PhEur: Glyceroli dibehenas

USPNF: Glyceryl behenate

2.5.1.2 Synonyms

Compritol 888 ATO; 2,3-dihydroxypropyl docosanoate; docosanoic acid, 2,3-dihydroxypropyl ester; E471; glycerol behenate; glyceryl monobehenate..

2.5.1.3 Chemical Name and CAS Registry Number

Docosanoic acid, monoester with glycerin [30233-64-8] (glyceryl behenate)

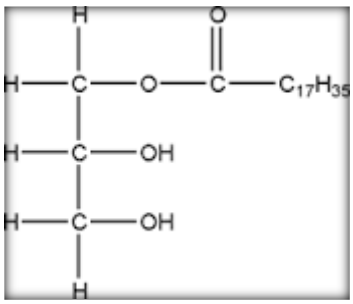
Docosanoic acid, diester with glycerin [94201-62-4] (glyceryl dibehenate)

Docosanoic acid, triester with glycerin [18641-57-1] (glyceryl tribehenate)

2.5.1.4 Empirical Formula and Molecular Weight

$C_{21}H_{42}O_4$ and 358.6

2.5.1.5 Structural Formula



2.5.1.6 Functional Category

Coating agent; tablet binder; tablet and capsule lubricant.

2.5.1.7 Applications in Pharmaceutical Formulation or Technology

Glyceryl behenate is used in cosmetics, foods, and oral pharmaceutical formulations. In cosmetics, it is mainly used as a viscosity-increasing agent in emulsions.

In pharmaceutical formulations, glyceryl behenate is mainly used as a tablet and capsule lubricant and as a lipidic coating excipient. It has been investigated for the encapsulation of various drugs such as retinoids. It has also been investigated for use in the preparation of sustained release tablets; as a matrix-forming agent for the controlled release of water-soluble drugs; and as a lubricant in oral solid dosage formulations, and it can also be used as a hot-melt coating agent sprayed onto a powder.

2.5.1.8 Description

Glyceryl behenate occurs as a fine white powder or hard waxy mass with a faint odor.

2.5.1.9 Stability and storage

Glyceryl behenate should be stored in a tight container, at a temperature less than 35 deg C.

2.5.1.10 Method of Manufacture

Glyceryl behenate is prepared by the esterification of glycerin by behenic acid (C22 fatty acid) without the use of catalysts. In the case of Compritol 888 ATO (Gattefosse'), raw materials used are of vegetable origin, and the esterified material is atomized by spray-cooling.

2.5.1.11 Safety

Glyceryl behenate is used in cosmetics, foods and oral pharmaceutical formulations and is generally regarded as a relatively nonirritant and nontoxic material.

LD50 (mouse, oral): 5 g/kg

2.5.1.12 Handling Precautions

Observe normal precautions appropriate to the circumstances and quantities of material handled. Glyceryl behenate emits acrid smoke and irritating fumes when heated to decomposition.

2.5.1.13 Regulatory Status

GRAS listed. Accepted for use as a food additive in Europe.

Included in the FDA Inactive Ingredients Guide (capsules and tablets). Included in the Canadian List of Acceptable Nonmedicinal Ingredients.

2.5.1.14 Related Substances

Glyceryl palmitostearate.

2.5.2 MEDIUM-CHAIN TRIGLYCERIDES

2.5.2.1 Nonproprietary Names

BP: Medium-chain triglycerides

PhEur: Triglycerida saturata media

USPNF: Medium-chain triglycerides

2.5.2.2 Synonyms

Bergabest; caprylic/capric triglyceride; Captex 300; Captex 355; Crodamol GTC/C; glyceryl tricaprylate/caprate; Labrafac CC; MCToil; Miglyol 810; Miglyol 812; Myritol; Neobee M5; Nesatol; oleum neutrale; oleum vegetable tenue; thin vegetable oil; Waglinol 3/9280.

2.5.2.3 Chemical Name and CAS Registry Number

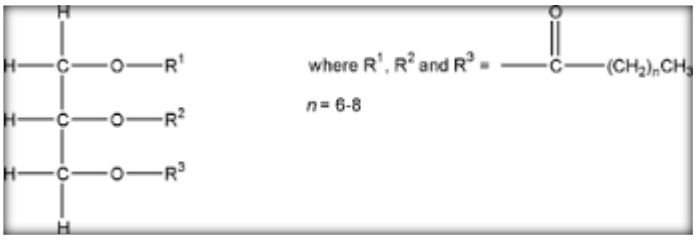
Medium-chain triglycerides [73398-61-5]

2.5.2.4 Empirical Formula and Molecular Weight

Approximately 500 (average)

The PhEur 2005 describes medium-chain triglycerides as the fixed oil extracted from the hard, dried fraction of the endosperm of Cocos nucifera L. or from the dried endosperm *of Elaeis guineenis Jacq*. They consist of a mixture of triglycerides of saturated fatty acids, mainly of caprylic acid and of capric acid. They contain not less than 95% of saturated fatty acids.

2.5.2.5 Structural Formula



2.5.2.6 Functional Category

Emulsifying agent; solvent; suspending agent; therapeutic agent.

2.5.3 INTRODUCTION TO SURFACTANTS

2.5.3.1. POLOXAMER 188

2.5.3.1.1 Nonproprietary Names

BP: Poloxamers

PhEur: Poloxamera

USPNF: Poloxamer

2.5.3.1.2 Synonyms

Lutrol; Monolan; Pluronic; poloxalkol; polyethylene–propylene glycol copolymer; polyoxyethylene–polyoxypropylene copolymer; Supronic; Synperonic.

2.5.3.1.3 Chemical Name and CAS Registry Number

a-Hydro-o-hydroxypoly(oxyethylene) poly(oxypropylene) poly(oxyethylene) block copolymer.

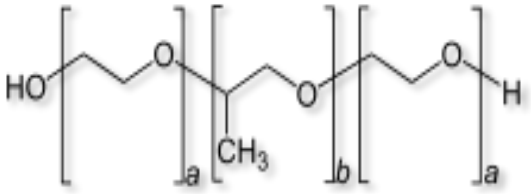
2.5.3.1.4 Empirical Formula and Molecular Weight

The poloxamer polyols are a series of closely related block copolymers of ethylene oxide and propylene oxide conforming to the general formula $HO(C_2H_{4O})_a(C_3H_{6O})_b(C_2H_{4O})_aH$.

The grades included in the PhEur 2005 and USPNF 23 are shown in Table I. The PhEur 2005 states that a suitable antioxidant may be added.

POLOXAMER	PHYSICAL FORM	a	b	AVERAGE MOLECULAR WEIGHT
124	Liquid	12	20	2090-2360
188	Solid	80	27	7680-9510
237	Solid	64	37	6840-8830
338	Solid	141	44	12700-17400
407	Solid	101	56	9840-14600

2.5.3.1.5 Structural formula



2.5.3.1.6 Applications in Pharmaceutical Formulation or Technology

Poloxamers are nonionic polyoxyethylene–polyoxypropylene copolymers used primarily in pharmaceutical formulations as emulsifying or solubilizing agents. The polyoxyethylene segment is hydrophilic while the polyoxypropylene segment is hydrophobic. All of the poloxamers are chemically similar in composition, differing only in the relative amounts of propylene and ethylene oxides added during manufacture. Their physical and surface-active properties vary over a wide range and a number of different types are commercially available.

Poloxamers are used as emulsifying agents in intravenous fat emulsions, and as solubilizing and stabilizing agents to maintain the clarity of elixirs and syrups.

Poloxamers may also be used as wetting agents; in ointments, suppository bases, and gels; and as tablet binders and coatings.

Poloxamer 188 has also been used as an emulsifying agent for fluorocarbons used as artificial blood substitutes and in the preparation of solid-dispersion systems.

More recently, poloxamers have found use in drug-delivery systems.

Therapeutically, poloxamer 188 is administered orally as a wetting agent and stool lubricant in the treatment of constipation; it is usually used in combination with a laxative such as danthron.

Poloxamers may also be used therapeutically as wetting agents in eye-drop formulations, in the treatment of kidney stones, and as skin-wound cleansers.

Poloxamer 338 and 407 are used in solutions for contact lens care.

USES	CONCENTRATION (%)
Fat emulsifier	0.3
Flavour solubilizer	0.3
Fluorocarbon emulsifier	2.5
Gelling agent	15-50
Spreading agent	1
Suppository base	1-5
Tablet coating	4-6
Tablet excipient	5-10
Wetting agent	0.01-5

2.5.3.1.7 Description

Poloxamers generally occur as white, waxy, free-flowing prilled granules, or as cast solids. They are practically odorless and tasteless. At room temperature, poloxamer 124 occurs as a colorless liquid.

2.5.3.1.8 Typical Properties

Acidity/alkalinity: pH = 5.0–7.4 for a 2.5% w/v aqueous solution.

Cloud point: >1008C for a 1% w/v aqueous solution, and a 10% w/v aqueous solution of poloxamer 188.

Density: 1.06 g/cm3 at 258C

Flash point: 260°C

Flowability: solid poloxamers are free flowing.

HLB value: 29 for poloxamer.

Melting point:

52–57 °C

Moisture content: poloxamers generally contain less than 0.5% w/w water and are hygroscopic only at relative humidity greater than 80%.

Solubility: solubility varies according to the poloxamer type

Surface tension: 19.8mN/m (19.8 dynes/cm) for a 0.1% w/v aqueous poloxamer 188 solution at 25 degrees C; 24.0mN/m (24.0 dynes/cm) for a 0.01% w/v aqueous poloxamer 188 solution at 25 degrees C; 26.0mN/m (26.0 dynes/cm) for a 0.001% w/v aqueous poloxamer solution at 25 degrees C.

Viscosity (dynamic): 1000 mPa s (1000 cP) as a melt at 77°C for poloxamer 188.

2.5.3.1.9 Method of Manufacture

Poloxamer polymers are prepared by reacting propylene oxide with propylene glycol to form polyoxypropylene glycol.

Ethylene oxide is then added to form the block copolymer.

2.5.3.1.10 Safety

Poloxamers are used in a variety of oral, parenteral, and topical pharmaceutical formulations and are generally regarded as nontoxic and nonirritant materials. Poloxamers are not metabolized in the body.

Animal toxicity studies, with dogs and rabbits, have shown poloxamers to be nonirritating and non-sensitizing when applied in 5% w/v and 10% w/v concentration to the eyes, gums, and skin.

In a 14-day study of intravenous administration at concentrations up to 0.5 g/kg/day to rabbits, no overt adverse effects were noted. A similar study with dogs also showed no adverse effects at dosage levels up to 0.5 g/kg/day. In a longer term study, rats fed 3% w/w or 5% w/w of poloxamer in food for up to 2 years did not exhibit any significant symptoms of toxicity. However, rats receiving 7.5% w/w of poloxamer in their diet showed some decrease in growth rate.

No hemolysis of human blood cells was observed over 18 hours at 258C, with 0.001–10% w/v poloxamer solutions.

Acute animal toxicity data for poloxamer 188:

LD50 (mouse, IV): 1 g/kg

LD50 (mouse, oral): 15 g/kg

LD50 (mouse, SC): 5.5 g/kg

LD50 (rat, IV): 7.5 g/kg

LD50 (rat, oral): 9.4 g/kg

2.5.3.1.11 Handling Precautions

Observe normal precautions appropriate to the circumstances and quantity of material handled. Eye protection and gloves are recommended.

2.5.3.1.12 Non-proprietary name and its commercial grade

NON-PROPRIETARY NAME	COMMERCIAL GRADE
POLOXAMER 124	L-44
POLOXAMER 188	F-68
POLOXAMER 237	F-108
POLOXAMER 407	F-127

3.1 Literature review on Colon Drug Delivery System:

Elkodairy et al.[20] aimed at formulating matrix tablets for colon-specific drug delivery (CSDD) system of indomethacin (IDM) by liquisolid (LS) technique. A CSDD system based on time-dependent polymethacrylates and enzyme degradable polysaccharides was established. Eudragit RL 100 (E-RL 100) was used as time-dependent polymer, while the LS system was presented by bacterial degradable polysaccharides. Indomethacin-loaded LS systems were prepared using different polysaccharides, viz, guar gum (GG), pectin (PEC), and chitosan (CH), as carriers separately or in mixtures of different ratios of 1:3, 1:1, and 3:1. LS systems that gave excellent results in terms of drug release rate in pH 1.2 and pH 6.8 when compressed into tablets after the addition of the calculated amount of E-RL 100 and lubrication with magnesium stearate and talc (1:9). The results proved improved flowability and compressibility. A sustain drug release over a period of 24 hours was obtained. Stability study proved that the LS system and matrix tablets prepared were stable for over one year and had a minimum shelf life of 2 years.

Newton et al.[21] investigated the effect of HPM E15 V LV in combination with pectin and chitosan on the in-vitro release profile and compression parameters of colon targeted matrix tablets. The test was performed in two dissolution models in various simulated fluids such as pH 1.2, 6, 6.8, 7.2, 5.5. The variation in colonic pH during IBD and less fluid content in colon may pose a limitation in expected drug release in the polysaccharide-based matrices when used alone. The Hydrophilic hydroxyl propyl methylcellulose ether premium polymer (HPMC E15 LV) was used for the formulation, which modified the physical and compression characteristics of granules significantly to improve significantly. The release profile revealed that prepared matrices could control the drug release until the dosage form reaches the colon and also the addition HPMC E15 LV showed the desirable changes in the dissolution profile by its hydrophilic nature since the colon is known for its less fluid content. The colonic fluids to enter into the matrix was found to improve by hydrophilic HPMC E15 LV and confirmed the drug release at the target site from a poorly water soluble polymer such as Chitosan and also from water soluble Pectin. Significant changes was reported in the drug release profile and physicochemical characteristics of the Pectin, Chitosan matrix tablets when a premium polymer HPMC E15 LV added in the formulation design in the optimized concentration. For the examination of drug release characteristics, various drug release mechanisms were used. Combined mechanism of diffusion, erosion, swelling and polymer entanglement was followed

in release profile. Treatment for IBD attracts many patents in novel treatment methods by using novel drug delivery systems.

Rebeiro et al.[22] introduced chitosan/layered double hydroxide (LDH) bio-hybrid beads coated with pectin for controlled release for treating IBD. The treatment involved 5-aminosalicylic acid (5ASA), the most used non-steroid-anti-inflammatory drug (NSAID) for site specific delivery in the colon. Co-precipitation method for the preparation of the hybrid material prepared by intercalation in a layered double hydroxide, were incorporated in a chitosan matrix in order to profit from its muco-adhesiveness. These materials were converted into beads and were further treated with the polysaccharide pectin for the formation of a protective coating that ensures the stability of both chitosan and layered double hydroxide at the acid pH of the gastric fluid. It was reported that the composite beads presenting the pectin coating were stable to swelling by water and a control release of drug was achieved along their passage through the simulated gastrointestinal tract in in vitro experiments, due to their resistance to pH changes. On the basis of the results, the pectin-chitosan/LDH-5ASA bio-nanocomposite beads was proposed as an effective candidates for the colon-targeted delivery of 5ASA which would treat IBD and least side effects.

Makhlof et al.[23] developed pH-Sensitive nanospheres using polymeric mixtures of poly (lactic-co-glycolic) acid (PLGA) and a pH-sensitive methacrylate copolymer. Budesonide, a non-halogenated drug which is a proven topically active corticosteroid, was used as a model drug. In-vivo studies were performed on Trinitrobenzenesulfonic acid (TNBS) colitis rat model, in comparison with conventional enteric microparticles. In addition, the colon targeting properties, systemic bioavailability, and specific uptake by the inflamed colon mucosa were evaluated using coumarin- 6 (C-6)-loaded nanospheres. The prepared nanospheres resulted in strongly pH-dependent drug release properties in acidic and neutral pH values followed by a sustained release phase at pH 7.4. Animal experiments revealed the efficient therapeutic efficiency of BSD-loaded nanospheres in alleviating the conditions of TNBS-induced colitis model. The in vivo studies using C-6-loaded nanospheres displayed higher colon levels and lower systemic availability of the fluorescent marker when compared with simple enteric coating. Moreover, quantitative analysis of the fluorescent marker and confocal laser scanning studies showed strong and specific adhesion of the nanospheres to the ulcerated and inflamed mucosal tissue of the rat colon. The proposed nanospheres system was concluded to have

improved properties of pH sensitivity, controlled release, and particulate targeting that could be useful for colon-specific delivery in inflammatory bowel disease.

Pertuit et al.[24] prepared 5-amino salicylic acid (5ASA) loaded nanoparticles in order to investigate their therapeutic potential in the treatment of inflammatory bowel disease. 5ASA was covalently bound to poly (caprolactone) and then the oil/water emulsification or nanoprecipitation methods were used for the nanoparticles formulation. In their study, the particle diameters were either 200 or 350 nm for emulsification or nanoprecipitation, respectively. In-vitro drug release demonstrated a significant drug retention inside the NP formulation. Caco-2 and HEK cell culture was used to evaluate toxicity of the different formulations was evaluated on Caco-2 and HEK cell culture which revealed slight increased adhesion for 5ASA grafted nanoparticle in comparison to blank nanoparticle. In-vivo, clinical activity score and myeloperoxidase activity was found to decrease after administration of all 5ASA containing formulations. NP formulations helped cut down the dose of 5ASA significantly. The oral nanoparticle formulations demonstrated their therapeutic potential and appear to be a promising approach for the therapy of inflammatory bowel disease.

Lamprecht et al.[25] developed bio-degradable nanoparticles for site specific drug delivery for the treatment of inflammatory bowel disease. Rolipram, an anti-inflammatory model drug, was used as model drug that was entrapped within poly (lactic-coglycolic acid) nanoparticles, with once a day oral dose for five consecutive days. A clinical activity score and myeloperoxidase activity assessed to check the severity of the inflammation, whereas an adverse effect index reflected the remaining neurotropic effect of rolipram resulting from its systemic absorption. Trinitrobenzenesulfonic acid colitis model was used and male Wistar rats were used for the experiment. The nanoparticle formulations proved to be as efficient as the drug in solution in treating the animals in the experimental colitis. The clinical activity score and myeloperoxidase activity decreased significantly after the oral administration of rolipram nanoparticles or solution. For the next 5 days when animals were kept without drug treatment the drug solution group, a strong relapse was observed, whereas the nanoparticle groups continued to show reduced inflammation levels. The rolipram solution group had a high adverse effect index, whereas the rolipram nanoparticle groups proved their potential to retain the drug from systemic absorption as proved by a significantly reduced index. This new delivery system enabled the drug to accumulate in the inflamed tissue with higher efficiency than when given

as solution. The nanoparticle deposition in the inflamed tissue should be given particular consideration in the design of new carrier systems for the treatment of inflammatory bowel disease.

Serpe et al.[26] prepared Dexamethasone loaded solid lipid nanoparticle SLN (dexa SLN) using warm microemulsion made up of stearic acid as lipidic matrix, sodium taurocholate as cosurfactant, Epikuron 200® (containing about 95% of soy phosphatidylcholine) as surfactant and water. The warm microemulsion was poured in cold water (2–3 °C) and the dexa SLN dispersion was washed by tangential filtration and then sterilized by autoclaving (30 min, 121 °C). Their data showed that dexamethasone loaded SLN determined a more than 90% decrease in the IL-1β and TNF-α mRNA expression compared to control cells, at the highest concentration, at 24 h. Also, the SLN formulation at the highest concentration stimulated TNF-α mRNA expression at 4 and 24 h, but inhibited protein secretion. When the two dexamethasone formulations were compared, the IL-1β and TNF-α production was significantly reduced, both at 4 and 24 h, with the SLN formulation, which was more evident for the highest concentration. Moreover, free dexamethasone led to a dose-dependent inhibition of TNF-α production, reaching its maximum at 250 nM with a 25% reduction with the free formulation and a 90% reduction with the SLN formulation, compared to the control. They concluded that the incorporation of butyrate and dexamethasone into SLN had the ability to enhance the anti-inflammatory efficacy of the drugs for treating IBD patients stimulated by LPS and that this effect may well be related to the favored uptake of the SLN formulation.

Dubey et al.[27] developed a colon targeted drug delivery system of 5 Aminosalicylic acid and Camylofine dihydrochloride. Chitosan microspheres were prepared separately for the drugs using emulsion method followed by enteric coating with EudragitS-100. The in vitro drug release was investigated in different simulated GIT medium. The drug release in PBS (pH7.4) and simulated gastric fluid showed almost similar pattern and rate, whereas a significant increase in drug) was observed in medium containing 3% rat caecal matter, after 24 h. Enzymatic induction on rats was done by orally administering 1 mL of 1% w/v dispersion of chitosan for 5 days and release rate studies were conducted in SCF with 3% w/v of caecal matter. An improved drug release (i.e., 92.3 +/- 3.81 and 95.5 +/- 3.52% 5-ASA and Camylofine, respectively) was observed after 24 h in dissolution medium containing 3% caecal content obtained from enzyme induced animals. In vivo data proved that microspheres

delivered most of its drug load (76.55 +/- 2.13%) to the colon after 9 h, which shows its site specific potential to the colon. It is concluded that orally administered microspheres of both drugs can be used together for the specific delivery of drug to the colon and reduce symptoms of ulcerative colitis.

Davis et al.[27] prepared Solutions, small pellets, and single units (matrix tablets and osmotic pumps). Solutions, small pellets, and single units (matrix tablets and osmotic pumps) were administered with different amounts of food in the stomach, ranging from fasted state to heavy breakfast and were compared. It was found that the gastric emptying was affected by the nature of the dosage form and the presence of food in the stomach. Solutions and pellets were emptied even when the stomach was in the digestive mode, while single units were retained for long periods of time, depending on the size of the meal. In contrast, measured intestinal transit times were independent of the dosage form and fed state. The small intestinal transit time of about three hours has implications for the design of dosage forms for the sustained release of drugs in specific positions in the gastrointestinal tract.

Bhol et al.[28] prepared Nanocrystalline silver (NPI 32101) to demonstrate its antimicrobial and anti-inflammatory properties. The purpose of this study was to evaluate the effect of NPI 32101 in a rat model of ulcerative colitis and the possible related mechanisms of action of the effects observed. NPI 32101, 4 mg/kg intracolonically or 40 mg/kg orally, significantly reduced colonic inflammation compared to the placebo and no-treatment groups. Sulfasalazine (100 mg/kg), either intracolonically or orally, also reduced colonic inflammation. NPI 32101 significantly suppressed the expression of matrix metalloproteinase (MMP)-9, tumor necrosis factor (TNF)-alpha, interleukin (IL)-1beta, and IL-12, whereas sulfasalazine suppressed MMP-9, IL-1beta, and TNF-alpha, but not IL-12, compared to placebo. MMP-9 activity was decreased by NPI 32101 and sulfasalazine. NPI 32101 administered intracolonically or orally decreases ulcerative colitis in a rat model and is as effective as sulfasalazine. NPI 32101 treatment suppresses the expression and activity of MMP-9 and the expression of TNF-alpha, IL-1beta, and IL-12, mechanisms by which NPI 32101 may exert its anti-inflammatory effects. NPI 32101 may have therapeutic potential for treatment of ulcerative colitis.

Laroui et al.[29] developed nanoparticles (NPs) of an anti-inflammatory tripeptide Lys-Pro-Val (KPV) using double-emulsion/solvent evaporation. KPV was loaded into the NPs during the first emulsion of the synthesis process. To target KPV to the colon, loaded NPs (NP-KPV) were encapsulated into a polysaccharide gel containing alginate and chitosan as polymers. The effect of KPV-loaded NPs on inflammatory parameters was evaluated in in vitro as well as in the dextran sodium sulfate-induced colitis mouse model. NPs (400 nm) did not harm cell viability or barrier functions. A swelling degree study showed that alginate-chitosan hydrogel containing dextran-fluorescein isothiocyanate-labeled NPs collapsed in the colon. Once delivered, NPs quickly released KPV on or within the closed area of colonocytes. The inflammatory responses to lipopolysaccharide were reduced in Caco2-BBE (brush border enterocyte) cells exposed to NP-KPV compared with those exposed to NPs alone, in a dose-dependent fashion. Mice given dextran sodium sulfate (DSS) followed by NP-KPV were protected against inflammatory and histologic parameters, compared with mice given only DSS. Nanoparticles are hence a versatile drug delivery system that could overcome physiologic barriers and target anti-inflammatory agents such as the peptide KPV to inflamed areas. KPV can be delivered at a concentration that is 12,000-fold lower than that of KPV in free solution, but with similar therapeutic efficacy by the use of NPs.

Tahara et al.[30] prepared Chitosan (CS)-modified poly(D,L-lactide-co-glycolide) (PLGA) nanospheres (NS) and evaluated the use with a nuclear factor kappa B (NF-κB) decoy oligonucleotide (ODN) oral delivery system in an experimental colitis model. Decoy ODN-loaded PLGA NS were prepared by an emulsion solvent diffusion method, and the physicochemical properties of NS were investigated. CS-modified PLGA NS (CS-PLGA NS) had a positive zeta potential, whereas the zeta potential of unmodified PLGA NS (plain-PLGA NS) was found negative. Decoy ODN uptake studies with Caco-2 cells using confocal laser scanning microscopy (CLSM) indicated that CS-PLGA NS were more effectively taken up by the cells than plain-PLGA NS. Decoy ODN-loaded CS-PLGA NS were able to improve the stability of ODN against DNase I or an acidic medium, such as gastric juice. Daily oral administration of CS-PLGA NS in a rat model significantly improved dextran sulfate sodium-induced diarrhea, bloody feces, shortening of colon length, and myeloperoxidase activity. Decoy ODN-loaded CS-PLGA NS were deposited specifically and adsorbed on the inflamed mucosal tissue of the UC model rat. Data suggested that CS-PLGA NS provide an effective means of colon-specific oral decoy ODN delivery in UC.

Moulari et al.[24] developed 5-Amino salicylic acid (5ASA) loaded silica nanoparticles (SiNP) proposed as drug delivery system for specific accumulation in inflamed colonic tissues allowing for selective medication delivery to such inflammation sites. The drug was covalently bound to SiNP by a four-step reaction process. In-vitro toxicity of modified SiNP was tested in appropriate cell culture systems, while targeting index and therapeutic efficiency were evaluated in a pre-existing colitis in mice. Particle size was found to be around 140 nm after final surface modification. In-vitro drug release demonstrated significant drug retention inside the NP formulation. Toxicity of the different formulations was studied on in-vitro cell culture exhibiting a lowered toxicity for 5ASA when bound to SiNP. In-vivo, oral SiNP were found to accumulate selectively in the inflamed tissues allowing for significant amounts of drug load. SiNP demonstrated their therapeutic potential by significantly lowering the therapeutically necessary drug dose when evaluating clinical activity score and myeloperoxidase. SiNP allow to combine advantages from selective drug targeting and prodrugs appearing to be a promising therapeutic approach for clinical testing in the therapy of inflammatory bowel disease.

Zhou et al.[31] prepared tripterine loaded nanostructured lipid carriers by the solvent evaporation method. The drug entrapment efficiency, particle size and zeta potential of the tripterine-loaded NLCs were 78.64 ± 0.37 %, 109.6 ± 5.8 nm and −29.8 ± 1.3 mV, respectively. The morphology of tripterine-loaded NLCs was found to be spherical morphology with smooth surface under the transmission electron microscope (TEM). The crystallization of drug in NLC was investigated by differential scanning calorimetry (DSC). The drug was confirmed to be in an amorphous state in the NLC matrix. Based on the in vitro release study, it was confirmed that the tripterine-loaded NLCs showed a delayed release profile of tripterine. To study the absorption of tripterine solution and tripterine-loaded NLCs, rat intestinal perfusion model was used. The results showed that the effective permeability of tripterine-loaded NLCs in the duodenum, jejunum, ileum and colon was approximately 2.1, 2.7, 1.1, 1.2-fold higher than that of tripterine solution, respectively. The percent absorption of 10 cm of intestine of tripterine-loaded NLCs in the duodenum, jejunum, ileum and colon was found to be approximately 2.2, 2.3, 1.2, 1.3-fold higher than that of tripterine solution, respectively. The intestinal toxicity of tripterine formulated in the NLCs was investigated and compared with the tripterine solution by the MTT assay with Caco-2 cell models. According to the result, the tripterine-loaded NLC greatly decreased the cytotoxicity of the drug. In conclusion, the NLC formulation remarkably improved the absorption of tripterine and showed a better biocompatibility.

Ispas et al.[32] used two drugs viz. mesalamine (MES) and sucralfate (SUC) and investigated the possible utilization as fixed-dose combination product. The anti-inflammatory action of MES in association with bio-adhesiveness and mucosal healing properties of SUC were considered promising for the development of a new compound containing both molecules, aimed as an improved treatment of ulcerative colitis. They investigated the ability of the two active agents to interact and generate a new and stable entity via self-assembling. Spray-drying was used to process the active ingredients from an aqueous mixture where the ratio MES:SUC was in the range 25:75, 50:50, and 75:25. The structural data had shown that MES and SUC interacted and led to formation of complexes with properties differing from those of each separate active agent and from their physical blends. ^{1}H NMR results indicated that complexation occurred when the aqueous suspensions of drugs were mixed, prior to spray-drying. Drug-drug self-assembling was the driving mechanism in the formation of the new entity. Based on the structural data, a hypothetical structure of the complex was proposed. Co-processing of MES and SUC represented a simple and useful procedure to prepare new self-assembled compounds by valorizing the ionic interactions between the two entities. Preliminary studies with oral solid dosage forms based on MES-SUC complexes tested in vitro had shown a controlled MES release, opening the perspective of a new colon-targeted delivery system and a novel class of compounds with therapeutic application in inflammatory bowel diseases.

Nandy et al.[33] designed, developed and optimized micro particulates system of Celecoxib, non-steroidal anti-inflammatory drug (NSAID), for colon specific delivery of celecoxib for both local (in prophylaxis of colorectal adreno-carcinoma) and systemic (in chrono-therapeutic treatment of arthritis) therapy. Their aim was to elucidate the effect of formulation variables e.g., amount of eudragit polymer, surfactant concentration and agitation speed on in-vitro release profiles, drug entrapment efficiency and particle size of micro-particulates system of celecoxib. A combination of ethyl cellulose (EC) and eudragit RS100/eudragit S100 was used to prepare the microspheres using a novel quasi emulsion solvent diffusion technique. Developed formulations were characterized and evaluated on the basis of FTIR, thermal, particle size, SEM and XRD analysis. The formulation variables were optimized by response surface methodology. Best optimized delayed release formulation was further subjected to the in vivo x-ray studies to evaluate the site specificity. It was found that in-vitro release decreased significantly ($p<0.05$) with increase in amount of eudragit polymer but increased significantly

($p<0.05$) with an increase in surfactant concentration and stirring speed. FTIR study indicated that no strong chemical interaction took place between the drug and excipients of prepared formulations. DSC and XRD studies indicated that drug was present in the amorphous state. The X-ray photographs were taken and were found to reveal that the swelling layer eroded from the outer surface and a size reduction was seen after 6 hours when optimized microspheres reached the site of colon. Therefore, their approach suggested that the combination of eudragit S100 and ethyl cellulose microspheres may be useful for the delivery of maximum amount of celecoxib in intact form to the colon.

3.2 Literature review on Budesonide:

Ali et al.[34] evaluated Budesonide loaded nanoparticles with pH-sensitive coating for targeted delivery in the treatment of inflammatory bowel disease. Budesonide was entrapped in poly (lactic-co-glycolic) acid nanoparticles by an oil in water (O/W) emulsion technique. A second batch of the same nanoparticles was prepared by an additional coat of pH-sensitive methyl-methacrylate-copolymer. The in-vitro studies were performed and it confirmed that the pH-sensitive coating prevented premature drug release at acidic pH and only releases the drug at neutral to slightly alkaline pH. The efficacy of both coated and plain nanoparticle formulations were studied in different acute and chronic colitis mouse models, also in comparison to an aqueous solution of the drug. It was found that delivery by coated PLGA nanoparticles alleviated the induced colitis significantly better than by plain PLGA particles, which was already more effective than treatment with the same dose of the free drug. The furnished data elaborated the potential of polymeric nano-carriers for targeted drug delivery to the inflamed intestinal mucosa.

Leonard et al.[35] screened Budesonide Nano-formulations for Treatment of Inflammatory Bowel Disease in an inflamed 3D Cell-Culture. Their laboratory had developed an in vitro model for the inflamed intestinal mucosa observed in chronic IBD, which allows high-throughput screening of anti-inflammatory drugs and their formulations. The in vitro model consisted of intestinal epithelial cells, human blood-derived macrophages, and dendritic cells that are stimulated by the inflammatory cytokine interleukin-1β. In their study, evaluation of the efficacy and deposition of budesonide, an anti-inflammatory drug was studied in three different pharmaceutical formulations: (1) a free drug solution, (2) encapsulated into PLGA nanoparticles, and (3) encapsulated into liposomes. The in vitro model of the inflamed intestinal mucosa demonstrated its ability to differentiate therapeutic efficacy among the formulations while maintaining the convenience of conventional in vitro studies and adequately representing the complex pathophysiological changes observed in vivo.

Crcarevska et al.[36] prepared a targeted delivery system for inflammatory bowel diseases, chitosan-Ca-alginate microparticles efficiently loaded with budesonide (BDS), designed by using one-step spray-drying process. The microparticles were eudragit coated and examined for *in vivo* efficacy. Experimental colitis was induced by rectal instillation of 2, 4, 6-trinitrobenzene sulphonic acid (TNBS) into male Wistar rats. Drugs were administered by oral

gavage daily for 5 days. Colon/body weight ratio, gross morphological and histological, evaluation, and clinical activity score were determined as inflammatory indices. Individual clinical and histological evaluation showed that colitis severity was suppressed the most greatly in order BDS < BDS/C-Ca-A < E-BDS/C-Ca-A. Clinical activity score decreased in the same order. Statistical analyses of total score points indicated that the incorporation of BDS in microparticles had significant differences in favor of efficacy of designed delivery system with muco-adhesive and controlled release properties.

Krishnamachari et al.[37] developed a pH and time dependent micro particulate system consisting of non-enzymatically degrading poly(d-lactide-*co*-glycolide) (PLGA) core and delivering budesonide site specifically to distal ileum and colon. Budesonide-loaded microparticles were fabricated using solvent evaporation technique and formulation variables studied included different molecular weight grades of PLGA polymer as well as concentration of polymer, surfactant and drug. Eudragit S-100, an enteric polymer, was then used to form a coating on the surface of budesonide-loaded PLGA microparticles for site specific delivery to the distal ileum and colon. Budesonide-loaded PLGA microparticles prepared from various formulation parameters showed mean encapsulation efficiencies ranging between 50% and 85% and mean particle size ranging between 10 and 35 micrometer. In vitro release kinetics studies showed a biphasic release pattern with an initial higher release followed by a slower drug release. Increasing polymer and surfactant concentrations exhibited sharply contrasting drug release profiles, with increasing polymer concentrations resulting in a lower drug release and vice versa. The budesonide-loaded PLGA microparticles coated with Eudragit® S-100 coating showed a decrease in entrapment efficiency with an accelerated *in vitro* drug release. Moreover, complete retardation of drug release in an acidic pH, and, once the coating layer of enteric polymer was dissolved at higher pH (7.4 and 6.8), a controlled release of the drug from the microparticles were observed. From the results of their investigation, the application of double microencapsulation technique employing PLGA matrix and Eudragit® S-100 coating shows promise for site specific and controlled delivery of budesonide in Crohn's disease.

Naeem et al.[9] designed colon targeted drug delivery of budesonide using dual pH and time dependent polymeric nanoparticles using oil-in-water emulsion/solvent evaporation method. Single pH-dependent drug delivery systems had been widely used for colon-targeted delivery, but their efficiency is often hampered by the variation in gut pH. To overcome the limitation

of single pH-dependent delivery systems, they developed and evaluated the therapeutic potential of budesonide-loaded dual pH/time-dependent nanoparticles (NPs) for the treatment of colitis. Eudragit FS30D was used as a pH-dependent polymer, and Eudragit RS100 as a time-dependent controlled release polymer. Single pH-dependent NPs (pH_NPs), single time-dependent NPs (Time_NPs), and dual pH/time-dependent NPs (pH/Time_NPs) were prepared using the oil-in-water emulsion method. The physicochemical properties and drug release profiles of these NPs in gastrointestinal (GI) tract conditions were investigated. The therapeutic potential and in vivo distribution of the NPs were evaluated in a dextran sulfate sodium (DSS)-induced colitis mice model. The pH/Time NPs prevented a burst drug release in acidic pH conditions and showed sustained release at a colonic pH. The in vivo distribution study in the mice GI tract demonstrated that pH/Time NPs were more efficiently delivered to the inflamed colon than pH NPs were. Compared to the single pH NPs-treated group, the pH/Time NPs-treated group showed increased body weight and colon length and markedly decreased disease activity index, colon weight/length ratios, histological damage, and inflammatory cell infiltration in colon tissue. Their results demonstrated that the dual pH/time-dependent NPs were an effective oral colon-targeted delivery system for colitis therapy.

3.3 Literature review on Nanostructured Lipid Carriers (NLCs)

Beloqui et al.[2] prepared Budesonide loaded nanostructured lipid carriers in order to reduce inflammation in murine colitis model. Size reduction technique used was high pressure homogenization. It was found that the levels of cytokines decreased. Cell line study was carried out to study the uptake of the nanoparticles. Coumarin 6 localization studies were performed to check the distribution of nanoparticles loaded with Coumarin 6 in murine model. It was found that blank NLCs or the drug suspension didn't have any significant effect while Budesonide (BDS)-loaded nanostructured lipid carriers reduced inflammation in murine DSS-induced colitis. Hence it can be concluded that the NLCs loaded with corticosteroid can effectly treat IBD.

Meissner et al.[23] fabricated tacrolimus-loaded PLGA nanoparticles. PLGA nanoparticles were found to substantiate the amount of drug that reached the inflamed site, with a lower total amount of drug delivered. Due to the risk for severe adverse effects and to achieve increased efficiency and tolerability, a selective delivery to the site of inflammation is of interest. acrolimus nanoparticles were tried for their productivity in neighborhood treatment of kindled inside tissue in IBD. Drug stacked NP were set up from either biodegradable poly (lactide-co-glycolide) (PLGA) or pH-touchy Eudragit P-4135F by utilizing a straightforward oil/water emulsification strategy. Tests on the remedial impact were led utilizing dextran sulfate model colitis as a part of mice accepting tacrolimus definitions every day for 12 days. Clinical movement score and myeloperoxidase action diminished while colon length expanded altogether after organization of all tacrolimus containing definitions. Oral NP details were less productive in moderating the test colitis contrasted with subcutaneous medication arrangement (PLGA: 7.88 ± 0.83; P-4135F: 7.48 ± 0.42; subcutaneous: 5.27 ± 0.68 U/mg) yet better than medication arrangement given by oral course (oral: 8.75 ± 1.34; untreated colitis control: 9.95 ± 0.92, all U/mg tissue). Tacrolimus arrangement bunches (oral/subcutaneous) displayed expanded levels of antagonistic impacts, while both NP bunches showed their capability to lessen nephrotoxicity. Both procedures indicated comparative alleviating impacts while nephrotoxic unfavorable impacts were somewhat less communicated with pH-sensitive NP.

4. MATERIALS AND METHODS

4.1 Materials used

Budesonide was received as a kind gift from Zydus Healthcare, Ahmedabad. Compritol ATO 888 was received as a gift from Hallstar, GmBH, Germany and Labrafac WL 1349 was gained as a gift sample from Abitech Corporation, Mumbai. Eudragit® S100 was received as a gift sample from Evonik Röhm GmbH, Darmstadt, Germany. Span 80, Stearyl amine and Polysorbate 80 were purchased from CDH, Mumbai. Poloxamer-188 was purchased from Torrent Research Centre, Ahmedabad. Lipo-PGO was purchased from Lipoid Group, Germany. Bovine serum albumin (BSA), Folin's reagent-ciocalteau reagent, Hexadecyltrimethylammoniumbromide (CTAB) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was purchased from Hi-media, Pune. D-mannose was purchased from Ottokemi, Mumbai. Oxazolone (4-Ethoxymethylene-2-phenyl-2-oxazolin-5-one) was bought from Sigma Aldrich Chemie, GmBH, USA.

4.2 METHODS

4.2.1 Identification of Budesonide

4.2.1.1 Melting point of Budesonide

The Thiel's tube method of melting point determination in liquid paraffin was used in the present investigation. The melting point of Budesonide was found to be **225-232° C.** The reported standard value of melting point of Budesonide is **226° C**. The observed value is closer to the standard value. This indicated good purity of drug sample.

Table 4.1 Melting point report of Budesonide

Standard melting point	Observed melting point
226° C	225-232° C

4.2.1.2 Determination by UV spectroscopy[38]

10µg/ml solution of the drug was prepared in methanol and resultant solution was scanned in the range of 200 nm to 350 nm using Schimadzu-1800 double beam UV/VIS spectrophotometer. The absorption maxima was found to be 243 nm. The reported absorption maxima value for Budesonide is 254 nm.

Table 4.2 λ_{max} report of Budesonide in methanol

Reported **λmax**	Observed **λmax**
254 nm	243 nm

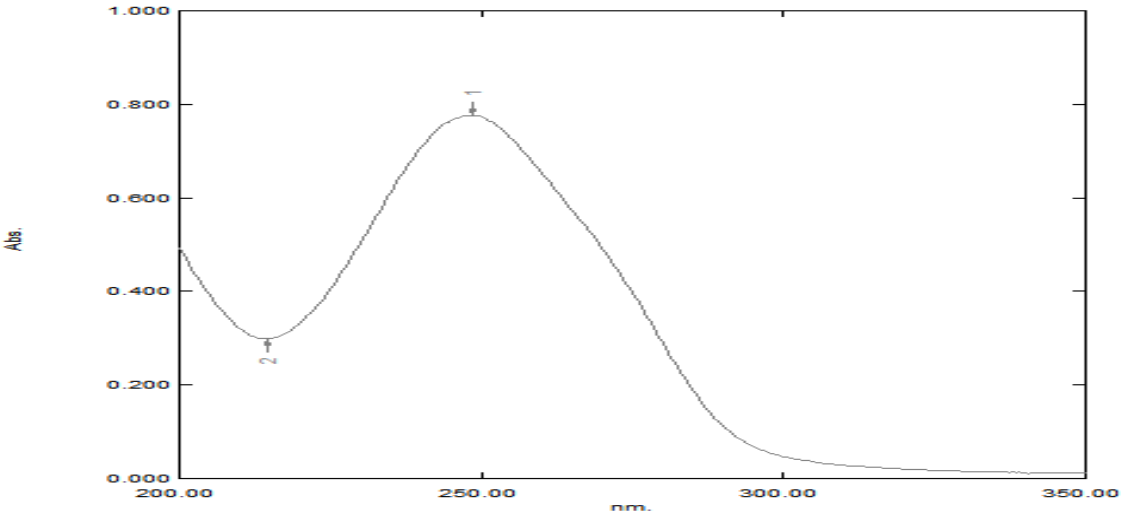


Figure 4.1 U.V spectra of Budesonide in methanol

4.2.1.3 Determination by IR spectroscopy (FTIR)

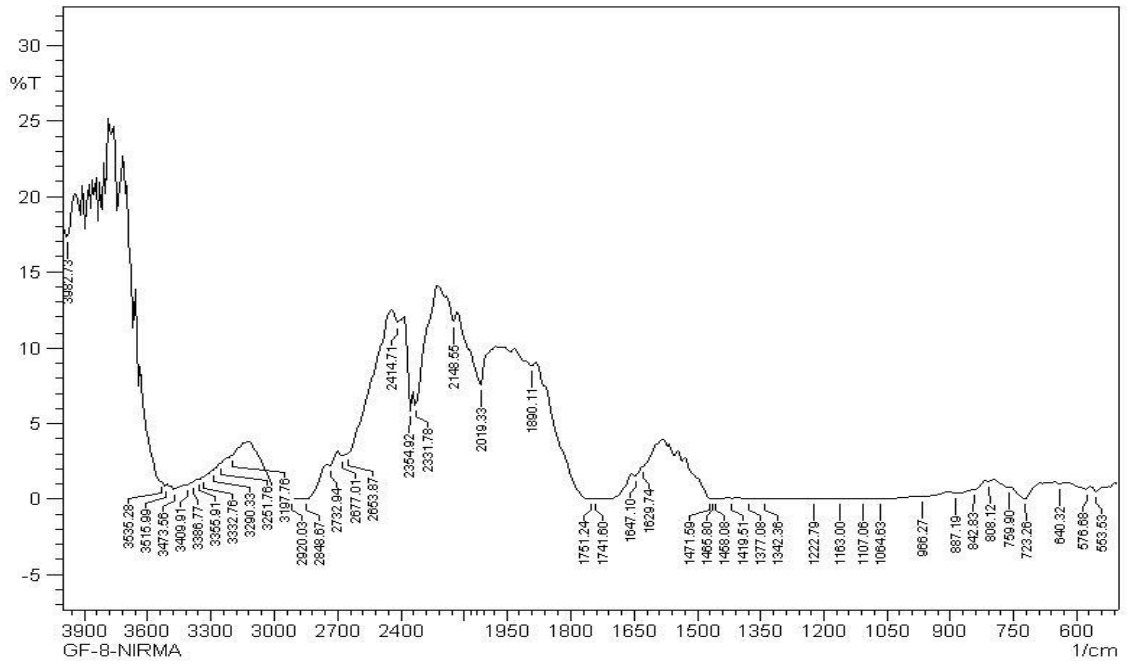


Figure 4.2 IR report of Budesonide

Table 4.3 Interpretation of IR spectra

Functional Groups	Wavenumber (cm-1)
C=O	1741.6
C=C	1647.10
C-H (Aliphatic)	2920.3
C-H (Aromatic)	3197.76
C-O	1064.63
O-H	3473.56
C-C	966.27

4.2.2 Establishment of calibration curve of Budesonide in methanol and different buffer media

4.2.2.1 Establishment of calibration curve of Budesonide in methanol

50 mg of drug was accurately weighed and dissolved in 50 ml of methanol. From this standard stock (1000µg/ml) different concentrations of solutions ranging from 6µg to 24µg were prepared. The absorbance of standard solutions were measured at 243 nm using double beam UV-VIS spectrophotometer and plot of the average absorbance vs concentration was established.

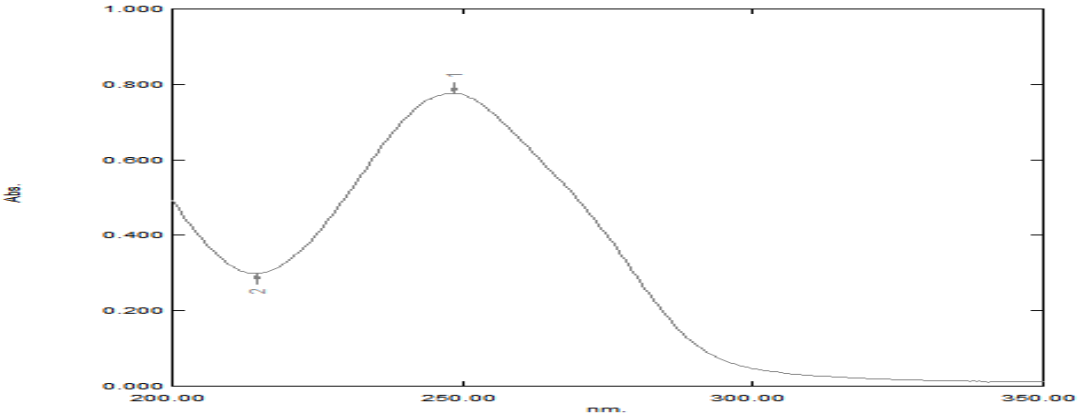


Figure 4.3 (a) U.V spectra of Budesonide in methanol

Table 4.4 Calibration curve of Budesonide in methanol

Sr No	Concentration (µg/ml)	Absorbance I	Absorbance II	Absorbance III	Average Absorbance
1	0	0	0	0	0
2	6	0.226	0.22	0.214	0.22
3	8	0.291	0.288	0.284	0.2875
4	10	0.37	0.370	0.36	0.365
5	12	0.437	0.441	0.438	0.4375
6	14	0.498	0.508	0.517	0.5075
7	16	0.588	0.593	0.598	0.593
8	18	0.668	0.660	0.651	0.6595
9	20	0.721	0.710	0.698	0.7095
10	22	0.78	0.801-	0.819	0.7995
11	24	0.865	0.869	0.873	0.869

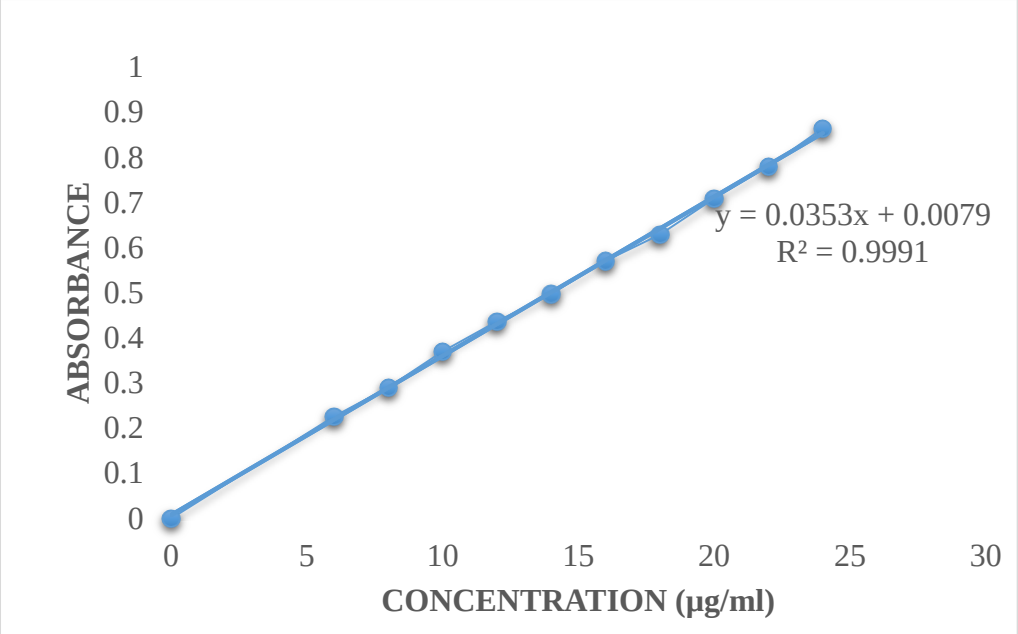


Figure 4.3 (b) Calibration curve of Budesonide in methanol

Regression Analysis:

Table 4.5 Regression analysis of Budesonide in methanol

Regression Parameters	**Values**
Correlation Co-efficient	0.9991
Intercept	0.0079
Slope	0.0353

4.2.2.2 Establishment of calibration curve of budesonide in pH 1.2 (0.1N Hydrochloride buffer)

50 mg of drug was accurately weighed and dissolved in 50 ml Methanol. 5 ml of this solution was withdrawn and diluted with 50 ml pH 1.2 buffer (0.1N hydrochloride buffer). Different concentration were prepared within a range of 6µg/ml to 24µg/ml. The absorbance of the prepared solutions were taken at a maximum wavelength 243 nm.

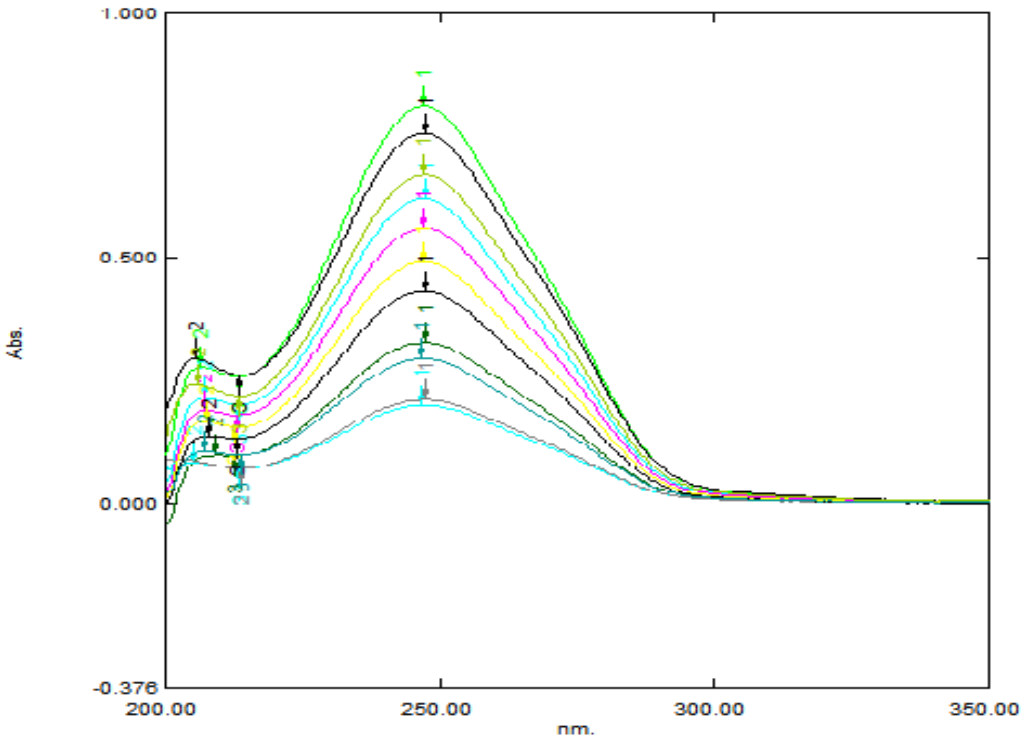


Figure 4.4(a) Calibration Curve of Budesonide in pH 1.2 (0.1 N HCl)

Table 4.6 Calibration curve of Budesonide in pH 1.2 (0.1 N HCl)

Sr No	Concentration (µg/ml)	Absorbance I	Absorbance II	Absorbance III	Average Absorbance
1	0	0	0	0	0
2	6	0.204	0.205	0.204	0.2045
3	8	0.276	0.287	0.282	0.2815
4	10	0.341	0.345	0.343	0.343
5	12	0.414	0.421	0.418	0.418
6	14	0.482	0.479	0.481	0.4805
7	16	0.542	0.546	0.544	0.544
8	18	0.596	0.604	0.603	0.601
9	20	0.678	0.671	0.674	0.675
10	22	0.744	0.745	0.746	0.745
11	24	0.835	0.828	0.832	0.832

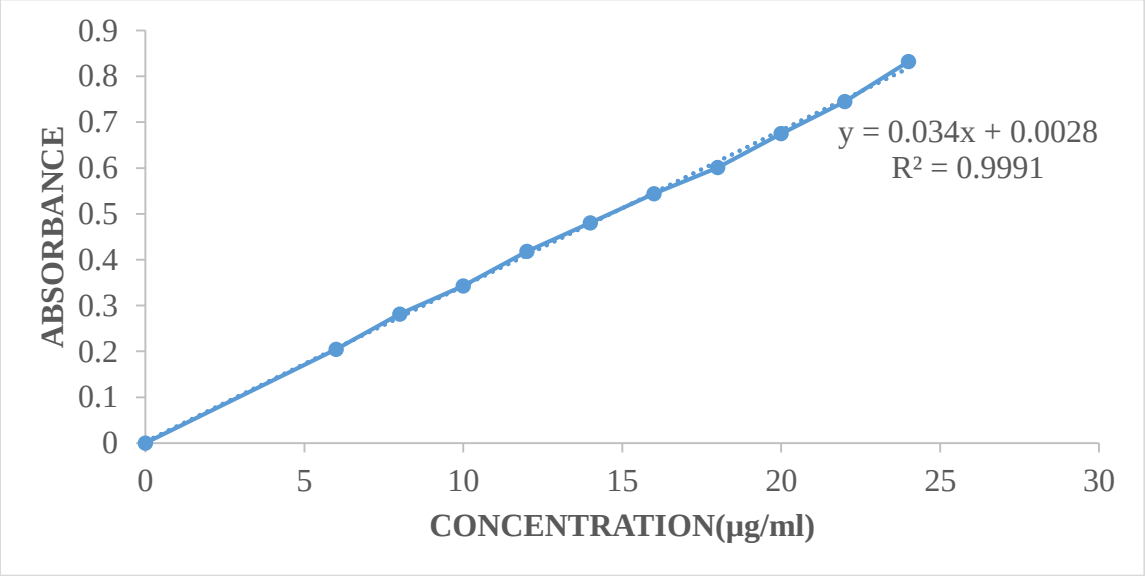


Figure 4.4(b) Calibration curve of Budesonide in pH 1.2 (0.1N HCl)

Regression Analysis

Table 4.7 Regression analysis of Budesonide in pH 1.2 (0.1N HCl buffer)

Regression Parameters	**Values**
Correlation Co-efficient	0.9991
Intercept	-0.0028
Slope	0.0343

4.2.2.3 Establishment of calibration curve of Budesonide in Phosphate buffer pH 6.0

50 mg of drug was accurately weighed and dissolved in 50 ml Methanol. 5 ml of this solution was withdrawn and diluted with 50 ml phosphate buffer pH 6.0. Different concentration were prepared within a range of 6µg/ml to 20µg/ml. The absorbance of the prepared solutions were taken at a maximum wavelength 243 nm.

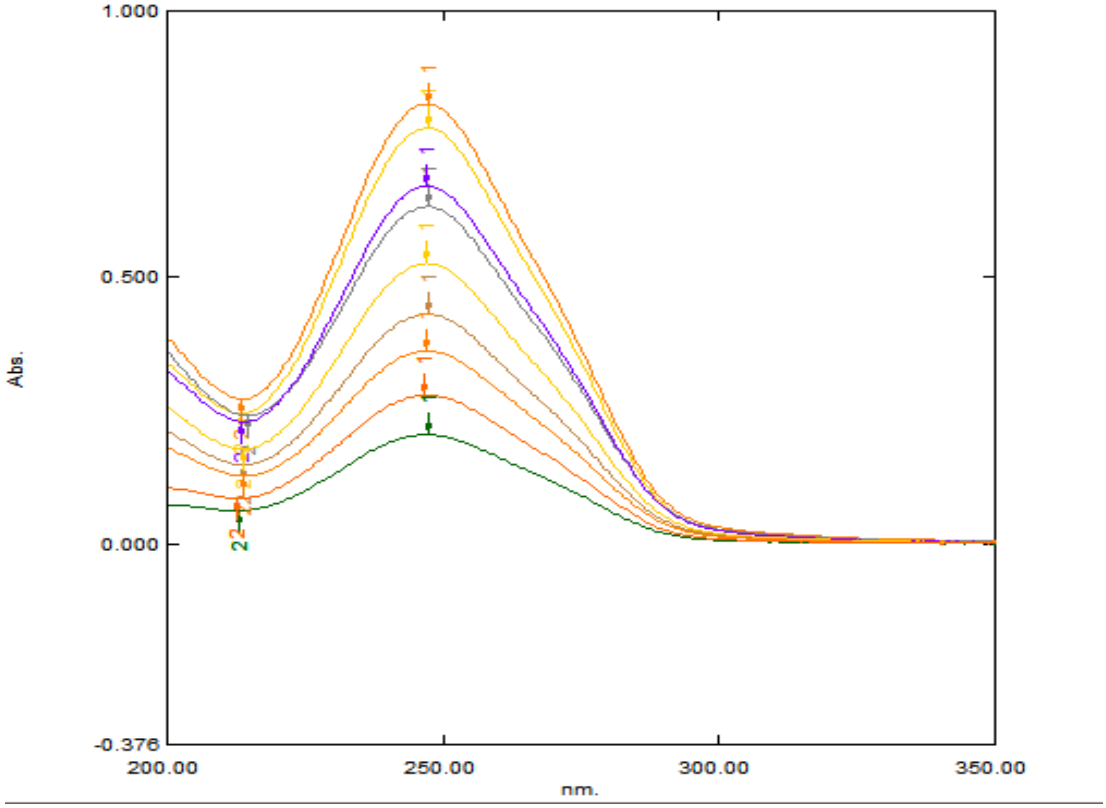


Figure 4.5(a) Calibration curve of Budesonide in phosphate buffer pH 6.0

Table 4.9 Calibration curve of Budesonide in phosphate buffer pH 6.0

Sr No	Concentration (µg/ml)	Absorbance I	Absorbance II	Absorbance III	Average Absorbance
1	0	0	0	0	0
2	6	0.242	0.243	0.249	0.248
3	8	0.332	0.331	0.339	0.336
4	10	0.419	0.408	0.410	0.412
5	12	0.512	0.49	0.509	0.499
6	14	0.563	0.534	0.559	0.562
7	16	0.653	0.632	0.629	0.643
8	18	0.71	0.723	0.724	0.722
9	20	0.804	0.795	0.802	0.8

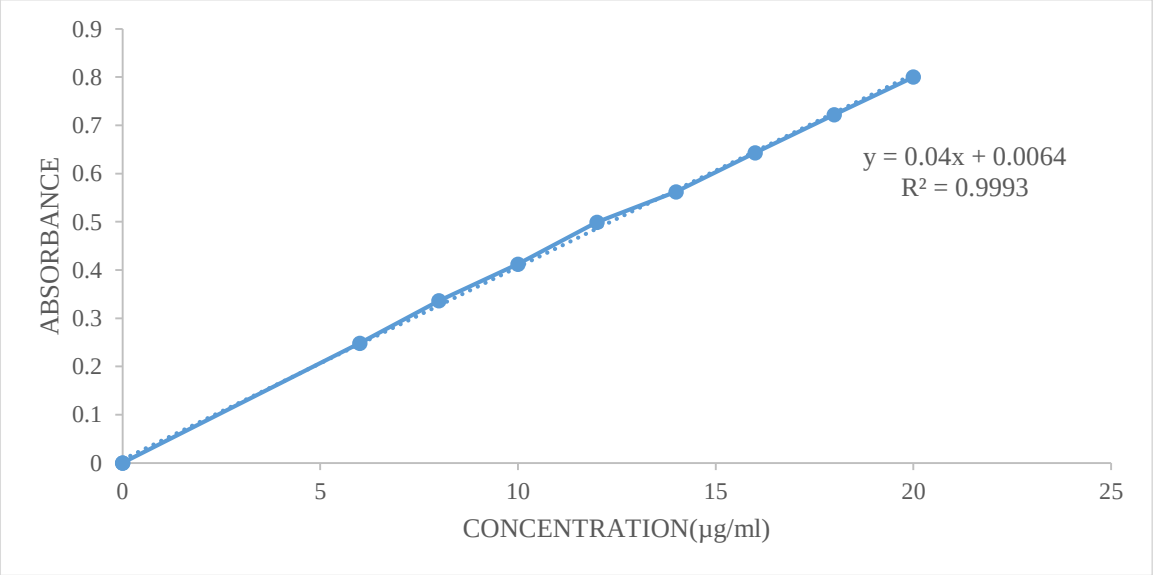


Figure 4.5(b) Calibration curve of Budesonide in phosphate buffer pH 6.0

Regression Analysis

Table 4.10 Regression analysis of Budesonide in phosphate buffer pH 6.0

Regression Parameters	Values
Correlation Co-efficient	0.9993
Intercept	0.0064
Slope	0.04

4.2.2.4 Establishment of calibration curve of Budesonide in phosphate buffer pH 7.2

50 mg of drug was accurately weighed and dissolved in 50 ml Methanol. 5 ml of this solution was withdrawn and diluted with 50 ml phosphate buffer pH 7.2. Different concentration were prepared within a range of 4 µg/ml to 20 µg/ml. The absorbance of the prepared solutions were taken at a maximum wavelength 243 nm.

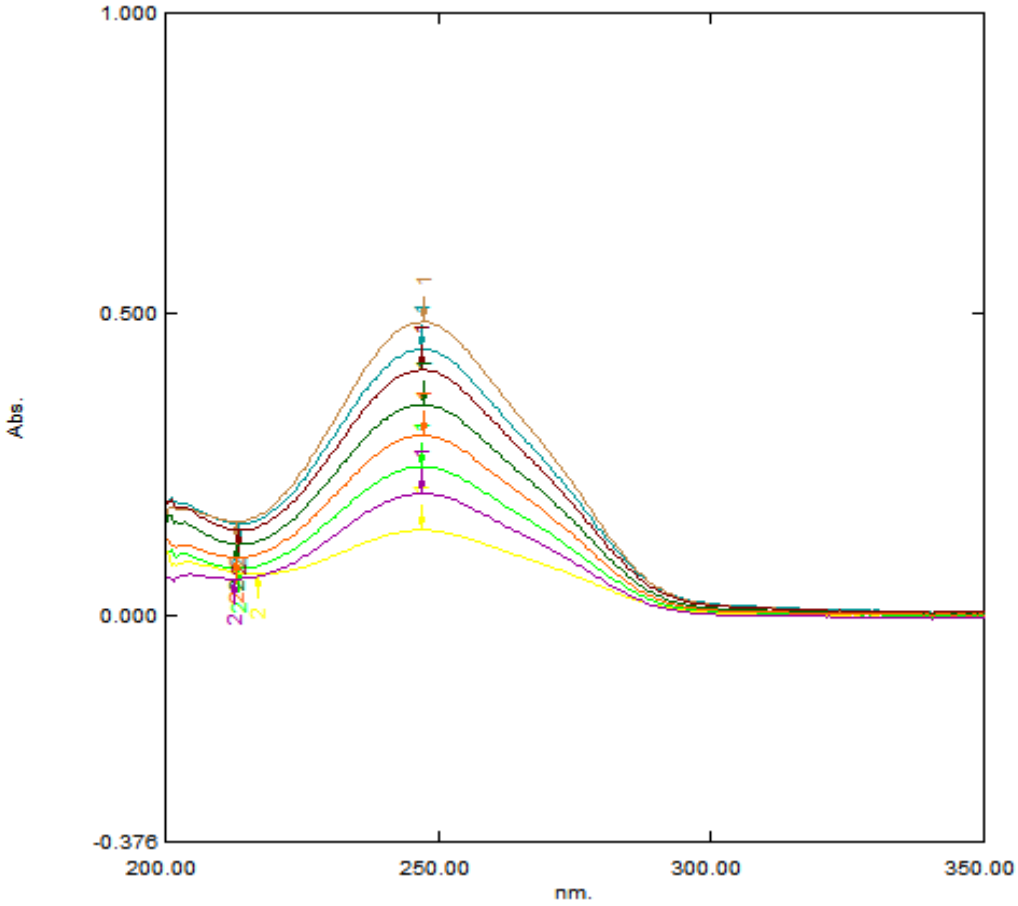


Figure 4.6(a) Calibration curve of Budesonide in phosphate buffer pH 7.2

Table 4.11 Calibration curve of Budesonide in phosphate buffer pH 7.2

Sr No	Concentration µg/ml	Absorbance I	Absorbance II	Absorbance III	Average Absorbance
1	0	0	0	0	0
2	4	0.202	0.203	0.202	0.1825
3	6	0.295	0.292	0.297	0.2545
4	8	0.351	0.355	0.341	0.328
5	10	0.434	0.432	0.435	0.4138
6	12	0.520	0.512	0.525	0.4885
7	14	0.581	0.576	0.583	0.5695
8	16	0.639	0.640	0.637	0.6405
9	18	0.719	0.721	0.714	0.7175
10	20	0.801	0.803	0.812	0.7975

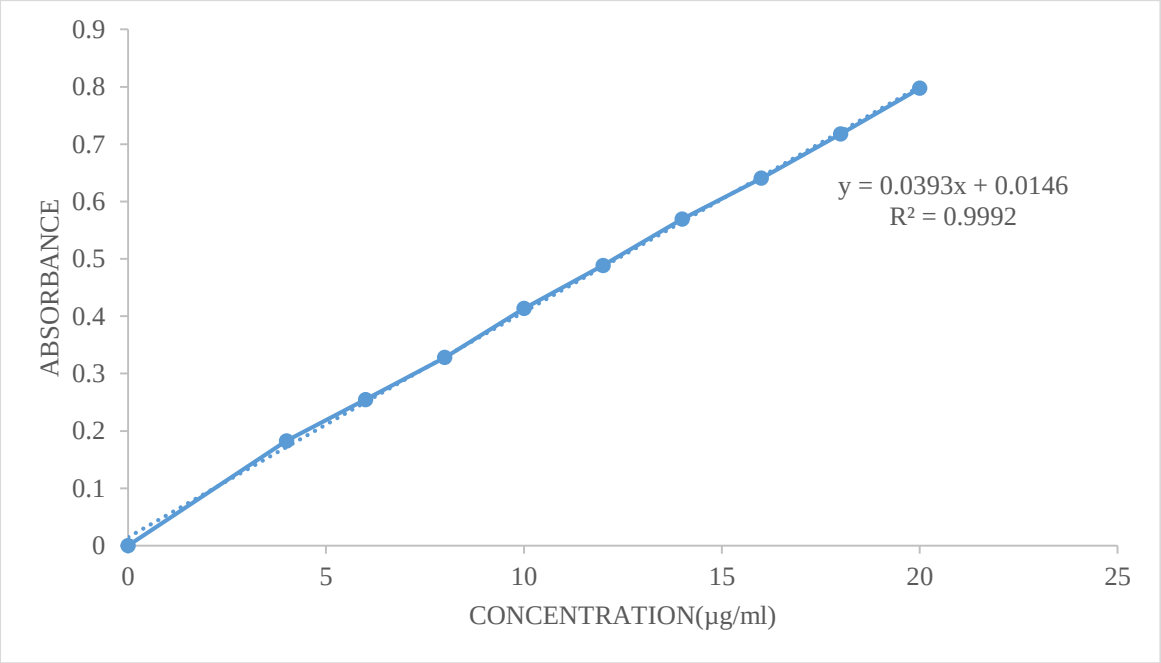


Figure 4.6(b) Calibration curve of Budesonide in phosphate buffer pH 7.2

Regression Analysis

Table 4.12 Regression analysis of Budesonide in phosphate buffer pH 7.2

Regression Parameters	**Values**
Correlation Co-efficient	0.9992
Intercept	-0.0146
Slope	0.0393

4.2.2.5 Establishment of calibration curve of Budesonide in acetate buffer pH 5.0

50 mg of drug was accurately weighed and dissolved in 50 ml Methanol. 5 ml of the stock solution was withdrawn and diluted with 50 ml phosphate buffer pH 5.0. Different concentration were prepared within a range of 8µg/ml to 24µg/ml. The absorbance of the prepared solutions were taken at a maximum wavelength 243 nm.

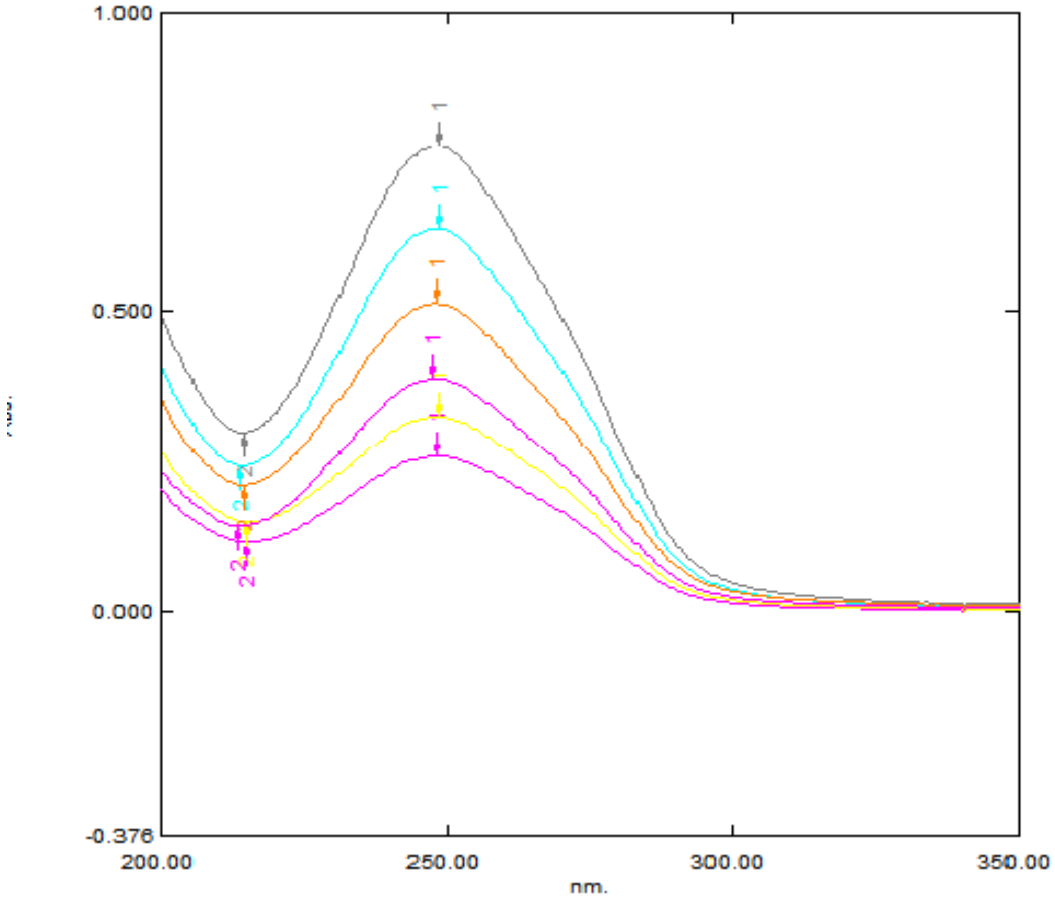


Figure 4.7(a) Calibration curve of Budesonide in acetate buffer pH 5.0 at λmax 243 nm

Table 4.13 Calibration curve of Budesonide in acetate buffer pH 5.0

Sr No	Concentration (µg/ml)	Absorbance I	Absorbance II	Absorbance III	Average Absorbance
1	0	0	0	0	0
2	8	0.244	0.248	0.242	0.245
3	12	0.376	0.375	0.377	0.376
4	16	0.495	0.494	0.495	0.4945
5	20	0.610	0.616	0.603	0.6095
6	24	0.756	0.748	0.763	0.7555

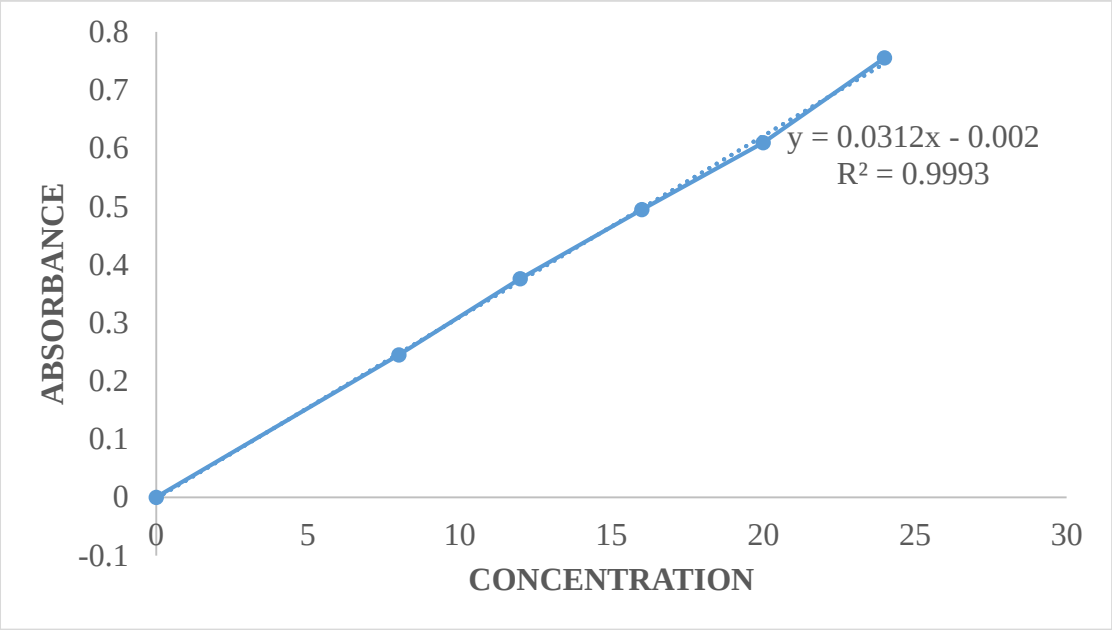


Figure 4.7(b) Calibration curve of Budesonide in acetate buffer pH 5.0

Regression Analysis

Table 4.14 Calibration curve of Budesonide in acetate buffer pH 5.0

Regression Parameters	Values
Correlation Co-efficient	0.9993
Intercept	-0.002
Slope	0.0312

4.4 Preparation and Evaluation of Budesonide loaded Nanostructured lipid carriers

4.4.1 Preparation of Budesonide loaded Nanostructured lipid carriers

Budesonide NLCs were prepared using High pressure homogenization technique. In brief, Compritol ATO 888 and Labrafac WL 1349 (85:15) were melted at 75ºC until a uniform and clear lipid phase was obtained. The aqueous phase was prepared by dissolving the surfactant in water (100ml) and was heated to the same temperature as the lipid phase. The melted lipid was added to surfactant solution under high shear at 6000 rpm, which was subsequently homogenized at 700 bar for 5 cycles using high pressure homogenizer (Panda Plus (HPH), Gea Neo Soavi).

4.4.2 Evaluation of Budesonide loaded Nanostructured lipid carriers

4.4.2.1 Particle size and Polydispersity index of NLC

NLC dispersions were characterized for average particle size (Z-average size) and polydispersity index using Malvern Zetasizer. The particle size diameters were determined (in triplicate) at 90th, 50th and 10th percentile of particles undersized.

4.4.2.3 In vitro drug release studies of NLCs

Dissolution study was performed by dialysis method. Typically, 5ml NLC suspension were placed in the dialysis membrane (Molecular weight cut off between 12000 to 14000). These dialysis bags were then placed in a beaker containing 50 ml of pH 5.0 acetate buffer containing 2% sodium lauryl sulphate to increase the solubility of hydrophobic drug in buffer medium, maintained at a temperature of 37 ± 0.5°C and stirred at 100 rpm. At predetermined time intervals (1, 2, 4, 6, 8, 12, 24, 48 and 72 hours), samples (5 mL) were taken from the beaker, and was refilled with the same volume of fresh medium. Drug release at different time points was calculated as a function of absorbance at 243nm.

4.4.2.4 Differential scanning calorimetry[40]

The possibility of any interaction between budesonide and excipients was assessed (DSC-60 Shimadzu Software TA-60). The thermogram of the samples were obtained at a scanning rate of 10ºC min-1 conducted over a range of 0-400 ºC under an inert atmosphere flushed with nitrogen at the rate of 20 ml/minute.

4.4.2.5 Fourier transmission infrared spectroscopy[41]

Drug-excipient interactions were studied by FTIR spetroscopy. The spectra was recorded for pure drug, drug loaded NLCs and ligand appended NLCs (Shimadzu FTIR spectrophotometer). The pellets were made with mixing 1 gram of drug and 100 gm of dried potassium bromide powder. Mixture was then compressed under 10 ton pressure in a hydraulic press to form a transparent pellet. The thin pellet was put on pellet disc to get IR spectra at the resolution of 2 cm-1. The spectra was then interpreted.

4.4.2.6 Transmission electron microscope

TEM was performed in Philips TEM to check the morphology of the prepared NLCs. Imaging was done at 200 kV at a magnification of 0.23 nm. A drop of NLC suspension (1 mg/ml) was placed on the Formvar copper grids followed by addition of a drop of 2% uranyl acetate. After 3 minutes of incubation, grid was air dried and images were taken.

4.7 Preparation of un-coupled and coupled pellets of NLCs[45]:

Pellets were prepared in the laboratory scale extrusion/ spheronization technique. The wet mass was produced by dry mixing the powders for 10 min. A powder mixture containing 30% nanoparticles, 15% cross-PVP and 5% SSG and 50% MCC were used as a raw material. Purified water was used as a binder. The wet powder mass was immediately extruded at 80 rpm through a radial screen extruder supplied with a 1 mm aperture screen. These extrudes were introduced in the next step of spheronization. The friction plate speed in the spheronizer was varied between 5000–5200 rpm. The extrudate was spheronized for 15 min. The wet pellets were dried in a hot air oven at 40°C for 24 h and then stored in sealed bags.

4.7.1 Evaluation of uncoupled nanostructured lipid carriers and ligand appended nanostructured lipid carriers loaded core pellets

4.7.1.1 Determination of the particle size and size distribution

Size analysis of pellets was carried out by sieve analysis using a nest of sieves containing mesh 10 # to 100 #. Total pellets collected in each batch were used and shaking time was kept 5 min. The pellets retained on each sieve were weighed. The pellets were assigned the mesh number of the screen through which it passed or on which it was retained. Mean particle size of the pellet was calculated using following formula:

Mean particle size (µm) = $\Sigma XiFi \times \Sigma Fi$

Where

$\Sigma XiFi$ = Weight size

ΣFi = Percent weight size

4.7.1.2 Determination of the aspect ratio

Aspect ratio (or elongation) measures the oblongation of the pellets. Aspect ratio decreases with higher spherecity. At least 50 pellets from each batch were randomly selected for measurement of aspect ratio. The maximum and minimum diameters of the pellets were measured using micrometer. Aspect ratio was measured using following formula:

Aspect Ratio = $(d\ max \div d\ min) \times 100$ (4.5)

Where,

dmax = Maximum diameter of the pellets

dmin = Minimum diameter of the pellets

4.7.1.3 Determination of friability

Roche friabilator was used for friability testing. Six gm accurately weighed pellets were taken and placed in friabilator. The test apparatus was rotated at 25 rpm for 4 minutes. After friability testing, the pellets were sieved through sieve no. 40 to remove fines generated. The weight loss (% F) after friability testing was calculated as:

% F = [(W1 – W2) ÷ W1] × 100 (4.6)

Where,

W1= Initial weight of the pellets,

W2 = Weight of pellets after friability

Percentage friability less than 1% would be acceptable.

4.7.1.4 Determination of % drug content

The amount of drug loaded in the pellets was determined as follows: The pellets were first crushed using a glass mortar pestle. An accurately weighed amount (100 mg) of powdered pellets was dispersed in 100 ml of methanol dichloro-methane (1:2). The resultant dispersion was exposed to ultrasonic treatment for 30 min. The ultrasonic treatment was repeated three times with a resting period of 15 min between the treatments. The sample was then kept for 24 h at room temperature. After centrifugation (4000 rpm for 10 min), the supernatant was diluted appropriately with methanol and the content was determined for each batch in triplicate. The % drug content was calculated using following equation:

% Drug content = (Df ÷ Da) × 100

At where,

Df - drug present in the final formulation

Da - amount of drug added

4.7.2. Enteric coating of core pellets

Pellets consisting of uncoupled and ligand appended nanoparticles (NLCs) were enteric coated using a combination of polymers namely EUDRAGIT S-100 using pan coating technique. Briefly, 5% of polymer solution was prepared in organic solvent acetone and isopropyl alcohol (70:30). An anti-adherent i.e. talc was added in a quantity of 50% with respect to polymer weight to prevent agglomeration of pellets along with anti-caking, Titanium dioxide (0.5% w.r.t polymer weight) and plasticizer , Tri-ethyl citrate (20% w.r.t polymer weight) to provide pellets a smooth and shiny texture. The pre-warmed pellets bed was used to coat the pellets by spraying methodology. The pellets were coated and dried using an inlet air temperature 30º – 35º C at 30 rpm. This process was carried out till the desired weight gain was achieved and then the pellets were cured for 15 minutes which were then dried overnight at 50º C. The percentage coating level of the pellets after coating was assumed to be indicative of the thickness of the coat.

5.1 PREPARATION OF NLCs

NLCs were prepared using High pressure homogenization (HPH) technique. Several methods of producing NLCs have been reported in the literature. These are HPH, solvent emulsification–evaporation, solvent emulsification–diffusion, double emulsion technique, high shear homogenization and ultra-sonication. The high shear homogenization and ultra-sonication are often not sufficient to achieve a smaller particle size (PS) or narrow size distribution. The HPH technique has been reported to be the most effective technique due to some advantages, such as narrow PS distribution, better dispersion of formulations with higher lipid content, avoidance or low volumes of organic solvents, acceptability of homogenization equipment by the regulatory authorities and feasibility of scale-up for large-scale production . The influence of pressure as well as the number of cycles during the HPH process on the physicochemical properties of various nano-particulate systems has been reported.

5.6 PRELIMINARY TRIALS

Factors related to process and formulation widely affect the formulation owing to its efficacy and stability. Hence, preliminary trials were conducted to evaluate the effect of process parameters like Cycles of homogenization and Homogenization pressure; formulation parameters like concentration and type of surfactant solution, concentration of lipid and concentration of drug on the preparation of NLCs.

5.6.1 SELECTION OF TYPE OF SURFACTANTS

On the basis of spherecity of particles and aggregation observed, the type of surfactant was selected.

Microscopy of the prepared batches revealed that in case of Poloxamer 188 completely spherical particles were formed showing intense aggregation. Also batches prepared using Poloxamer 407 showed considerable spherecity in particles with aggregation. While in case of batches prepared using Polysorbate 20 and Polysorbate 40 showed no aggregated particles but spherecity was compromised. Batch GS5 prepared using Polysorbate 80 showed good spherecity and no aggregation within the particles.

Since batch GS5 showed good spherecity and no aggregation, Polysorbate 80 was elected as surfactant for further studies.

5.6.3 SELECTION OF COMBINATION OF SURFACTANTS

A series of combination of surfactants were tried. From previous batches no aggregation was observed with the use of Polysorbate 80 and spherecity was noticed with the use of Poloxamer 188. Hence with a constant concentration of Polysorbate 80, batches were prepared by varying the concentration of Poloxamer to control the spherical appearance of NLCs which tends to affect the polydispersity index in terms of uniformity of the NLCs formed.

From preliminary screening of surfactant it was concluded that when Poloxamer 188 was used as surfactant, excellent spherecity of the particles were observed. But since it showed high degree of aggregation, Poloxamer-188 was not selected as surfactant. On the other hand, Polysorbate 80 showed no aggregation of particles with good spherecity of particles. Polysorbate 80 was selected for the preparation of NLCs. Since Polysorbate 80 when used alone was not able to control the spherecity and PDI of NLCs, a combination of surfactants were evaluated.

Different batches were prepared by keeping concentration of Polysorbate 80 at 6% w/w and by varying the concentration of Poloxamer 188 at 0.1, 0.25, 0.5, 1 and 2% w/w. It was found that with the use of Poloxamer 188 even at the lowest concentration i.e. 0.05%, NLCs obtained were spherical in shape. At higher concentrations of Poloxamer 188, slight aggregations were observed, also batches tend to gel within 24hours.

Thus concentration of Poloxamer 188 was fixed at 0.05% and a range of concentrations of Polysorbate 80 were again assessed in order to obtain desired NLCs.

5.6.5 EFFECT OF CONCENTRATION OF SURFACTANT

Batches GS1, GS2 and GS3 were prepared to elucidate the effect of concentration of surfactant on characteristics of formulation after addition of co-surfactant (10% w/w w.r.t lipid).

Concentration of drug and concentration of lipid were kept constant at 2% and 1% respectively.

Polysorbate 80 and 0.05% Poloxamer 188 as surfactant and Span 80 (as co-surfactant) possessed Z-average of 559.85 nm which was beyond the desirable range. In terms of PDI, the batch exhibited good uniformity in particles. The zeta potential was observed to be -3.7 and the % EE was found to be 93.67%. Highest amount of drug was found to be incorporated in the NLCs when lowest amount of surfactant is used. This is because when low concentration of surfactant is used, it stabilizes the system to a low extent thus yielding huger particles as a result of which higher amount of drug is entrapped.

To achieve further reduction in particle size Batch GS2 with 4% Polysorbate 80 and 0.05% Poloxamer 188 was evaluated. Improved Z-average was noticed whereas no significant change was observed on ZP and with %EE of 89.18%. Batch GS3 was also prepared using 6% T80 and P188 and a Z-average of 402.3 nm was achieved depicting the decrease in particle size with increase surfactant concentration. As compared to Batch GS1, the entrapment efficiency was observed to decrease with increase in the surfactant concentration owing to low particle size formation and possible leaching of drug.

From the above results it can be concluded that as the concentration of surfactant was increased the particle size decreases. With the decrease in particle size the %EE decreases. This could be attributed to the fact that leaching of drug from formulation might occur with high surfactant concentration.

As a result of these observations, 6% tween 80, 0.05% Poloxamer and 1% co-surfactant (span80) of lipid were selected for further studies.

5.6.6 EFFECT OF CONCENTRATION OF DRUG

To achieve maximum entrapment efficiency, concentration of drug was varied keeping rest of the factors constant. The effect was then studied.

Batch GD2 and GD3 were prepared by varying amount of drug added from 40 mg to 80 mg keeping amount of lipid as 1000 mg. Z-Average was noticed to be increasing as the drug concentration was increased. Although no significant rise was noted Z-Average value and ZP. The ZP of Batch GD3 was highest (towards positive) which contained highest amount of drug than Batch GD2 and GD1.

From the above results, it can be concluded that the higher the amount of drug, higher amount of drug localizes over the lipid shell and as hence a significant increase in Z-average and %EE is observed.

As it is a shell enrich model, more the amount of drug that accumulates on the shell higher rapid release is observed.

5.6.7 EFFECT OF CONCENTRATION OF LIPID

Lipid concentration was screened from 1% to 3% to evaluate if this factor affected any characteristic of NLCs.

5.7 BOX-BEHNKEN DESIGN

Box-Behnken is a response surface design devised by George Box and Donald Behnken for optimization purpose. It provides a quadratic response surface approach. It is suitable for multi-variant optimization characterized by set of points lying at the mid-point of each edge of multi-dimensional cube and center point replicates (n=3). There are three level fractional factorial designs of 3 (k-p) runs. These designs do not have simple design generators. They have complex confounding of interaction.

Advantage of using BBD:

1. It requires fewer experimental runs (15 runs for 3 factors) and hence it provides a far more cost-effective technique and less time consuming too.
2. It does not have to deal with axial points and hence it ensures that all the factors are never simultaneously set at their higher or lowest levels. Thus it is beneficial in avoiding experiments to be performed under extreme conditions for which unsatisfactory results might occur.

On the basis of preliminary trials, the independent variable selected were Concentration of Surfactant, Concentration of Co-surfactant and concentration of Drug. The dependent variable were the Z-Average of particles, PDI and % EE.

Mathematical model for deriving equations indicating relationship between independent and dependent variables are as follows:

$Y= B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2X_3 + B_{123}X_1X_2X_3 + B_{11}X_1^2 + B_{22}X_2^2 + B_{33}X_3^2$

Where B_0 = intercept = constant

B_1, B_2, B_3 = Co-efficient of X_1 and X_2 variables

B_{12}, B_{23}, B_{13}, B_{123} = Co-efficient of interactions

B_{11}, B_{22} = Co-efficient of quadratic terms = non linearity

X_1 and X_2 = Variables

Y = Measured response

Y = Constant term + Main effect + Interaction effect + Quadratic effect

5.9 DIFFERENTIAL SCANNING CALORIMETRY (DSC)

It is a technique that shows the change in the thermal behavior of the drug and other excipients occurring due to the formulation of NLCs. Thus to check and confirm the interactions between the drug, lipid and other excipients, DSC characterizes the stability of formulation significantly.

As observed in the Figure 5. , the transition peak of pure Budesonide was observed at 260.97°C. The DSC analysis of lipid component (Compritol ATO 888: Labrafac WL 1349) exhibited a peak 71.44 °C. When a blank batch was analyzed, a transition peak at 63.94 °C appeared that indicated change in melting point of lipid owing to the addition of other component like stearyl amine.

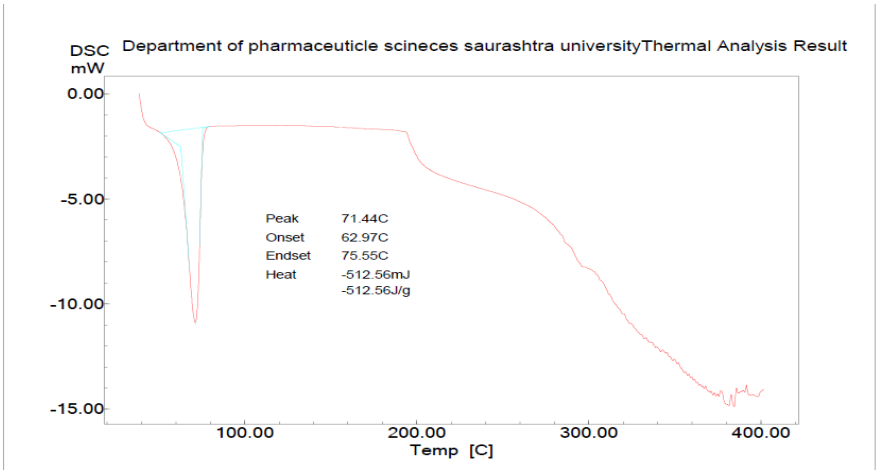


Figure 5.21 DSC analysis of lipid mixture

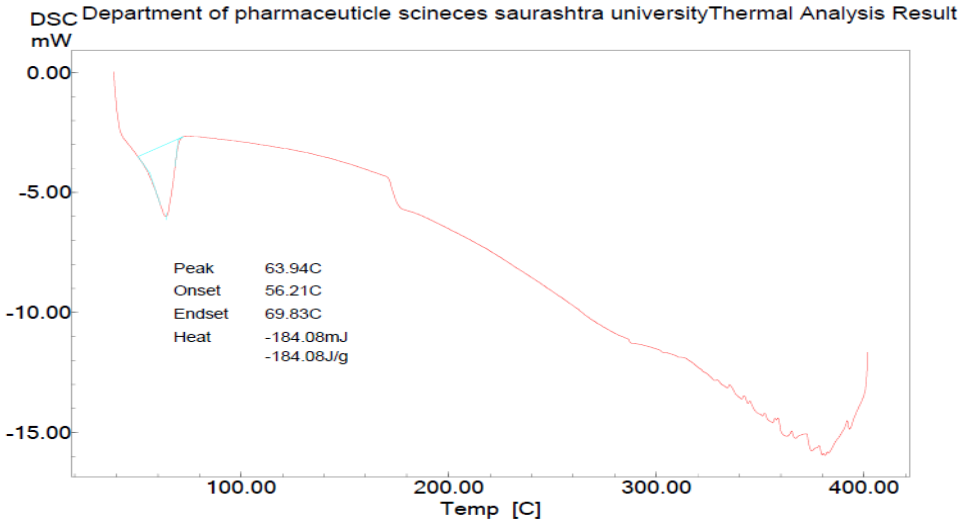


Figure 5.22 DSC analysis of lipid mixture

A slight change in transition peak on addition of drug was observed at 64.04 ℃

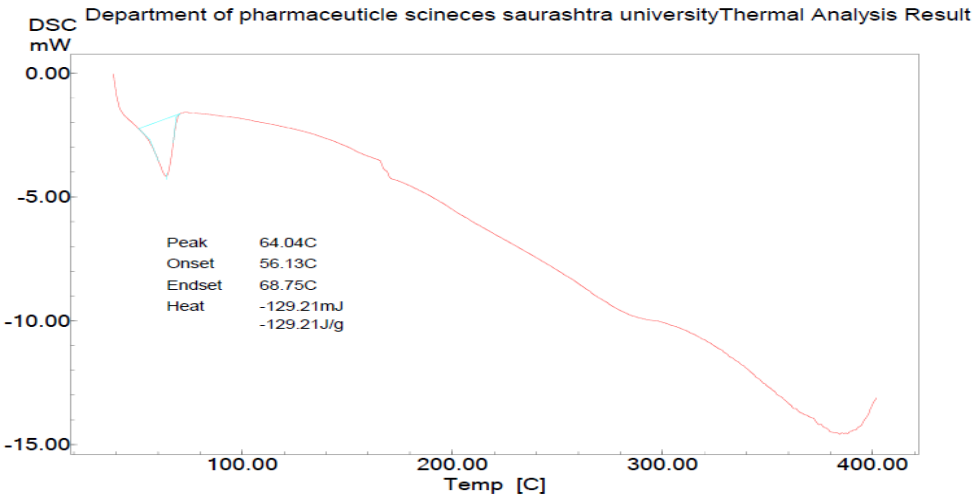


Figure 5.23 DSC of Budesonide loaded NLCs

5.11 PREPARATION OF UN-COUPLED AND COUPLED PELLETS OF NLCs

Agglomeration through extrusion and spheronization is one of the oldest widely used process for preparation of pellets. The process comprises of four vital steps:

- Moistening the powder mixture
- Formation of cylinder shaped agglomerate through extrusion
- Breaking the extrudes to form round pellets through spheronization
- Drying the finished product

The dry, mixed, powderly ingredients were kneaded into dough with a liquid which is then forced through an extruder to get extrudes.

The pellets of Budesonide were prepared by extrusion and spheronization technique. Budesonide loaded NLCs, MCC, SSG, Cross PVP and water were passed through sieve no. 40 prior to pelletization and mixed uniformly. Water was added in sufficient quantity to the powder blend and mixed properly. The obtained dough mass was extruded using a piston extruder (1.5 mm orifice). The extrudates were immediately spheronized in 80 mm diameter friction plate with groove spaces of 3 mm for 45 min at a rotation speed of 4500 rpm. The pellets were dried overnight at room temperature.

Table 5.15 Trials taken for the preparation of pellets

Ingredient	Batch A	Batch B	Batch C	Batch D	Batch E
NLCs (30%)	4.5 gm	4.5 gm	4.5 gm	4.5 gm	**4.5 gm**
MCC (50%)	7.5 gm	7.5 gm	7.5 gm	7.5 gm	**7.5 gm**
SSG (5%)	0.75 gm	0.75 gm	0.75 gm	0.75 gm	**0.75 gm**
CROSS PVP (15%)	2.25 gm	2.25 gm	2.25 gm	2.25 gm	**2.25 gm**
WATER	4 ml	8 ml	10ml	12ml	**14 ml**
Shape	Dumb bell shaped	Dumb bell shaped	Dumb bell shaped	Dumbbell + spherical	**Spherical and uniform**

As shown in table 5.16, Batch A was formulated with minimum quantity of water was found to have insufficient moisture required to gain spherical shape under the extrusion-spheronization process. A higher volume of water was incorporated in Batch B and it was observed that the pellets formed were still dumb-bell shaped.

Batches with higher volume of water were formulated (Batch C, Batch D and Batch E). Batch D had a water content of 12 ml which gives mixed type of pellets (spherical + dumb-bell) were almost spherical in shape. Batch E was developed with 14 ml of water volume and it possessed completely spherical shape and uniform. The disintegration time of these pellets were found to be 69 seconds. Particle size of pellet was found to be around 0.91 mm and friability was tested yielding a result of ≤ 0.01% which was desirable so as to maintain its integrity during coating in Pan Coater. Aspect ratio which indicates the degree of elongation was within desirable range i.e. 1.12-1.14.The drug release and drug content was found to be 84.93% and 99.91% respectively. Evaluation parameters of Batch E is shown in

Figure 5.28(a) Unconjugated uncoated pellets

Table 5.16 Evaluation parameters of Batch E

Sr.No	Parameters	Result
1	Average particle size	0.91 mm
2	Pellet size distribution	1.0-1.2 mm
3	Aspect ratio	1.12-1.14
4	Friability	≤ 0.01%
5	%Drug content	99.91%
6	Drug release (72hours)	84.93%
7	Disintegration time	69 seconds

5.12 EVALUATION OF UNCOUPLED NANOSTRUCTURED LIPID CARRIERS AND LIGAND APPENDED NANOSTRUCTURED LIPID CARRIERS LOADED CORE PELLET

The table 5.25 depicts the characteristic properties of pellets formulated.

Table 5.17 Characterization of uncoated pellets

PARAMETERS	UNCONJUGATED BATCH	FA-NLCs	Mn-NLCs	Tf-NLCs
Average Particle size(mm)	0.91	1.1	1.1	1.1
Pellet size distribution(mm)	1.0-1.2	1.0-1.2	1.0-1.2	1.0-1.2
Friability	≤ 0.01%	≤ 0.01%	≤ 0.01%	≤ 0.01%
Aspect ratio	1.12-1.14	1.12-1.14	1.14-1.16	1.14-1.16
% drug content	99.91	99.88	99.86	99.85
%Drug release (72hours)	84.93	82.74	82.75	81.92
Disintegration time(seconds)	69	72	65	67

The particle size distribution of all the formulations was found to be around 1.0 to 1.2 mm. Friability of the prepared batches was noted to be less than 0.01% which was important in order to undergo coating process without losing its integrity while rotation in pan coater. Aspect ratio is the measure of elongation and signifies the uniformity of the formed pellets. For unconjugated batch of pellets and FA-NLCs, the aspect ratio was found to be 1.12-1.14 and for Mn-NLCs and Tf-NLCs aspect ratio varied from 1.14-1.16. %Drug release at the end of 72 hours is depicted in Figure 5.25(a)

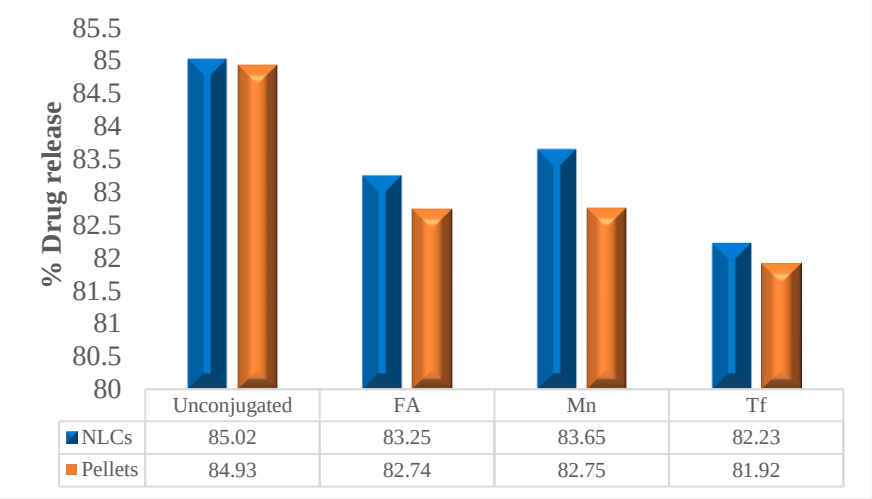


Figure 5.28(b) % Drug release of NLCs and Pellets

Drug content was determined to ensure no effect on the drug loaded NLCs occurs during the pelletization process that included extrusion-spheronization. Drug content was found to be sufficient in the prepared batches.

Figure 5.28(c) Uncoated FA-NLCs

5.13 ENTERIC COATING OF CORE PELLETS

Enteric coating of pellets was done with Eudragit® S100 (ES) using a pan coating technique. Approximately 5, 7.5, 10, 15 and 20% (w/w) (Batches PG1–PG5) of coating was applied to study the effect of concentration of ES on drug release in the gastric fluid. Drug content of enteric coated pellets (99.56%, 99.33%, 99.18%, 99.26% and 99.34%) was found to be similar to that of uncoated pellets, which revealed that there was no loss of drug during the coating process of pellets. In vitro dissolution of Batches PG1–PG5 was carried out as discussed earlier, which reveals that a 5% (w/w) of coating level was required to impart an enteric effect. At 5% (w/w) coating level, the percentage of drug released at the end of 5th hour was found to be 26.43. Increasing the coating thickness to 7.5, 10, 15 and 20% (w/w) (Batches PG2, PG3, PG4 and PG5 respectively) reduced the drug release to 23.53%, 17.75%, 4.23% and 1.23% respectively after a period of 5 hours. Further, it was observed that the dissolution rate was inversely proportional to the thickness of the coat applied. These results are in good agreement that an increase in the coat thickness of enteric polymer, exhibited a decrease in the dissolution rate of drug. This can be explained by the fact that increasing the coat concentration makes the coat more impermeable and drug release is retarded. Slowly as the coating solubilizes, drug dissolution is facilitated.

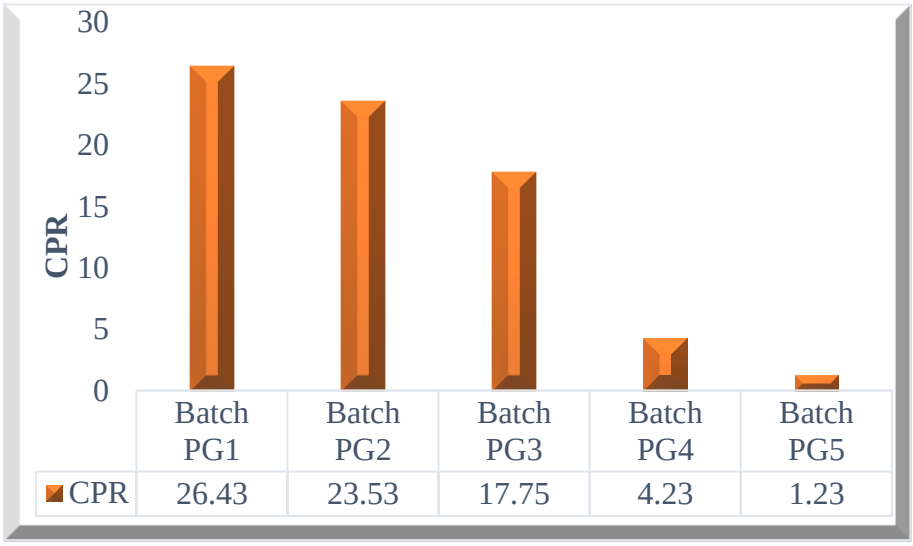


Figure 5.29(a) In-vitro drug release (at the end of 5 hours) of Batches PG1-PG5

The lag time profile of Batches PG1–PG5 revealed that by increasing the coating level of ES, the lag time for drug release increases. It was observed that the lag time for drug release at 5 and 7.5% (w/w) coating level was found to be 2 hour, which increases to 3

hour at 10% (w/w) and 5 hour at 15 and 20% (w/w) coating level. It was observed that the lag time was same at 5 and 7.5% (w/w) coating level, and at 15 and 20% (w/w) coating level with a difference in the % drug released from the respective batches. As Batch PG4 and PG5 showed maximum lag time, the optimum batch was selected on the basis of their drug release. No significant difference in the % drug release was observed and hence Batch PG4 was selected as optimum batch.

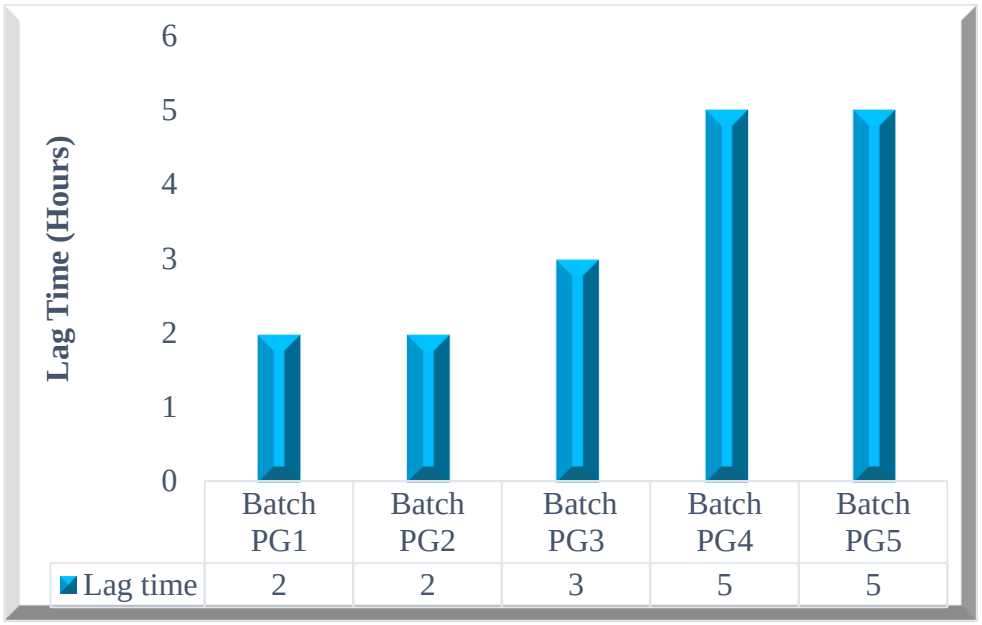


Figure 4.29(b) Effect of coating on Lag time

Figure 5.29(c) Unconjugated coated pellets

SUMMARY:

Inflammatory bowel disease (IBD) is a communal term for a group of idiopathic intestinal conditions that includes two main features Ulcerative colitis and Crohn's disease. IBD is considered to be chronic relapsing disorder allied with uncontrolled inflammation within the gastrointestinal tract which may lead to the development of colorectal cancer later in life. Patients suffering from IBD require life-long anti-inflammatory and immunosuppressive therapy. Major limitation in the drug therapy to treat IBD is a failure to deliver the drug molecules selectively at the site of inflammation.

Budesonide is a non-halogenated corticosteroid with high topical efficacy and minimal systemic activity owing to its low oral bioavailability and an extensive hepatic first pass effect which allows only 10-15% of the drug to reach systemic circulation. Based on these effect, Budesonide is proved to have better adverse effect profile than the conventional steroids and hence it is suggested as the first choice for the induction of emission of IBD. Marketed formulations available include controlled release formulations of Budesonide such as Entocort or Budenofalk which is prescribed widely for topical delivery of the drug. However due to long term side effects accompanied by its therapy makes it incompatible therapy for maintenance of remission. Hence Budesonide was studied for its anti-inflammatory activity in chronic colonic inflammation. Reduction in the rise of pro-inflammatory cytokines, TNF-α and IL-1β was observed proving its role in treatment of IBD.

Hot high pressure homogenization technique was selected for the formulation as it ensures desired size reduction and has easy industrial scale up. Glyceryl behnate (Compritol ATO 888) and Medium chain triglyceride (Labrafac WL 1349) were studied to achieve nanoparticles of desired features.

Preliminary trial batches were prepared to check the effect concentration of surfactant, concentration of combination of surfactants, type and concentration of co-surfactant, concentration of drug and concentration of lipid to achieve NLCs within desired size range and maximum % entrapment efficiency. Based on the preliminary trials, concentration of drug, concentration of surfactant and concentration of co-surfactant were found to affect the properties of formulation significantly and hence Box-benhken was applied for

optimization procedure. Batch GB8 was selected as the optimized batch as it has a Z-Average of 289.3 nm, %EE of 92.66% and a drug release of 85.02% at the end of 72 hours.

Nano particulate drug delivery systems have led to ground breaking progressions in therapeutic selectivity. Although it enables the drug formulation to deliver the drug at the inflamed site, major portion of loaded drug is eliminated without imparting a beneficial effect. Therefore to overcome this limitation of the drug delivery, ligand appended nanostructured lipid carriers were prepared comprising of folate, mannose and transferrin covalently bound to the surface of nanostructured lipid carrier and were characterized for local drug release in colon.

NLCs pellets were prepared by extrusion-spheronization technique and in-vitro release profiles of NLCs and NLCs pellets were compared to ensure the integrity of NLCs. In-vitro release profile were studied and it was found that a premature release of drug in the stomach and small intestine occurs. As enteric coating of nanostructured lipid carriers is difficult to achieve, pellets were prepared and were enteric coated using Eudragit L-100 using pan coater. Drug release studies of nanostructured lipid carriers coated pellets revealed that the formulation remained intact with drug during the pelletization and coating procedure.

To ensure the efficacy of the formulation, animals studies were also carried out in oxazolone induced colitis rat models. Clinical activity score, MPO activity, inflammatory markers level, macroscopic and histopathological scoring were performed. Based on the results, it was observed that ligand appended NLCs played a promising role in the treatment of IBD.

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