Frontiers in Health InformaticsISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 3
Open Access

Optimizing Colon Targeted Drug Delivery: Deep Insights into Microsphere-Based Methods

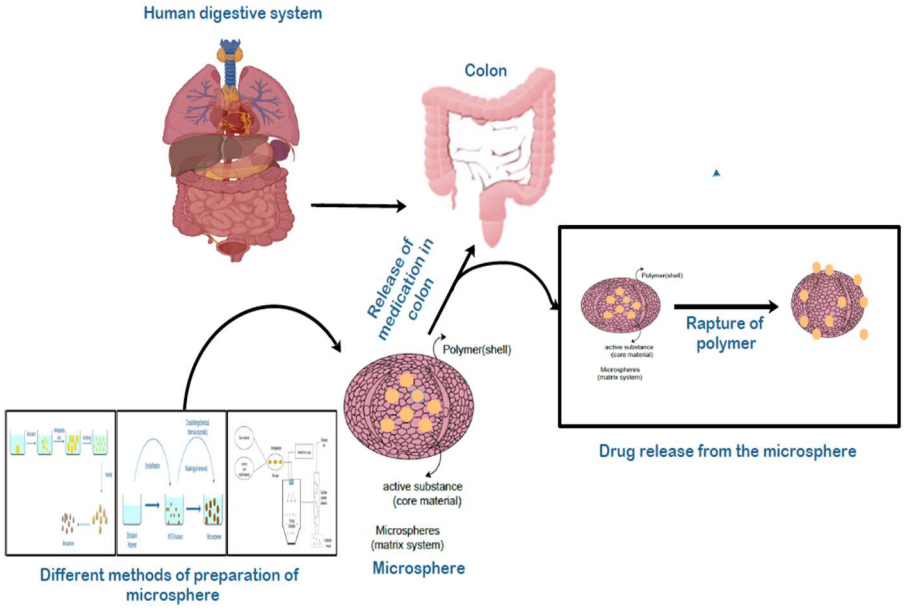
Priyanka Venkatesan[1], Samarpan Sarangi[2], P N Remya[3*], N Damodharan[4]

[1,2,3,4]Department of Pharmaceutics, SRM College of Pharmacy, SRM Institute of Science & Technology, Kattankulathur, India, 603203

Corresponding Author's: Dr. P N Remya,
Department of Pharmaceutics, SRM College of Pharmacy, SRMIST, Kattankulathur, Chennai, India 603203
E-mail: remyan@srmist.edu.in

Cite this paper as: Priyanka Venkatesan, Samarpan Sarangi, P N Remya, N Damodharan (2024) Optimizing Colon Targeted Drug Delivery: Deep Insights into Microsphere-Based Methods. *Frontiers in Health Informatics, 13* (3), 8117-8143

GRAPHICAL ABSTARCT:



A B S T R A C T

Colon-targeted drug delivery systems have gained significant attention for the treatment of localized colon disorders such as Crohn's disease, ulcerative colitis, and irritable bowel syndrome. Oral administration is favored for its high patient acceptance and ease of use. Among various delivery methods, microparticulate systems, particularly microspheres, are the most effective for controlled and sustained drug release to specific inflammatory sites. Microspheres, defined as particles with dimensions between 1 and 1000 µm, are produced from proteins or synthetic polymers and are biodegradable. They can be coated with various compounds to optimize drug delivery and reduce

nonspecific interactions. Understanding the colon's anatomy, including its unique pH levels, microflora, and transit time, is essential for effective drug targeting. Various prodrug approaches, such as azo bond conjugates, glycoside conjugates, and cyclodextrin conjugates, have been explored to ensure that drugs are selectively released in the colon. These systems provide a promising avenue for enhancing treatment outcomes while minimizing systemic side effects.

KEYWORDS: *Colon Targeted Drug Delivery, Oral Drug Delivery, Microspheres, Ulcerative Colitis, Targeted Release.*

1. INTRODUCTION

The most practical and crucial way to take medication to produce systemic effects is orally. Oral administration is the intended usage for around half of the medication delivery systems now on the market. These systems offer numerous advantages, chief among them being high patient acceptance and ease of use[1].Research has focused a lot of attention on colon-targeted medication delivery recently due to its potential to improve treatment outcomes for localised colon disorders while lowering systemic side effects. These developments concentrate on conditions that damage the colon, including such as irritable bowel syndrome(IBS),ulcerative colitis (UC),and crohns disease (CD). Medications that are frequently used to treat these disorders include prednisolone, metronidazole, hydrocortisone, sulfasalazine, and dexamethasone [2,3].Numerous strategies, including the conjugate approach, probiotic method, pH-dependent system, time-released system, multiparticulate system, and nanoparticulate system, are employed for suitable targeting. Of all the methods, the microparticulate system is the most effective for delivering controlled and sustained medication to a particular inflammatory location [3]

Microspheres are defined as particles with dimensions between 1 and 1000 μm. Produced from proteins or synthetic polymers, these particles are spherical, free-flowing, and biodegradable. Microcapsules and micromatrices are the two different forms of microspheres; they are defined as, Microcapsules are defined as having a distinct capsule wall enclosing the substance that is entrapped. as well as micromatrices, which distribute the material that is trapped throughout the matrix[4]

Microspheres have significantly contributed to the advancement of prolonged or controlled release drug delivery methods. Microspheres have caught the attention of the pharmaceutical industry specifically because they have the ability to provide continuous and regulated medication delivery[5].

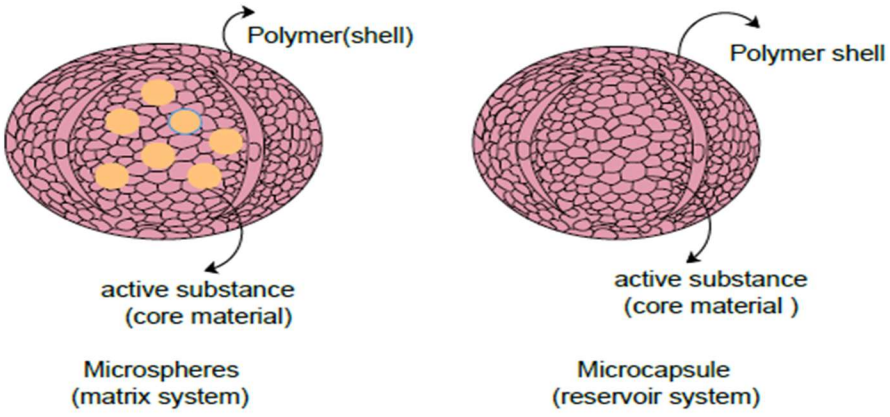


FIGURE 1: Microspheres and Microcapsule

2. CHARACTERISTICS OF MICROSPHERES

In certain instances, the size of microspheres is vital for assay functionality, while in other cases, it might be less critical than other properties. Particle size is often determined by the test format in conventional diagnostic techniques. For instance, larger spheres (~4–10µm) that mimic cells are used in bead-based flow cytometric research, whereas minuscule spheres (~0.1–0.4µm) are used to enable efficient wicking in lateral flow tests. Poly(methyl methacrylate) (PMMA), polystyrene (PS), and silica are frequently used to create microspheres. Depending on the application, the distinct optical and physical qualities of different materials may be beneficial or detrimental. For example, polymer beads often have significant protein binding characteristics due to their hydrophobic nature. However, they often require adding a surfactant, like Tween® 20 or sodium dodecyl sulfate, to the storage buffer at concentrations ranging from 0.01% to 0.1% in order to facilitate handling.
Microspheres can be coated with substances like peptides, oligonucleotides, antibodies, and other such substances for use in separation or diagnostic applications. The primary objective of microsphere coating optimization is to reduce nonspecific interactions while achieving the desired specific activity. The required stability, the timetable for development, financial constraints, and the type of biomolecule to be coated are all taken into account. The selection of the optimal coating strategy for both short- and long-term objectives is influenced by these factors. Conventional microsphere products typically offer three primary coating techniques: affinity binding, covalent coupling, and adsorption [6]. In biological sciences applications, different uses demand beads with distinct characteristics, such as fluorescence, coloration, or magnetic attributes. To achieve these characteristics, polymer spheres are frequently dyed internally using organic solvents. This enables the modification of dye concentrations to create beads with different intensities, meeting specific requirements such as multiplexed flow cytometric assays or imaging applications. Furthermore, fluorescent beads designed expressly for use as standards in flow cytometry are available [7].

3. ANATOMY OF COLON

Understanding the distinct functional characteristics of the colon in relation to the entire gastrointestinal tract and its associated organs is crucial for effectively directing drugs to this specific location. More broadly, the colon contains proximal and distal sections, spanning approximately 1.6 meters in length within the overall 6-meter gastrointestinal tract (with average lengths of 1.66 ± 0.36 meters for males and 1.55 ± 0.29 meters for females)[8]. The proximal colon refers to the first and middle segments of the large intestine. The cecum, ascending colon, and transverse colon are the three main areas that make up this segment. Distal colon is the last part of colon, which in turn splits into four parts: the descending colon, sigmoid colon, rectum, and anus. The hepatic flexure connects the ascending and transverse colon, while the splenic flexure connects the transverse and descending colon. Importantly, the diameter of the colon is much greater than that of the small intestine (with an average terminal ileum diameter of 1.87 ± 0.36 cm). At the caecum, its widest point measures 4.7 ± 0.9 cm in males and 4.8 ± 0.8 cm in females. It progressively narrows to measure 3.4 ± 0.6 cm in men and 3.2 ± 0.6 cm in females at the sigmoid colon, then widens again at the rectum, reaching 4.0 ± 1.0 cm in males and 3.5 ± 1.0 cm in females. The caecum, transverse colon, and sigmoid colon reside in the abdominal peritoneal cavity, supported by mesentery, and demonstrate some degree of mobility. The rectum, ascending colon, and descending colon, on the other hand, are retroperitoneal and hence

fixed in their placements [9]. Eickhoff et al [10]CT colonography to gather data on the number of colonic flexures (sharp angles less than 90 degrees) and the degree of twisting (measured on a 10-point visual scale). Their research revealed that the typical number of bends in a colon averages around 9.6, with a standard deviation of 2.4. Furthermore, the visual analog scale employed to evaluate twisting recorded an average rating of 3.7, with a standard deviation of 1.9. Patients with a large number of sharp angles in their colon and significant twisting are challenging to examine using colonoscopy, which increases the likelihood of an incomplete procedure. Additionally, Alazmani et al [11] observed that colon twisting is more noticeable when patients are supine (laying on their backs) compared to when they are resting in the prone posture, or on their stomachs.. The colon performs various essential physiological roles, such as absorbing water, minerals, and vitamins; compacting feces; breaking down polysaccharides during digestion; and regulating the immune system within the intestines [11]. The appendix connects to the caecum approximately 1 to 2 centimeters beneath the junction of the ileum and the caecum. Sadahiro et al[12]presented diameter measurements of the large intestine, as depicted in the table below.

Diameter (Cm) Of Distended Large Intestine [12,13]

	Male	Female
Rectum	4.0±1.0	3.5±1.0
Colon sigmod	3.4±0.6	3.2±0.6
Descendant colon	3.4±1.2	3.2±0.6
Transverse colon	4.2±1.2	4.2±0.7
Ascendant colon	4.8±1.2	5.0±2.0
Cecum	4.7±0.9	4.8±0.8

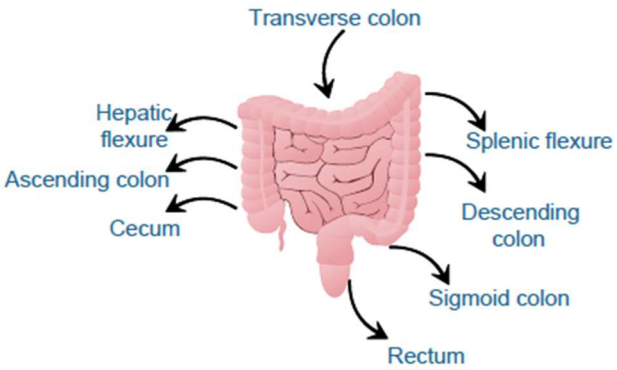


FIGURE 2: Anatomy of Colon

4. APPROACHES FOR COLON DRUG TARGETING

4.1prodrug Approaches

4.1.1 Azo Bond Conjugate

The medication is connected in this method via an azo bond. The gut microbiota produces enzymes called azoreductases, which cleave (break) the azo bond in the colon after the medication passes through the upper gastrointestinal tract (GIT). This targeted delivery mechanism allows the drug to be

selectively released in the colon. The bacteria that live in the intestines substantially break down the azo compounds through extracellular reduction as well as intracellular enzymatic activities. Sulfasalazine, a medication used to treat inflammatory bowel diseases (IBD), is composed of two linked components - sulfapyridine, which has antibacterial properties, and 5-aminosalicylic acid (5-ASA), which has anti-inflammatory effects. These two drug components are connected via an azo bond. The azo link is broken in the colon by enzymes known as azoreductases, which releases the active ingredient in the medication along with the carrier molecule sulfapyridine[14]

4.1.2 Glycoside Conjugated Prodrug

The human gut microbiome produces various enzymes called glycosidases, including β-D-galactosidase, α-L-arabinofuranosidase, β-D-xylopyranosidase, and β-D-glucosidase. These glycosidase enzymes are located in the colon's brush edge, which is the surface layer of cells lining the colon. Many naturally occurring drugs contain a glycoside structure, composed of a sugar moiety (glycoside) and a pharmacologically active non-sugar component (aglycon). When these drugs are administered orally and reach the colon, the glycosidase enzymes act on the glycoside part, cleaving it off and releasing the pharmacologically active aglycon component. Glycosides are soluble in water and hydrophilic substances that are poorly absorbed from the gastrointestinal tract (GIT). Glycosides have the ability to transport medications to the colon because of these characteristics. Glucosides, galactosides, and cellobiosides of the corticosteroids dexamethasone, prednisolone, hydrocortisone, and fludrocortisone are a few examples of medications that are targeted using this method. One specific example is dexamethasone-21-β-glucoside. Studies were conducted in rats comparing two prodrugs (dexamethasone-21-β-glucoside and prednisolone-21-β-glucoside) to the unmodified steroid forms (dexamethasone and prednisolone). According to the findings, the unmodified analogues were absorbed in the small intestine, while both modified steroid versions reached the cecum[15]

4.1.3 Glucuronid Conjugation Prodrug

A key drug metabolic route is the conjugation of glucuronides. β-Glucuronidases, enzymes secreted by the large intestine, can deglucuronidate (remove the glucuronide group from) various drugs. This allows the drugs to be reactivated and potentially reabsorbed in the colon. Drugs are delivered to the colon by using this idea of drug metabolism. The medication is joined to a glucuronide group, a typical metabolic conjugation. When a medication is taken orally and enters the colon, the β-Glucuronidases in the colon selectively break the glucuronide conjugation.This releases the active drug molecule, allowing it to be delivered to the target site in the colon. Colon targeting studies have been conducted using glucuronide conjugation of the narcotic antagonist drugs naloxone and nalmefene. These studies suggest that glucuronide conjugation is a useful approach for delivering such drugs to the colon. This could be beneficial in treating constipation caused by opioid medications, as the glucuronide-conjugated forms would be selectively released in the colon to counteract the constipating effects of the opioids[16]

4.1.4 Dextran Conjugated Prodrug

Dextran is a carbohydrate that serves as an energy source for the colonic microbiome. Dextran hydrogels have been used to deliver drugs to the colon at precise sites.Non-steroidal anti-inflammatory medications (NSAIDs) and dextran have been joined via ester bonds to create a variety of prodrugs.When these dextran-NSAID conjugates are administered orally, upon reaching the colon, the enzyme dextranase produced by the human gut flora breaks down the ester linkage, liberating the free drug molecule[17]

4.1.5 Cyclodextrine Conjugation

Cycle-like oligosaccharides, cyclodextrins are made up of six to eight glucopyranose units connected by α(1-4) glucosidic linkages, organised in a cyclic configuration. Because their surface is hydrophilic

and their inside is lipophilic, they can form inclusion complexes with a variety of medicines. Because cyclodextrins are large, non-toxic molecules that absorb little from the gastrointestinal tract (GIT), they are useful as carriers for medications that change during absorption in the stomach and intestinal milieu.

α-cyclodextrin, β-cyclodextrin, and γ-cyclodextrin are the three primary analogues of cyclodextrins. Both the β- and γ-cyclodextrin forms exhibit greater stability against gastric pH as well as pancreatic, salivary, and stomach enzymes. While the colonic microbiota fully breaks them down, they are only partially broken down in the small intestine.

Research has been carried out on rats using cyclodextrin conjugates of the anti-inflammatory medication biphenyl acetic acid.It was found that the cyclodextrin form of biphenyl acetic acid selectively reached the colon and released the drug without being absorbed in the upper GIT[18]

4.1.6 Protein Conjugate

Proteins are hydrophilic by nature because they include polar functional groups such as amino (-NH2) and carboxyl (-COOH) groups. The membrane permeability of some proteins can be decreased by these polar groups. Consequently, such polar amino acids have been conjugated to create prodrugs, which allow for site-specific medication delivery to the colon. Within two hours of oral treatment, salicylic acid was found in the blood. Blood samples were observed to contain the intact salicylic acid-glycylglycine combination after intravenous administration. However, free salicylic acid was found in the blood samples after intracaecal injection. These results indicate that cleaving of such amino acid conjugates is carried out by the intestinal microbiota. This concept of utilizing intestinal microflora to cleave prodrug conjugates is useful for delivering drugs specifically to the colon[19]

5. CRITERIA FOR SELECTION OF DRUG FOR TARGETTING COLON

Controlled delivery of drug are particularly beneficial for medications that have poor absorption in the stomach or intestines, such as peptides. Because these systems allow for targeted delivery to the colon, they are particularly beneficial for medications used to treat disorders such as diarrhoea, ulcerative colitis, inflammatory bowel disease (IBD), and colon cancer.

The choice of drug carrier plays a significant role in Controlled Drug Delivery Systems (CDDS). The targeted ailment and the drug's physicochemical properties are taken into consideration while choosing the right carrier for a certain medication.

The partition coefficient, stability, chemical characteristics, and kind of absorption enhancer employed all have an impact on the carrier selection. Moreover, the functional groups found in the drug molecule have an impact on the choice of drug carrier.

5.1 PH

pH levels across the gastrointestinal tract span from 1 to 8, with the stomach residing in the acidic range (PH 1-3) and the small intestine transitioning from acidic to neutral (pH 5.9-7.8) [20,21]. This acidic environment in the colon plays a pivotal role in directing certain drugs to the colon, thus emphasizing its significance in colon drug delivery. Leveraging this pH dependency can safeguard drugs from degradation in the acidic stomach environment by utilizing polymers such as Eudragit S100 to coat the drug, which remains stable under these conditions[22]. However, challenges arise due to variations in pH values among individuals, both within and between them. Additionally, patients with Crohn's disease and colitis often exhibit lower colonic pH levels, casting uncertainty on the effectiveness of pH-dependent drug delivery[23].

5.2 PH Ranges of Various Segments in the Colon

Dew et al [18] were pioneers by incorporating the enteric polymer Eudragit®S into capsule dosage

forms, gastrointestinal pH can act as a trigger for medication release in the distal gut. Any pH level above 7 causes Eudragit S, a copolymer of methyl methacrylate and methacrylic acid, to disintegrate. Following the initial research, a subsequent study was conducted involving tablets coated with Eudragit S. This study focused on patients with ulcerative colitis, examining the efficacy and safety of the coated tablets in this specific patient population [24].PH of caecum ranges from 6.4±0.4,PH of Ascending colon ranges from 6.37±0.58,PH of transverese colon ranges from 6.61±0.83,PH of descending colon ranges from 7.04±0.67[25],PH of sigmoid colon ranges from 7.38±0.59,PH of rectum ranges from 7.15±0.44[26]

5.3 Colonic Microflora

The human colon harbors over 300 bacteria species, employing hydrolytic and reductive enzymes to break down polysaccharides for their energy needs[27]. This unique bacterial environment can influence the behavior of both drug dosage forms and drugs themselves. Consequently, polysaccharides and prodrugs like pectin, guar gum, and chitosan are often chosen for colon delivery, as they can be degraded into active drugs by bacterial enzymes[28]. However, drug metabolism by bacteria may lead to toxicity or ineffectiveness. Furthermore, factors such as drug, diet, and disease can perturb the balance of colonic microflora[29]. These observations underscore the importance of considering how these variables can affect drug release when designing colon-specific drug delivery systems reliant on bacterial enzymes.

5.4 Transit Time

Transit time refers to the duration it takes for food to pass through the digestive system, from the mouth to the anus. Transit time can significantly differ among people and is influenced by the specific nutrients and ingredients present in the meal. Fryne *et al* [30]Krevsky et al[31] employed a distinct method to determine the duration of food transit through various parts of the gastrointestinal tract. Time-dependent colon-targeted delivery systems make use of the gastrointestinal tract's transit time as a tactic. In the colon, the transit time ranges from 6 to 70 hours, whereas in the small intestine, it is 2 to 6 hours[32]. Patients with colitis exhibit quicker transit times compared to others[33], whereas there is a decrease in transit time for formulations in individuals with bowel disease[34]. This reduction in transit time can diminish the effectiveness of therapeutic agents due to shorter exposure periods to the affected areas.

5.5 Mucus Barrier

Mucus is a hydrogel layer composed mostly of big glycoproteins called mucins [35]It acts as the initial physical barrier that oral drug formulations face during absorption in the gastrointestinal tract [36]In humans, the intestinal mucus layer is made up of a loose, luminal layer and a thinner, more tightly adherent basal layer[37]. The total thickness of the mucosal layer varies between 10 and 200 micrometers, depending on its location in the gastrointestinal tract, ranging from the jejunum to the colon [38]

The primary roles of GI mucus are:

- Providing lubrication to chyme (partially digested food)
- Shielding the epithelium from mechanical harm
- capturing microorganisms and keeping them from entering the epithelial cells [35]

Most foreign particles—including those from conventional medication delivery systems—are efficiently absorbed via adhesion into the human GI mucosal layer and subsequently eliminated in stool. This can greatly restrict the duration of sustained local drug delivery and potentially lead to suboptimal therapeutic effects

6. FUNCTIONS OF COLON

A fitting location and conditions for the development of colon-dwelling microorganisms.
The bacteria found in the large intestine are abundant in cytochrome and contribute to the body's normal flora, which inhibits the growth of harmful bacteria and provides benefits. Additionally, certain bacteria have the ability to break down cellulose, and individuals with constipation have been found to break down cellulose more effectively, thereby reducing the excess bulk in their system.
Creation of feces and a repository for waste materials. The process of absorbing potassium and water from the digestive tract leads to the formation of feces, and this section of the intestine is particularly efficient at absorbing substances like saline, glucose, certain anesthetics, and amino acids. The release and elimination of potassium and bicarbonate, along with metals such as bismuth, mercury, and arsenic, are carried out in this area. In the colon, microorganisms perform synthesis functions by producing vitamin K and folic acid. While these microorganisms also synthesize a significant quantity of vitamin B12, it is not absorbed by the body[39]

7. TYPES OF MICROSPHERES

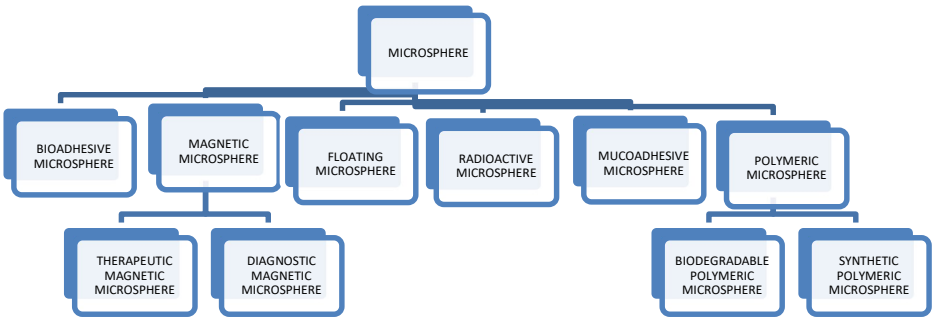


FIGURE 3: Types of Microspheres

7.1 bioadhesive Microspheres

The process of attaching a medication delivery device to a mucosal membrane—such as those in the mouth, eye, rectum, or nose—is known as bioadhesion."Bioadhesion" refers to the properties of substances that adhere to biological surfaces, such as mucous membranes. By adhering to mucosal tissue, bioadhesive drug delivery devices can establish a close and sustained contact at the administration site. This prolonged contact can enhance drug absorption and improve patient compliance by reducing the need for frequent medication administration. By attaching a medication to carrier particles like liposomes, nanospheres, microspheres, or nanoparticles, carrier technology offers a tactical way to control the release and absorption of drugs.Microspheres are frequently preferred in these techniques because of their compact size and excellent capacity for carrying drugs[40,41]

Researchers Ahmad et al [42]created a bioadhesive microsphere (BAM) using Assam Bora rice starch to deliver metronidazole specifically to the colon. It has been observed that the bioadhesive microparticles (BAMs) stayed in the colon for a longer amount of time and improved medication absorption. Studies conducted in vitro showed that while over 90% of the metronidazole was released in the cecal content, just a minor portion was released in the stomach and small intestine. Additionally, in vivo investigations demonstrated that the medication exhibited comparable pharmacological effects to commercial formulations and was only released when the BAMs reached the colon.
Example of Bioadhesive Microsphere Metronidazole

7.2 Magnetic Microspheres

These microspheres possess a valuable property for drug delivery, as they can localize medication to the site of disease. The main objective of these microspheres is to minimize the reliance on freely circulating drugs by substituting them with a reduced amount of magnetically targeted drugs Magnetic microspheres, smaller than 4 micrometers, can move efficiently through blood capillaries without causing any obstructions. These microspheres can be guided to the site of disease using an external magnetic field ranging from 0.5 to 0.8 tesla, as illustrated in the extraction of neuroblastoma cells from bone marrow using monoclonal antibodies linked to magnetic microspheres.[43–46]

Example of magnetic microspheres targeting colon

Karkar et al[47] have been formulated Mesalamine microsphere as magnetic microsphere

There are two types of Magnetic Microspheres

7.2.1 Therapeutic Magnetic Microsphere

The primary objective of these microspheres is to target liver tumors by delivering a chemotherapy drug to the affected area. To provide targeted therapy, these microspheres are usually filled with medications based on proteins or peptides.

7.2.2 Diagnostic Magnetic Microsphere

These microspheres are mostly used to image liver metastases, but they may also be modified to create nanoparticles like supramagnetic iron oxides, which help identify intestinal loops from other abdominal structures [48].

7.3 Floating Microspheres

The density of the floating types is lower than that of stomach fluid, which allows them to remain buoyant regardless of the stomach pace of emptying. These systems release the medication gradually while remaining buoyant on the contents of the stomach. This prolongs the time the drug is in the stomach and causes fluctuations in plasma concentration. Furthermore, by decreasing the likelihood of a sudden dose release, this approach ensures a long-lasting therapeutic effect. Moreover, it reduces the risk of dose hitting and dumping, thereby prolonging the therapeutic effect and reducing the requirement for frequent dosing [49–51] Subhash *et al*[52] have been formulated 5-Florouracil using ethyl cellulose as a floating microspheres targeted to the colon

7.4 Radioactive Microspheres

Microspheres used in radioembolization treatment are bigger than capillaries, usually ranging in size from 10 to 30 nanometers, and they lodge in the first capillary bed upon contact. They are injected into the arteries that supply the intended tumour, preserving the surrounding healthy tissues while providing a targeted dosage of radiation. This technology differs from conventional drug delivery techniques because the radioactivity remains contained within the microspheres and operates within the radioisotope's typical range. There are three distinct types of radioactive microspheres: gamma, beta, and alpha emitters[53,54]

7.5 Mucoadhesive Microspheres

Adding mucoadhesive properties to microspheres offers further advantages, including improved drug absorption and increased bioavailability. This is accomplished by precisely directing medication to the absorption site, interacting with the mucus layer more closely, and having a larger surface-to-volume ratio. Mucoadhesive microspheres can be made to stick to many mucosal tissues, including those in the gastrointestinal tract, nasal cavity, urinary tract and eye. This capability allows for controlled drug release, targeting both localized and systemic delivery.This is accomplished by engineering the microspheres to adhere to mucosal tissue, enabling precise and controlled delivery of medications.[55]

Nalini et al[56]have been formulated Con-A conjugated mucoadhesive microspheres for the colonic delivery of diloxanide furoate

7.6 Polymeric Microspheres

Different forms of polymeric microspheres exist, including synthetic and biodegradable varieties[57]

7.6.1 Biodegradable Polymeric Microspheres

Because they are compatible with biological systems, naturally decay, and stick to biological surfaces, natural polymers like starch are used in many applications. Their significant ability to swell in water-based environments extends their interaction with mucosal membranes, resulting in the gel's creation. The polymer concentration controls the rate and volume of medicine release as well as the sustained release pattern. However, in clinical applications, the main obstacle is the complex drug loading efficiency of biodegradable microspheres, which makes drug release management more difficult[58,59]

7.6.2 Synthetic Polymeric Microspheres

Synthetic polymeric microspheres are widely used as embolic particles, fillers, bulking agent and drug delivery vechicle in a variety of therapeutic contexts. It has been shown that they are safe and work well with biological systems. However,there is a significant disadvantage to these microspheres :they have tendency to drift away from the injection site,which raises the risk of embolism and the subsequent organ damage. [60,61]

8. PREPARATION TECHNIQUES

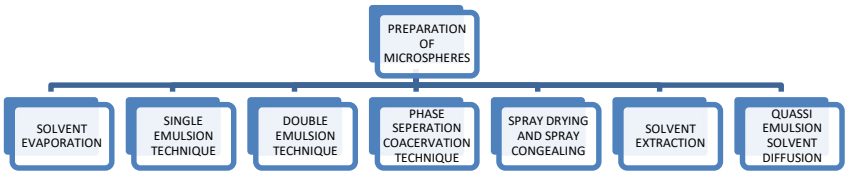


FIGURE 4: Microspheres Preparation Techniques

8.1 Solvent Evaporation

The microencapsulation procedure occurs within a liquid manufacturing environment, where the coating of the microcapsule is blended with a volatile solvent that does not blend with the vehicle phase of the liquid manufacturing process. The coating polymer solution dissolves or disperses a material intended for microencapsulation. To produce microcapsules of the appropriate size, the liquid production vehicle phase is mixed with the core material combination and agitated. To allow the polymer of the core material to distribute throughout the polymer solution and shrink around the core, the mixture may need to be heated in order to evaporate the solvent. Matrix-type microcapsules are created when the core material dissolves in the covering polymer solution. Substances that are either soluble in water or insoluble in water may make up these basic materials. Vapourization of the solvent causes the polymer solution to form an emulsion with a continuous phase that can be either aqueous (o/w) or non-aqueous[62–65]. Kishori et al[66]have been formulated tinidazole microspheres by using this solvent evaporation method.

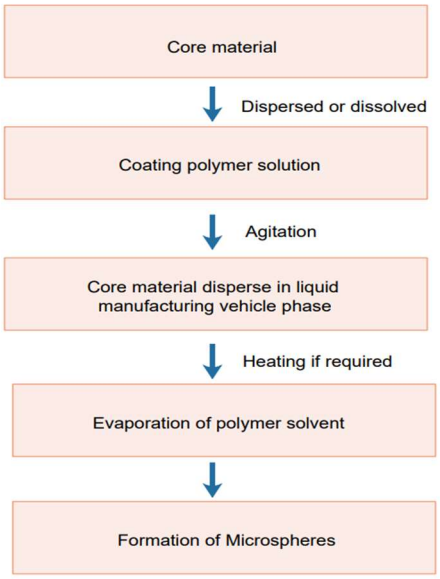


FIGURE 5: Formation of Microspheres by Using Solvent Evaporation

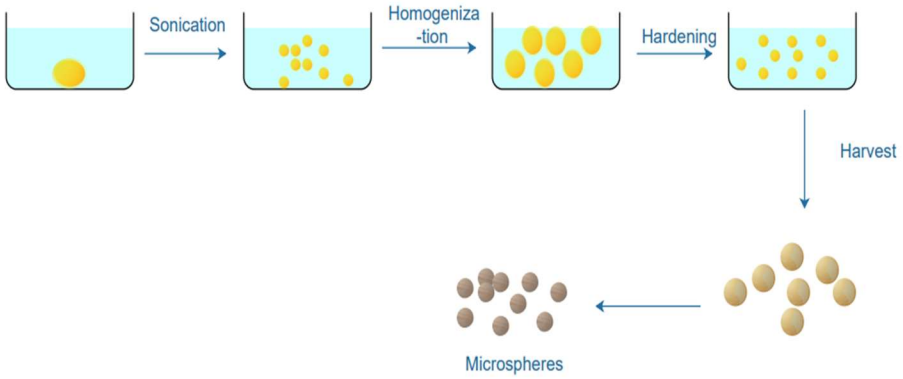


FIGURE 6: Process of Solvent Evaporation

8.2 Single Emulsion Technique

One common method for creating micro-particulate carriers from natural polymers, such proteins and carbohydrates, is the single emulsion process. The natural polymers are first dissolved or dispersed in an aqueous solution, and then they are dissolved in a non-aqueous media, such as oil.The dispersed globules are then cross-linked, achieved through either heat treatment or chemical cross-linkers. Materials that are susceptible to high temperatures should not be subjected to heat denaturation. However, if the active component is applied during preparation and then goes through procedures like centrifugation, washing, and separation, chemical cross-linking may expose it to an excessive amount of chemicals. The final multiparticulate product's size, distribution, surface features, drug loading, drug release, and overall performance are all greatly influenced by the surfactants chosen to stabilise the emulsion phases.[67–70]

S. C. Dhawale et al[71] have been formulated 5- Fluorouracil microspheres targeted to colon by using

single emulsion method.

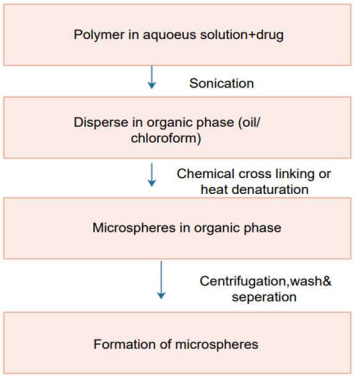


FIGURE 7: Formation of Microspheres by Using Single Emulsion Method

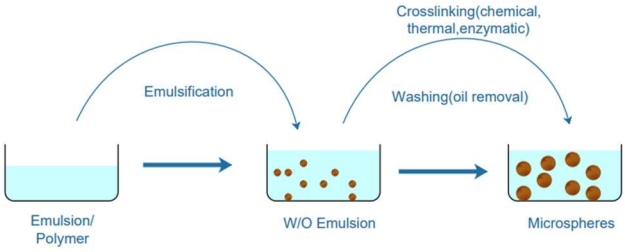


FIGURE 8: Process of Single Emulsion

8.3 Double Emulsion Technique

In order to produce microspheres, the double emulsion approach involves creating double emulsions, usually of the w/o/w kind, which works well for water-soluble medications, peptides, proteins, and vaccines. This method works with both synthetic and natural polymers. A lipophilic organic continuous phase contains a protein solution that may contain active substances. This continuous phase usually consists of a polymer solution that is meant to encapsulate the protein in the aqueous phase that is disseminated. A double emulsion is produced by sonicating the original emulsion and combining it with an aqueous polyvinyl alcohol (PVA) solution. After that, the emulsion is treated to extract the solvent, usually by evaporation or extraction. This method has been used to effectively incorporate a wide variety of hydrophilic drugs into microspheres, including vaccines, proteins, peptides, LH-RH agonists, and traditional small molecule drugs.[72–74]

Ogawa et al[75]have been formulated Eudragit Microspheres by this double emulsion method

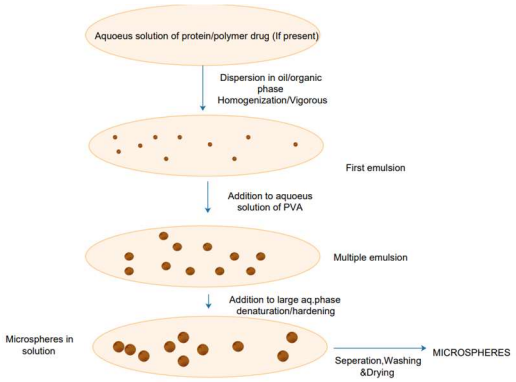


Figure 9: Schematic Representation of Double Emulsion Method of Microspheres Preparation

8.4 Phase Seperation Coacervation Technique

The phase separation technique is specifically tailored for creating reservoir-type systems, primarily for encapsulating water-soluble drugs like peptides and proteins. However, some formulations may take on a matrix structure, especially when dealing with hydrophobic drugs such as steroids. By decreasing the polymer's solubility in the organic phase, this technique produces a polymer-rich phase known as coacervates.Drug introduction involves first dissolving the polymer in a suitable solvent, followed by the drug's introduction into an aqueous solution (if hydrophilic) or its dissolution in the polymer solution (if hydrophobic). By altering the conditions of the solution, for as by adding salt, introducing non-solvents, integrating incompatible polymers, or regulating pH, phase separation can be caused. Throughout this process, continuous stirring is maintained to control the size of the micropartices.[76]

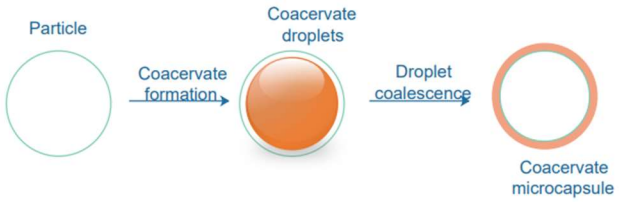


FIGURE 10: Formation Process of Coacervate Microsphere

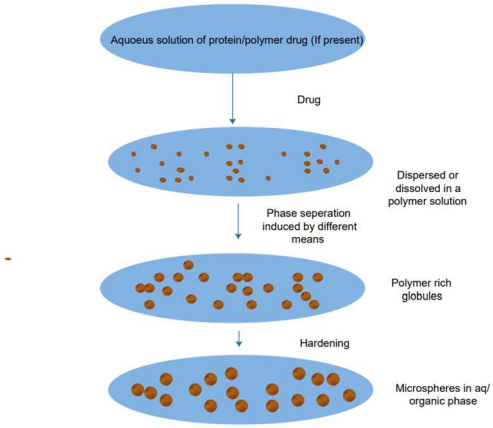


FIGURE 11: Schematic Representation of Microspheres Formulation by PHASE SEPERATION METHOD

8.5 Spray Drying and Spray Congealing

This method uses spray drying and spray congealing to air-dry a polymer and drug combination while removing the solvent or chilling the solution. During spray drying, the medication is disseminated into the polymer solution by high-speed homogenization after the polymer has been dissolved in a volatile organic solvent like dichloromethane or acetone. After the mixture is atomized in a hot air stream, the solvent quickly evaporates, generating tiny droplets or a fine mist that eventually become microspheres with a typical size range of 1 to 100 µm. A cyclone separator is used to separate the microparticles from the heated air, and vacuum drying is used to eliminate any remaining solvent. An advantage of this method is its applicability in sterile conditions [77,78]. The example of microspheres developed by this technique Somatropin, Triptorelin, Lanreotide.

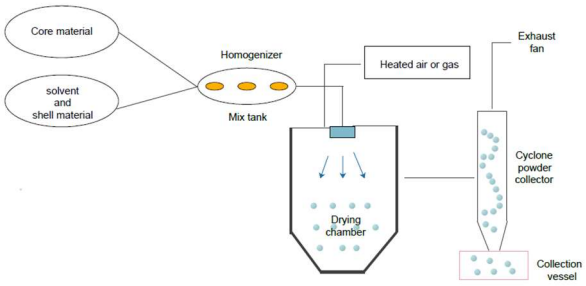


FIGURE 12: Schematic Representation of Spray Drying and Spray Congealing

8.6 Solvent Extraction

An organic solvent is used to disperse the polymer, and the drug is either dissolved or distributed inside the polymer solution. After that, the product-containing solution is emulsified into an aqueous phase using necessary additives (such polymers or surfactants) to create an oil-in-water emulsion. Following emulsification, the organic solvent is extracted using either constant stirring or heating under pressure. When the solvent is removed, the polymer precipitates at the oil-water droplet interface and forms cavities [79–83]

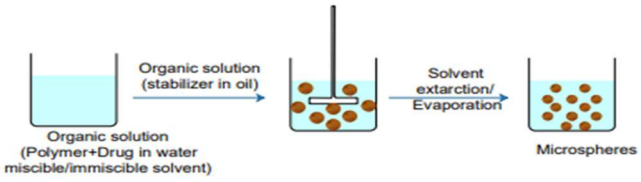


FIGURE 13: Schematic Representation of Solvent Extraction for the Formation of Microspheres

8.7 quassi Emulsion Solvent Diffusion Method:

Academic sources describe the production of drug-controlled release microspheres from acrylic polymers using a novel quasi-emulsion solvent diffusion process.This method, known as Quassi emulsion solvent diffusion, makes microsponges by using an external phase made of distilled water and polyvinyl alcohol(81). Polymers, ethanol, and the drug make up the interior phase. The exterior phase comprising polyvinyl alcohol and distilled water is added after the internal phase, which is first prepared at 60°C. An emulsion is formed by constantly stirring the two phases for a duration of two hours. Filtration is followed by the recovery and separation of the microsponges. [84]

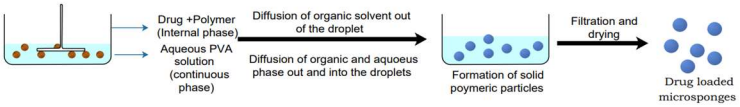


FIGURE 14: Process of Formation of Microspheres by Using Quassi Emulsion Solvent Diffusion Method

9. ADVANTAGES OF MICROSPHERES

Microspheres offer the advantage of consistent drug absorption, ensuring a steady level of medication in the bloodstream. Additionally, the release of drugs in the stomach is impeded, preventing premature degradation and enhancing stability. This controlled release mechanism contributes to enhanced therapeutic efficacy, even with a brief duration of action, as the medication is delivered more efficiently to the target site[85]

Microspheres, often used in drug delivery systems, provide a sustained and consistent therapeutic impact due to their ability to control the release of medication over an extended period. This can be particularly beneficial in enhancing patient adherence to treatment regimens. By decreasing the dosing frequency, patients are less likely to miss doses, thereby improving overall treatment efficacy. By increasing a drug bioavailability, microspheres can make sure that a larger proportion of the active component reaches the intended place in the body. This combination of controlled release, reduced dosing frequency, and improved bioavailability makes microspheres a valuable technology in the field of medicine.

Microspheres reduce the occurrence of dose dumping, providing a more controlled and steady release of medication. They also protect drugs from harsh environmental conditions, preserving their stability and efficacy. Microspheres can conceal the taste and smell of medications, making them more palatable for patients. Furthermore, they help overcome initial metabolic breakdown, ensuring that a higher percentage of the active ingredient remains effective when it reaches the target site [86–88]

10. DISADVANTAGES OF MICROSPHERES

Variability in drug release from diverse formulations within the gastrointestinal tract (GIT) can lead to differences in the rate of release between different doses. This uneven drug release can potentially cause toxicity, highlighting the importance of following dosage instructions carefully. It is crucial not to crush or chew the tablet, as this can alter the controlled release mechanism and lead to unexpected side effects or reduced efficacy[89]

Variations in temperature, pH variations, the addition of solvents, agitation, or evaporation can all impact the stability of the core particles meant for encapsulation, resulting in a reduction in uniformity in drug release. [90]

11. REGULATORY ASPECT

Quality by Design (QbD) is integral to the International Conference on Harmonization (ICH) Quality guidelines and offers a significant chance for the pharmaceutical sector to propel manufacturing sciences forward into the future. Establishing a quality target product profile (QTPP) is the first step in the QbD approach. This defines the critical quality attributes (CQAs). Next, a critical material

attribute (CMA) and critical process parameter (CPP) analysis should be conducted methodically.The QbD approach highlights a methodical and proactive approach to product development, ensuring that quality is integrated into the product right from the start. QbD is described by ICH Q8 (R2) as a methodical approach to product development that starts with predetermined goals, stresses comprehending and controlling the product as well as the process, uses sound scientific concepts, and incorporates risk management. The guideline emphasizes the importance of designing the product to meet the requirements and expected performance desired by patients. Recently, the QbD approach has been widely applied in developing numerous pharmaceutical products. While companies and products may vary in their approaches to product development, QbD implementation typically starts with defining the Quality Target Product Profile (QTPP) in many instances. Based on risk assessment, the QTPP offers a proactive overview of the drug product's features that are normally met to guarantee its intended quality, accounting for safety and efficacy. This profile delineates various factors crucial to the safety and efficacy of the drug product. Once established, CQAs are identified during the drug development process. According to ICH Q8, these Critical Quality Attributes (CQAs) are the physical, chemical, biological, and microbiological traits or qualities that have to adhere to specified bounds, ranges, or distributions in order to guarantee the intended product quality. During the formulation of microspheres, various quality characteristics must be considered. However, the Critical Quality Attributes (CQAs) are particularly crucial because their failure to meet specifications could pose serious risks to patients. Long-acting injectables utilizing microspheres have been extensively used for delivering peptides and protein therapeutics, benefiting from their capacity to load high amounts and sustain the release of biologically active macromolecules over extended periods. Researchers have investigated numerous biocompatible and biodegradable polymers, both natural and synthetic, to develop optimal microsphere formulations [91]

12. CHARACTERIZATION OF MICROSPHERES

12.1 Physiochemical Evaluation

12.1.1 Size and Shape of Particle

Scanning electron microscopy (SEM) and light microscopy (LM) have historically been the methods most commonly employed to observe microspheres. To ascertain the form and surface organisation of these microspheres, both techniques can be utilised. Double-walled microsphere coating parameters can be controlled using light microscopy, however scanning electron microscopy offers a greater resolution than light microscopy. Microsphere surfaces may be examined using scanning electron microscopy. Moreover, cross-sectioning particles allows for the investigation of double-walled structures. Multiwalled microspheres are characterized by their structure using confocal fluorescence imaging. Coulter counters and laser light scattering are two methods used in addition to experimental approaches to ascertain the form, size, and shape of microspheres [92]

12.1.2 Drug Entrapment Efficiency

A specific quantity of microspheres were dissolved in methanol, they were sonicated for fifteen minutes. The following equation was then used to filter, appropriately dilute, and use a spectrophotometer to determine the drug concentration in the mixture [93]

% Entrapment = (Actual content/Theoretical content) ×100

12.1.3 Percentage Yield

It is calculated by dividing the weight of the microspheres made from each batch by the total weight of the drug and polymer needed for that batch, and then multiplying the result by 100.

12.1.4 Angle of Contact

The contact angle is evaluated to assess the wetting properties of the micro-particle carrier and determine whether the microspheres are hydrophilic or hydrophobic. Each component that has been adsorbed has an impact on this intrinsic solid characteristic. In order to measure the advancing and retreating angles of the contact angle, a droplet is put in a circular cell above the objective of an inverted microscope and the contact angle is measured at the solid/air/water interface. At 20°C, the contact angle is measured one minute after the microspheres are deposited [94]

12.1.5 The Percentage of Entrapment Efficiency

The following formula was used to determine the encapsulation efficiency.

Entrapment efficiency = AQ/TQ×100

AQ = Acutal drug present in microsphere

TQ = Theoretical amount of drug in microsphere [95]

12.1.6 Swelling Studies

After being dried, the microspheres were carefully measured. They were then put in a USP dissolving apparatus II with 900 ml of phosphate buffer (pH 6.8) at 37 ± 2 °C and allowed to swell until their weight stabilised. The microspheres were properly dried with filter paper after removal, and the weight difference was recorded. Three times over, these steps were repeated.

The given formula was then used to calculate the swelling index.

Swelling index = (Wg –Wo) × 100 / Wo

Where, W o = Microspheres initial weight

Wg = the weight of microspheres at equilibrium swelling in the medium[96–98]

12.1.7 Density Determination

A multivolume pycnometer is utilized to ascertain the microspheres' thickness. First, the sample is carefully placed in a vessel, and then introduced into the multivolume pycnometer. Helium is introduced into the chamber at a consistent pressure, allowing for expansion. In this development, the results hold less significance within the group. The initial weight is recorded when two consecutive weight readings differ. The volume, determined by two weight readings, can ascertain the thickness of the microsphere's transporter[99]

12.1.8 Electron Spectroscopy in Chemical Analysis

The microspheres surface chemistry may be ascertained by electron spectroscopy for chemical analysis, or ESCA[100]

12.1.9 Fourier Transform-Infrared Spectroscopy

The polymeric structure of the carrier framework is altered by the application of FT-IR. Measurements of Attenuated Total Reflectance (ATR) infer the microspheres' exposed surface. The ATR cell allows the infrared beam to pass through, and the sample is usually reflected back, giving rise to IR spectra that are mostly focused on the surface material. Microsphere surface structure is shown using ATR-FTIR, which takes into account environmental influences and manufacturing processes [101]

12.2 Invitro Method

The drug release is established using the following techniques.

12.2.1 Beaker Method

An overhead stirrer is used to continuously mix the dosage form so that it remains in the medium at the bottom of the beaker. Previous research has employed medium volumes ranging from 50 to 500 cc and stirrer speeds from 60 to 300 rpm[102,103]

12.2.2 Interface Diffusion Method

This method, which has four separate portions, was created by Dearden and Tomlinson. First, a suitable

dosage of medication in buffer solution is kept in Compartment A, which is designed to resemble the mouth. Compartment B resembles the buccal membrane and contains 1-octanol, while compartment C simulates physiological fluids and includes 0.2M HCl. Compartment D is used to illustrate protein binding and contains 1-octanol. Prior to use, the aqueous and 1-octanol phases must be saturated with one another. Using a syringe, samples are taken out of compartment A and then put back in

12.2.3 Dissolution Apparatus Method

Both of the spinning parts of a normal USP or BP dissolving apparatus—the paddle and the basket—have been used to evaluate the in vitro release characteristics. The research used a dissolving media that has a volume ranging from 100 to 500 ml with rotating speeds between 50 and 100 rpm[104]

12.3 In Vivo Method

In order to measure intact mucosal permeability, techniques that evaluate an organism's biological response, either locally or systemically, as well as those that directly measure drug absorption or accumulation at the mucosal surface, are utilised. The most widely used in vivo research techniques usually involve the use of animal models and buccal absorption tests [105]

13. APPLICATION OF MICROSPHERES IN PHARMACEUTICAL INDUSTRY

13.1 Vaccine Delivery through Microspheres

The formation of immunity against infectious illnesses is the fundamental concept behind the protective effects of vaccines. Examples of such vaccinations, like those for tetanus, diphtheria, and cholera, are encapsulated within microspheres[106]Microspheres that contain vaccines prolong the release of antigens over weeks or even months, thereby boosting the immune response[107,108]Enclosing the vaccine within an appropriate carrier and retaining it until its release prevents vaccine degradation. When administering a vaccination under control, it is possible to accommodate both the adjuvant and the antigen within a single carrier, which may lessen the likelihood of systemic side effects. Biodegradable polymers are employed to ensure a stable release of encapsulated antigens while they decompose into harmless, minuscule constituents inside the organism. Chitosan microspheres can encapsulate a range of antigens. It is frequently chosen to use synthetic biodegradable polymers, such poly-lactic-co-glycolic acid, to encapsulate antigens in single-dose vaccinations [109]

13.2 Delivery of Opthalmic Drug through Microspheres

Polymers used in administering ocular medications provide adhesive properties and enhance permeability. Polymer hydrogels are more effective than other forms of eye drug delivery, like ointments or solutions, due to their elasticity [110].A chitosan gel prolongs the presence of drugs in the precorneal area by preventing their escape through tear flow, spreading across the conjunctiva, and enhancing adherence to the eye's mucous membrane. Drug-loaded microspheres can be incorporated into polymer hydrogel systems to enable controlled or continuous medicine delivery in the eye [111]

13.3 Delivery of Genes Using Microspheres

Recombinant adenoviruses are often employed for gene transfer because they are very efficient and can target many kinds of cells. However, when used in vivo, they can cause immunological reactions and can cause cancer. Viral vector-based gene therapy usually requires recurrent delivery. In order to distribute genes continuously without the risk of infection, genes are encased in microspheres. Microspheres offer advantages such as scalability, reproducibility, stability, simplicity of production, and precise targeting of cells or tissues [112]

13.4 Microspheres Containing Monoclonal Antibodies

When it comes to antigen molecules that are present at the targeted region, monoclonal antibodies

exhibit a high degree of specificity. Microspheres exploit this selectivity to target pharmacologically active substances [113] .There are several ways to attach monoclonal antibodies to microspheres: chemical coupling, nonspecific adsorption, covalent bonding, and selective adsorption. The free carboxyl, aldehyde, amino, or hydroxyl groups on the surface of microspheres can be bound by these antibodies. For as long as six months, monoclonal antibodies against vascular endothelial factor were persistently released from microspheres [114]

13.5 Microspheres for Delivery of Protein and Peptide

Studies have looked at the regulated release of peptides and proteins from microspheres composed of biodegradable polymers [115]. Plasma proteins or peptides can be preserved in these microspheres for extended periods of time at steady-state concentrations. Protein/peptide therapies can be produced using chitosan microspheres, polylactic-co-glycolic acid, and biodegradable polylactic acid. Microsphere-based distribution is used by commercially marketed peptide drugs such as abarelix, buserelin, lanreotide, and triptorelin[116]

13.6 Microspheres Utilized In Cancer Treatment

Microspheres that contain radioactive substances like yttrium-90 are utilized to treat liver cancers. After being injected into the hepatic artery, these 30-micron-diameter microspheres go to the tumor vasculature, where radiation exposure destroys the tumor cells while sparing the nearby healthy cells. Colon cancer can be treated with the drug in polymeric microspheres. 5-fluorouracil. These microspheres stop the drug from breaking down in the stomach[117]

13.7 Porous Microspheres for Topical Use

Numerous active substances, including volatile oils, emollients, and perfumes, can be encapsulated in these porous microspheres. They are used as carriers for topical therapies and can be added to products like creams, lotions, and powders[118,119]

13.8 Drug Delivery through Vaginal Route

A polymer that has had its major amino groups changed with thioglycolic acid encapsulates clotrimazole, an imidazole derivative that is frequently used to treat fungal infections of the genitourinary tract. The polymer's capacity to stick to mucosal surfaces is significantly improved by the addition of thiol groups. This allows the polymer to stay on vaginal tissue for up to 26 times longer than it would have otherwise and allows for controlled medication release to treat fungal infections. Together with metronidazole and acriflavine, this polymer has been used in vaginal tablets that have shown good adhesion qualities and efficient drug release[120]

13.9 Colonic Drug Delivery

Insulin has been developed with the goal of delivering it straight to the colon by the use of polymers. Insulin, together with a variety of absorption boosters and enzyme inhibitors, was put into chitosan capsules with an enteric coating (hydroxy propyl methyl cellulose phthalate). The capsules were shown to dissolve exclusively in the colonic area; this may have been caused by lower pH levels in the ascending colon relative to the terminal ileum or by the activity of bacterial enzymes that may break down the polymer[120]

13.10 Delivery Through Buccal

Because polymer has muco/bio adhesive qualities that improve absorption, it's a great option for buccal distribution. Through prolonged drug release in the buccal cavity, buccal tablets containing chitosan microspheres and chlorhexidine diacetate show increased antibacterial efficacy. Because of the nature of the polymer, even drug-free polymer microparticles have antibacterial qualities. Buccal bilayer devices have remarkable potential for regulated distribution in the oral cavity. These devices include palavered tablets and bilaminated films, with or without anionic crosslinking polymers such gellan gum, sodium alginate, and polycarbophil. These drugs can include propranolol hydrochloride and

nifedipine [120]

13.11 Gastrointestinal Drug Delivery

When placed in acidic and neutral conditions, polymer granules with internal gaps created by deacidification float, allowing prednisolone to be released gradually. The controlled-release, gastroretentive action of melatonin is facilitated by the use of floating hollow microcapsules. With times varying from 1.75 to 6.7 hours in artificial gastric juice, these microcapsules greatly extend the time that the drug is released. For more than ten hours, the majority of mucoadhesive microcapsules stay in the stomach, even those laden with glipizide and methoclamide[120]

14. FUTURE ASPECT

The future of microspheres targeted to the colon holds immense potential, driven by advancements in materials science, drug delivery mechanisms, and personalized medicine. Emerging biodegradable and biocompatible polymers, along with stimuli-responsive materials, will enhance the specificity and safety of these drug delivery systems. Innovations in pH-sensitive, enzyme-sensitive, and bacteria-triggered release mechanisms will ensure precise drug release at the target site.

Therapeutically, microspheres will offer more effective treatments for conditions such as inflammatory bowel disease, colon cancer, and colonic infections, with minimal systemic side effects. Advances in drug loading techniques and controlled release profiles will further improve therapeutic outcomes. Manufacturing scalability will benefit from cutting-edge technologies like 3D printing and microfluidics, ensuring consistent quality and efficacy. Patient compliance will improve with taste-masking techniques and convenient dosage forms. Regulatory and safety considerations will remain paramount, with ongoing studies to ensure long-term biocompatibility. Personalized medicine will thrive with customizable microsphere systems tailored to individual genetic and microbiome profiles. Integration with nanotechnology, bioprinting, and artificial intelligence will drive the creation of sophisticated, highly targeted delivery systems.

Finally, extensive clinical trials and real-world evidence will validate and refine these innovations, ensuring that microsphere-based therapies become a cornerstone of targeted treatment for colonic diseases.

15. CONCLUSION

In conclusion, the future of microspheres targeted to the colon is poised to revolutionize drug delivery and therapeutic efficacy for colonic diseases. Advancements in biodegradable and biocompatible materials, coupled with sophisticated release mechanisms triggered by colonic-specific stimuli, will enable precise and safe drug delivery. These innovations promise significant improvements in treating conditions such as inflammatory bowel disease, colon cancer, and infections, with reduced systemic side effects.

Enhanced drug loading capacities and controlled release profiles will optimize therapeutic outcomes, while advanced manufacturing techniques will ensure scalable production of high-quality microspheres. Patient compliance will benefit from improved taste-masking and user-friendly dosage forms, and the integration of personalized medicine will allow for tailored therapies based on individual patient profiles.

As regulatory frameworks evolve to accommodate these novel systems, and as clinical trials and real-world applications validate their efficacy, microsphere-based drug delivery to the colon is set to become a cornerstone of modern medical treatment, offering targeted, efficient, and patient-centric

solutions.

16. CONFLICT OF INTEREST

The authors are not having any conflict of interest.

17. ACKNOWLEDGEMENT

The authors are immensely thankful to the Management of SRM College of Pharmacy, SRMIST, Kattankulathur for their never ending help and support.

18. DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

In the process of preparation of the manuscript, the authors utilised "Quill Bot Premium" tool to enhance the language and readability. Upon utilising this tool, the authors thoroughly assessed and revised the text as required, assuming complete accountability for the publication's content.

19. REFERENCE

1. Pachore A, Dubey BK, Basediya DK, Shrivastava A. FORMULATION, DEVELOPMENT AND EVALUATION OF COLON TARGETING MICROSPHERES FOR EFFECTIVE TREATMENT OF COLONTITIS. International Journal of Pharmaceutical Sciences and Medicine 2022;7:1–16. https://doi.org/10.47760/ijpsm.2022.v07i12.001.
2. Amidon S, Brown JE, Dave VS. Colon-Targeted Oral Drug Delivery Systems: Design Trends and Approaches. AAPS PharmSciTech 2015;16:731–41. https://doi.org/10.1208/s12249-015-0350-9.
3. Nidhi, Rashid M, Kaur V, Hallan SS, Sharma S, Mishra N. Microparticles as controlled drug delivery carrier for the treatment of ulcerative colitis: A brief review. Saudi Pharmaceutical Journal 2016;24:458–72. https://doi.org/10.1016/j.jsps.2014.10.001.
4. N.R K. Microsphere: A Brief Review. Asian Journal of Biomedical and Pharmaceutical Sciences. 2015; 05(47):13-19. doi:10.15272/ajbps.v5i47.713 n.d.
5. Deore KL, Thombre NA, Gide PS. Formulation and development of tinidazole microspheres for colon targeted drug delivery system. J Pharm Res 2013; 6:158–65. https://doi.org/10.1016/j.jopr.2012.11.034.
6. Mohammad Khalid, Archana Gautam. Microsphere A Novel Drug Delivery System – A Review. Int J Sci Res Sci Technol 2023:406–11. https://doi.org/10.32628/IJSRST2310155.
7. Kumaresh S. Soppimath, Anandrao R. Kulkarni, Walter E. Rudzinski, Tejraj M. Aminabhavi, Drug Metabolism Reviews, Volume 33, Issue 2, April 2001, Page no.- 149-160 n.d.
8. Barducci L, Norton JC, Sarker S, Mohammed S, Jones R, Valdastri P, et al. Fundamentals of the gut for capsule engineers. Progress in Biomedical Engineering 2020;2. https://doi.org/10.1088/2516-1091/abab4c.
9. McCoubrey LE, Favaron A, Awad A, Orlu M, Gaisford S, Basit AW. Colonic drug delivery: Formulating the next generation of colon-targeted therapeutics. Journal of Controlled Release 2023; 353:1107–26. https://doi.org/10.1016/j.jconrel.2022. 12.029.
10. Eickhoff A, Pickhardt P J, Hartmann D and Riemann J F 2010 Colon anatomy based on CT colonography and fluoroscopy: impact on looping, straightening and ancillary manoeuvres in colonoscopy Digestive Liver Dis. 42 291–6 n.d.

11. Cai S, Fan Y, Zhang B, Lin J, Yang X, Liu Y, et al. Appendectomy Is Associated With Alteration of Human Gut Bacterial and Fungal Communities. Front Microbiol 2021;12. https://doi.org/10.3389/fmicb.2021.724980.
12. Sadahiro S, Ohmura T, Yamada Y, Saito T and Taki Y 1992 Analysis of length and surface area of each segment of the large intestine according to age, sex and physique Surg. Radiol. Anat. 14 251–7 n.d.
13. Helander H F and F¨andriks L 2014 Surface area of the digestive tract – revisited Scand. J. Gastroenterol. 49 681–9 n.d.
14. Macromolecular Prodrug of 4-Aminosalicylic Acid for Targeted Delivery to Inflamed Colon n.d.
15. Friend DR, Chang GW. A colon-specific drug-delivery system based on drug glycosides and the glycosidases of colonic bacteria. J Med Chem. 1984; 27:3:261-6 n.d.
16. Simpkins JW, Smulkowski M, Dixon R, Tuttle R. Evidence for the delivery of narcotic antagonists to the colon as their glucuronide conjugates. J Pharmacol Exp Ther. 1988; 244:1:195-205 n.d.
17. Harboe E, Larsen C, Johansen M, Olesen HP, Macromolecular prodrugs. XV. Colontargeted delivery--bioavailability of naproxen from orally administered dextran-naproxen ester prodrugs varying in molecular size in the pig. Pharm Res. 1989; 6:11:919-23 n.d.
18. Minami K, Hirayama F, Uekama K. Colon-specific drug delivery based on a cyclodextrin prodrug, release behavior of biphenylylacetic acid from its cyclodextrin conjugates in rat intestinal tracts after oral administration. J Pharm Sci. 1998; 87:6:715-20 n.d.
19. Nakamura J, Asai K, Nishida K, Sasaki H. A novel prodrug of salicylic acid, salicylic acid-glycylglycine conjugate, utilizing the hydrolysis in rabbit intestinal microorganisms. J Pharm Pharmacol. 1992, 44:9:713- 6. n.d.
20. Collnot E-M, Ali H, Lehr C-M. Nano- and microparticulate drug carriers for targeting of the inflamed intestinal mucosa. Journal of Controlled Release 2012; 161:235–46. https://doi.org/10.1016/j.jconrel.2012.01.028.
21. Eva-Maria Collnot HA. Investigation of pH and Temperature Profiles in the GI Tract of Fasted Human Subjects Using the Intellicap(®) System n.d.
22. Kim JH, Shin DH, Kim J-S. Preparation, characterization, and pharmacokinetics of liposomal docetaxel for oral administration. Arch Pharm Res 2018;41:765–75. https://doi.org/10.1007/s12272-018-1046-y.
23. Very low intraluminal colonic pH in patients with active ulcerative colitis n.d.
24. Dew MJ, Ryder REJ, Evans N, et al. Colonic release of 5-ami- enterocytes, normal colorectal tissues, and colorectal adeno- nosalicylic acid from an oral preparation in active ulcerative carcinomas. Drug Metab Dispos 2002; 30: 4-6 colitis. Br J Clin Pharmacol 1983; 16: 185-7 n.d.
25. D.F. Evans, G. Pye, R. Bramley, A.G. Clark, T.J. Dyson, J.D. Hardcastle, Measurement of gastrointestinal pH profiles in normal ambulant human subjects, Gut 29 (1988) 1035–1041 n.d.
26. C.J. McDougall, R. Wong, P. Scudera, M. Lesser, J.J. DeCosse, Colonic mucosal pH in humans, Dig. Dis. Sci. 38 (1993) 542–545 n.d.
27. Gut Fermentation of Dietary Fibres: Physico-Chemistry of Plant Cell Walls and Implications for Health n.d.
28. Polysaccharide-based micro/nanocarriers for oral colon-targeted drug delivery n.d.
29. Gut instincts: explorations in intestinal physiology and drug delivery n.d.
30. Worsøe J, Fynne L, Gregersen T, Schlageter V, Christensen L A, Dahlerup J F, Rijkhoff N J, Laurberg S and Krogh K 2011 Gastric transit and small intestinal transit time and motility assessed by a magnet tracking system BMC Gastroenterol n.d.
31. Krevsky B, Maurer A H, Niewiarowski T and Cohen S 1992 Effect of verapamil on human intestinal transit n.d.

32. Application of a biomagnetic measurement system (BMS) to the evaluation of gastrointestinal transit of intestinal pressure-controlled colon delivery capsules (PCDCs) in human subjects n.d.
33. Limited exposure of the healthy distal colon to orally-dosed formulation is further exaggerated in active left-sided ulcerative colitis n.d.
34. Objective evaluation of small bowel and colonic transit time using pH telemetry in athletes with gastrointestinal symptoms n.d.
35. Pelaseyed T, Bergstrom JH, Gustafsson JK, Ermund A, Birchenough GM, Schutte A, van der Post S, Svensson F, Rodriguez-Pineiro AM, Nystrom EE, Wising C, Johansson ME, Hansson GC (2014) The mucus and mucins of the goblet cells and enterocytes provide the frst defense line of the gastrointestinal tract and interact with the immune system. Immunol Rev 260:8–20 n.d.
36. Ghosh D, Peng X, Leal J, Mohanty RP (2018) Peptides as drug delivery vehicles across biological barriers. J Pharm Investig 48:89–111. n.d.
37. Lai SK, Wang YY, Hanes J (2009) Mucus-penetrating nanoparticles for drug and gene delivery to mucosal tissues. Adv Drug Deliv Rev 61:158–17 n.d.
38. Johansson ME, Ambort D, Pelaseyed T, Schutte A, Gustafsson JK, Ermund A, Subramani DB, Holmen-Larsson JM, Thomsson KA, Bergstrom JH, van der Post S, Rodriguez-Pineiro AM, Sjovall H, Backstrom M, Hansson GC (2011) Composition and functional role of the mucus layers in the intestine. Cell Mol Life Sci 68:3635–3641 n.d.
39. Akhil Gupta1 2, Anuj Mittal1, Alok Kumar Gupta1. COLON TARGETED DRUG DELIVERY SYSTEMS — A REVIEW n.d.
40. Patel J. Bioadhesion is a topic of current interest in the design of controlled or targeted drug delivery system n.d.
41. Thanou M, Nihot MT, Jansen M, Coos Verhoef J, Junginger HE. Mono N- carboxymethyl chitosan (MCC),a polyampholytic chitosan derivative enhances the intestinal absorption of low molecular weight heparin across intestinal epithelia in vitro and in vivo. J.Pharm Sci.2001; 90: 38- 46 n.d.
42. Ahmad MZ, Akhter S, Ahmad I, Singh A, Anwar M, Shamim M, et al. In vitro and in vivo evaluation of Assam Bora rice starch-based bioadhesive microsphere as a drug carrier for colon targeting. Expert Opin Drug Deliv. 2012;9(2):141–9. doi: 10.1517/17425247.2012.633507 n.d.
43. Chandna A, Batra D, Kakar S, Singh R. A review on target drug delivery: Magnetic microspheres. J Acute Dis 2013; 2:189-95 n.d.
44. Zhang J, Zhang S, Wang Y, Zeng J. Composite magnetic microspheres: Preparation and characterization. J Magn Magn Mater 2007; 309:197-201. n.d.
45. Chein YW. Oral Drug Delivery and Delivery systems. In Novel drug delivery systems. Vol. 50, Marcel Dekker, Inc., New York. P-139- 177(1992) n.d.
46. Francesca Maestrelli, Marzia Cirri, Giovanna Corti, Natascia Mennini, Paola Mura. Dept of Pharmaceutical sciences, University of Florence. Italy (EJBP 2008; 69: 508-518) n.d.
47. Preparation of magnetic microspheres of mesalamine by phase separation emulsion polymerisation technique Satinder Kakar1* and Ramandeep Singh n.d.
48. MOUSAMI S SAMANTA* DGMWCGSKS. A REVIEW ON MICROSPHERES AS A NOVEL CONTROLLED DRUG DELIVERY SYSTEM n.d.
49. Najmuddin M., Ahmed A., Shelar S, Patel V, Khan T. Floating Microspheres Of Ketoprofen: Formulation and Evaluation, International Journal Of Pharmacy and Pharmaceutical sciences. 2010; 2(2):83-87. n.d.
50. Tusharkumar Gautam Ingle 1 SDPRSDP. The Current Trends in Microspheres: A Review n.d.
51. DESIGN AND EVALUATION OF MICROSPHERES: A REVIEW Chitra Singh1, Suresh Purohit2, Madhu Singh3, B.L.Pandey4 1. Ph.D scholar,Department of pharmacology, BHU,Varanasi 2.

Associate professor, Department of pharmacology, BHU,Varanasi 3. Assistant professor,Bundelkhand Govt. Ayurvedic college, Jhansi 4. Professor Department of Pharmacology, IMS,BHU,Varanasi n.d.
52. Stomach-specific drug delivery of 5-Fluorouracil using ethylcellulose floating microspheres Subhash VaghaniDepartment of Pharmaceutics, PES College of Pharmacy, BangaloreCorrespondencesubhashvaghani@gmail.com , S. VasantiDepartment of Pharmaceutics, PES College of Pharmacy, Bangalore , Kiran ChaturvediDepartment of Pharmaceutics, PES College of Pharmacy, Bangalore , C.S. SatishDepartment of Pharmaceutics, PES College of Pharmacy, Bangalore n.d.
53. Yadav AV, Mote HH. Development of Biodegradable Starch Microspheres for Intranasal Delivery, Indian Journal of pharmaceutical Sciences. 2008; 70(2):170-74 n.d.
54. Lachman LA, Liberman HA, Kanig JL. The Theory and Practice of Industrial Pharmacy. 3rd edition 1991; Varghese Publishng House, Mumbai, India: P-414-415. n.d.
55. Chowdary KPR, Yarraguntla SR. Mucoadhesive microsphere for controlled drug delivery. Biol. Pharm. Bull. 2004; 1717-24. n.d.
56. Con-A conjugated mucoadhesive microspheres for the colonic delivery of diloxanide furoate Nalini M. Anandea , Sunil K. Jaina,∗ , Narendra K. Jainb n.d.
57. Ando S, Putnam D, Pack DW, and Langer R. PLGA Microspheres Containing Plasmid DNA: Preservation of Super coiled DNA via Cry preparation and Carbohydrate Stabilization. J. Pharmaceut. Sci.1998; 88(1): 126– 130. n.d.
58. Polymeric Microspheres for Medical Applications by Ketie Saralidze *, Leo H. Koole andMenno L.W. Knetsch n.d.
59. Development of poly-(d,l-lactide–coglycolide) microsphere formulations containing recombinant human vascular endothelial growth factor to promote local angiogenesis n.d.
60. Trivedi P, Verma AML, Garud N. Preparation and Characterization of Acclofenac Microspheres, Asian Journal of pharmaceutics. 2008; 2(2): 110-15 n.d.
61. Jain NK. Controlled and Novel drug delivery, CBS Publishers New Delhi, India; 4th Edition, 236- 237. n.d.
62. Kadam N. R. and Suvarna V. MICROSPHERES: A BRIEF REVIEW n.d.
63. Patel B., Modi V., Patel K., Patel M., Preparation and evaluation of ethyl cellulose microspheres prepared by emulsification - solvent evaporation method, International Journal For Research In Management And Pharmacy. 2012;1(1):83-91. n.d.
64. Venkatesan P.,. Manavalan R., . Valliappan K., Preparation and evaluation of sustained release loxoprofen, J of Basic and Clinical Pharmacy.2011;2(3):159-162 n.d.
65. Lakshmana P.S., Shirwaikar A., Kumar A., Formulation and evaluation of sustained release microspheres of rosin containing aceclofenac, Ars Phar.,2009;50(2):51-62 n.d.
66. Formulation and development of tinidazole microspheres for colon targeted drug delivery system n.d.
67. Patel N. R., Patel D. A., Bharadia P.D., Pandya V., Modi D.,Microsphere e as a novel drug delivery, Int. j. pharm. life sci. 2011;2(8):992-7 n.d.
68. Vyas and Khar. Targeted and Controlled drug delivery (CBS Publishers and distributors, 2001). n.d.
69. Patel N.R. , Patel D.A., Bharadia P.D., PandyaV., Modi V., Microsphere as a novel drug deliver, Int J of Pharm & Life Sci.2011;2(8):992-997 n.d.
70. Prasanth V.V., Moy A.C., Mathew S.T., MathapanR., Microspheres: an overview, Int J of Pharm & Biomedical Sci.2011;2(2):332-338 n.d.
71. Formulation and Evaluation Porous Microspheres of 5- Fluorouracil for Colon Targeting S. C. Dhawale1 , A. S. Bankar2 , *M. N. Patro3 n.d.

72. Microspheres As A Novel Drug Delivery System - A Review Athira K*, K Vineetha, Krishnananda Kamath K, A R Shabaraya n.d.
73. Gurung BD, Kakar S. An overview on microspheres. Int J Health Clin Res. 2020;3(1):11-24 n.d.
74. Jain D., Panda A.K.,. Majumdar D.K., Eudragit S100 Entrapped Insulin Microspheres for Oral Delivery, AAPS Pharm Sci Tech. 2005;6(1):101-107. n.d.
75. Influence of Preparation Conditions on the Properties of Eudragit Microspheres Produced by a Double Emulsion Method Pe´ter Sipos,1 Ildiko´ Cso´ka,1 Stane Src˘ic˘, 2 Kla´ra Pintye-Ho´di,1 and Istva´n Eros+ 1n n.d.
76. Bib Dolma Gurung* SK. An overview on microspheres n.d.
77. Athira K* KVKKKARS. Microspheres As A Novel Drug Delivery System - A Review n.d.
78. Mathew Sam T, Devi Gayathri S, Prasanth VV, Vinod B. NSAIDs as microspheres, The Internet Journal of Pharmacology. 2008;6: 11-15. n.d.
79. Sunny Patel1* RPKPADCCG. A Review on Microspheres: Types, Methods of Preparation, Effects of Process Variables and Applications n.d.
80. Trivedi P, Verma AML and Garud N: Preparation and Characterization of Aceclofenac Microspheres. Asian Journal of Pharmaceutics 2008; 2:110-115 n.d.
81. Pandey K, Prajapati G and Patel MR: A Review on Microspheres. International Journal of Pharmaceutical Sciences 2012; 2:53-57 n.d.
82. Prasanth VV, Chakraborty A, Mathew ST, Mathappan R and Kamalakkannan V. Formulation and evaluation of Salbutamol sulphate microspheres by solvent evaporation method. Journal of Applied Pharmaceutical Science 2011; 1:133-137 n.d.
83. Sahu M, Satapathy T, Bahadur S, Saha S, Purabiya P, Kaushik S, et al. Preparation methods for nanoparticle: A smart carrier system for treatment of cancer. March 2018; 7(19): 216- 26. n.d.
84. Yadav R, Bhowmick M, Rathi V, Rathi J, Design and characterization of floating microspheres for rheumatoid arthritis, Journal of Drug Delivery and Therapeutics 2019; 9(2-s):76-81 n.d.
85. Herfarth H, Obermeier F, Andus T, Rogler G, Nikolaus S, Kuehbacher T, Schreiber S. Improvement of arthritis and arthralgia after treatment with infliximab (Remicade) in a German prospective, open-label, multicenter trial in refractory Crohn's disease. The American journal of gastroenterology. 2002 Oct 1; 97(10):2688 n.d.
86. Sharma N, Purwar N. and Gupta PC: Microspheres as Drug Carriers for Controlled Drug Delivery: A Review. Int J Pharm Sci Res 2015; 6(11): 4579-87 n.d.
87. D'Souza SS, Deluca PP. Development of a dialysis in-vitro release method for biodegradable microspheres. Aaps Pharm Sci Tech. 2005; 6(2): E323-8 n.d.
88. Kamalakkannan V, Kumaran KA, Kannan C, Bhama S, Kumar RS. Effects of Permeability Characteristics of Different Polymethacrylates on the Pharmaceutical Characteristics of Diltiazem Hcl-Loaded Microspheres. Indian J Res in Pharmacy and Biotechnology. 2013 Jul 1; 1(4): 504 n.d.
89. Prasad BSG, Gupta VRM, Devanna N, Jayasurya K. Microspheres as Drug Delivery System – A Review. 2014 5(3): 1961-1972. n.d.
90. Sree Giri P B, Gupta V.R.M, Devanna N, Jayasurya K. Microspheres as drug delivery system - A review. JGTPS. 2014; 5(3):1961 -72. n.d.
91. Quality by design thinking in the development of long-acting injectable PLGA/PLA-based microspheres for peptide and protein drug delivery Chengqian Zhanga , Liang Yangb , Feng Wanc , Hriday Beraa , Dongmei Cuna , Jukka Rantanenc , Mingshi Yanga,c,⁎ n.d.
92. Preparation and characterization of microspheres containing the anti-inflammatory agents, indomethacin, ibuprofen, and ketoprofen n.d.

93. Chowdary KP, Babe JS. Permeability of ethylene vinyl acetate copolymer microcapsules: Effect of solvents. Indian journal of pharmaceutical sciences. 2003; 65(1):62 n.d.
94. Preparation of multiple unit hollow microspheres (microballoons) with acrylic resin containing tranilast and their drug release characteristics (in vitro) and floating behavior (in vivo) n.d.
95. Gavhane P, Deshmukh M, Khopade AN, Kunjir VV, Shete RV. A review on microsphere. Journal of Drug Delivery and Therapeutics. 2021 Jan 15; 11(1):188-94. https://doi.org/10.22270/jddt.v11i1.4501 n.d.
96. Verma R, Verme S, Kumar S. Microsphere-a novel drug delivery system. Research Chronicle in Health Sciences. 2019 Jul 30; 5(1):5-14 n.d.
97. Thota S, Kusuma B, Rarevati M, Narendra P, Babu SM. Formulation and Evaluation of ethyl cellulose microspheres containing diclofenac sodium. International Journal of Research in Pharmaceutical Sciences and Technology. 2021 Oct 25; 2 n.d.
98. Kakkar V, WaniSu, Gautam Sp, Qadrie Zl. Role of microspheres in novel drug delivery systems: preparation methods and applications. International Journal of Current Pharmaceutical Research. 2020 May 15:10-5 n.d.
99. Sinha VR, Agrawal MK, Kumria R. Influence of formulation and excipient variables on the pellet properties prepar ed by Sinha VR, Agrawal MK, Kumria R. Influence of formulation and excipient variables on the pellet properties prepar ed by Sinha VR, Agrawal MK, Kumria R. Influence of formulation and excipient variables on the pellet properties prepar ed by n.d.
100. Mahale MM, Saudagar RB, Microsphere: a review, Journal of drug delivery and therapeutics 2019; 9(3-s):854-856 n.d.
101. More S, Gavali K, Doke O, Kasgawade P, Gastroretentive drug delivery system, Journal of drug delivery and therapeutics 2018; 8(4):24-35 https://doi.org/10.22270/jddt.v8i4.1788 n.d.
102. Tanaka, W; Akito, E; Yoshida, K.; Terada, T.and Ninomiya, H. (1977) “pharmaceutical preparations for oral cavity administration” US patent No.4059686 n.d.
103. Venkatesh H. (1989) “A buccal delivery system of Salbutamol Sulphate” M.Pharm; Budrinarayan, N. (1991) “Utilisation and Evaluation of plant products in pharmaceutical formulations” M.Pharm n.d.
104. Modified Dissolution Method for Rifampin Samir A. Gharbo,Mary M. Cognion &Martin J. Williamson n.d.
105. Kushwaha N, Jain A, Jain PK, Khare B, Jat YS, An Overview on Formulation and Evaluation Aspects of Tablets. Asian Journal of Dental and Health Sciences 2022; 2(4):35-39 https://doi.org/10.22270/ajdhs.v2i4.23 n.d.
106. Lin CY, Lin SJ, Yang YC, Wang DY, Cheng HF, Yeh MK. Biodegradable polymeric microsphere-based vaccines and their applications in infectious diseases. Hum Vaccin Immunother 2015; 11:650-6. https://doi.org/10.1080/21645515.2015.1009345 n.d.
107. Kohn J, Niemi SM, Albert EC, Murphy JC, Langer RS, Fox JG. Singlestep immunization using a controlled release, biodegradable polymer with sustained adjuvant activity. J Immunol Methods 1986; 95:31-7. https://doi.org/10.1016/0022-1759(86)90314-5 n.d.
108. Gupta RK, Singh M, O’Hagan DT. Poly (lactide-co-glycolide) microparticles for the development of single-dose controlledrelease vaccines. Adv Drug Delivery Rev 1998; 32:225- 46. https://doi.org/10.1016/S0169-409X (98)00012-X n.d.
109. Nellore RV, Pande PG, Young D, Bhagat HR. Evaluation of biodegradable microspheres as vaccine adjuvant for Hepatitis B surface antigen. J Parenteral Sci Tech 1992; 46:176-80. 42. Desai KG,

Schwendeman SP. Active self-healing encapsulation of vaccine antigens in PLGA microspheres. J Controlled Release 2013; 165:62-74 https://doi.org/10.1016/j.jconrel.2012.10.012 n.d.

110. Sailaja AK, Anusha K, Jyothika M. Biomedical applications of microspheres. J Modern Drug Discovery Drug Delivery Res 2015; 4:1-5 n.d.

Biodegradable Microsphere-Hydrogel Ocular Drug Delivery System for Controlled and Extended Release of Bioactive Aflibercept In Vitro n.d.

111. Poly (D, L-lactide-co-glycolide)/DNA microspheres to facilitate prolonged transgene expression in airway epithelium in vitro, ex vivo and in vivo n.d.
112. Monoclonal antibodies: formulations of marketed products and recent advances in novel delivery system Yanan Cui,Ping Cui,Binlong Chen,Suxin Li &Hua Guan n.d.
113. Adamson P, Wilde T, Dobrzynski E, Sychterz C, Polsky R, Kurali E, et al. Single ocular injection of a sustained-release anti-VEGF delivers 6 mo pharmacokinetics and efficacy in a primate laser CNV model. J Controlled Release 2016; 244:1-13. https://doi.org/10.1016/j.jconrel.2016.10.026 n.d.
114. Stern M, Ulrich K, Geddes DM, Alton EWFW. Poly (D, L-lactidecoglycolide)/DNA microspheres to facilitate prolonged transgene expression in airway epithelium in vitro, ex vivo and in vivo. Gene Ther 2003; 10:1282-8. https://doi.org/10.1038/sj.gt.3301994 n.d.
115. Thundimadathil J. Peptide therapeutics: strategy and tactics for chemistry manufacturing and controls. In: Srivastava V. editor. Formulations of microspheres and nanoparticles for peptide delivery. 1st edition. Cryodon: Royal Society of Chemistry; 2019. p. 503-30. https://doi.org/10.1039/9781788016445-00503 n.d.
116. Rajput MS, Agrawal P. Microspheres in cancer therapy. Indian J Cancer 2010; 47:458-68. https://doi.org/10.4103/0019- 509X.73547 n.d.
117. Capron A.C., Locht C. and Fracchia G.N. Vaccine, 1994; 12:66 https://doi.org/10.1016/0264-410X (94)90275-5 n.d.
118. Nachts S. and Martin K. In: The microsponges a novel topical programmable delivery formulation, Marcel Dekker Inc.,Newyork., 1990; 299. n.d.
119. Kalyan Shweta, Sharma Parmod Kumar et al. Recent Advancement In Chitosan Best Formulation And Its Pharmaceutical Application. Pelagia Research Library. 2010; 1(3):195-210 n.d.