Available online on www.ajper.com

Asian Journal of Pharmaceutical Education and Research

Vol -1, Issue-2, October-December 2012

ISSN: 2278 – 7496

REVIEW ARTICLE

COLON TARGETING MICROSPHERE: A REVIEW

Manish Kumar Chakradhari*, Anurag Sharma, Dilip Agrawal, M.P. Khinchi, Natasha Sharma, M.K. Gupta

Department of Pharmaceutics, Kota College of Pharmacy, Kota, Rajasthan

Article Received on 4 September 2012.

Revised on 15 September 2012,

Accepted on 19 september 2012

***Correspondence for Author:**

Manish Kumar Chakradhari

Dept. of Pharmaceutics

Kota College of Pharmacy, Kota, Rajasthan

Mobile No: 09887879986

Email: Chakradhari.manish2612@gmail.com

Abstract:

Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers which are biodegradable in nature and ideally having a particle size less than 200 µm. A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. Day by day there are new developments in field of colon specific drug delivery system. Colonic drug delivery has gained increased importance not just for the delivery of the drugs for the treatment of local diseases associated with the colon like cancer,Crohn's disease, ulcerative colitis, etc but also for the systemic delivery of proteins, therapeutic peptides, anti-asthmatic drugs, antihypertensive drugs and anti-diabetic agents. Various routes of administration have been explored for the effective delivery of the drug to the target site. The oral route is considered to be most convenient for the administration of drugs to patients. But it has a serious drawback in conditions where localized delivery of the drug in the colon is required. Colon target aimed mainly because of less enzymatic activity, longer transit time so it is suitable to deliver the protien and peptide drugs. This review article discusses, in brief, anatomy and physiology of colon, factor affecting colonic absorption, polymer used in preparation of colon targeting microsphere and various techniques used in preparation of microsphere etc.

Keywords: Microsphere, Colon targeting, Colorectal Cancer, Gastro retentive Tract.

Introduction

A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. To obtain maximum therapeutic efficacy, it becomes necessary to deliver the agent to the target tissue in the optimal amount in the right period of time there by causing little toxicity and minimal side effects[1].

There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microspheres as carriers for drugs. The colon specific drug delivery system (CDDS) should be capable of protecting the drug in route to the colon i.e. drug release and absorption should not occur in stomach as well as small intestine, and neither the bioactive agent should be degraded either of the dissolution sites, but only released absorbed once the system reaches the colon. Formulations for colonic delivery are also suitable for delivery of drugs, which are polar and / or susceptible to chemical and enzymatic degradation in upper GIT; in particular, therapeutic proteins and peptides are suitable for colonic deliveries. Proteins and peptides such as insulin, calcitonin and vasopressin may be delivered systematically via colonic absorption. Other examples include novel peptides such as cytokine inhibitors and antibiotics, which are useful in treatment of IBD and GI infections respectively.

Apart from protecting these labile molecules, colon also offers an opportunistic site for oral delivery of vaccines because it is rich in lymphoid tissue. A colonic targeted approach found to be effected in minimizing uncertain side effects .So, the colon, as a site for drug delivery, offers distinct advantages on account of near neutral pH, a much longer transit time, relatively low proteolytic enzymatic activity and offers a much greater responsiveness to absorption enhances. Colon specific delivery systems should prevent the release of drug in upper part of GIT and require a triggering mechanism to release the drug on reaching the colon [2].

Materials used in preparation of microsphere

A. Polymers: are classified into two types:

1. Synthetic Polymers

2. Natural polymers

1. Synthetic polymers are divided into two types.

a. Non-biodegradable polymers

E.g. Poly methyl methacrylate (PMMA) [3], Acrolein [4], Glycidyl methacrylate,

b. Biodegradable polymers

E.g. Lactides, Glycolides & their co polymers[5], Poly alkyl cyano acrylates

2. Natural polymers

Proteins: Albumin, Gelatin, and Collagen

Carbohydrates: Agarose, Carrageenan, Chitosan, Starch

Chemically modified carbohydrates: Polydextran, Poly starch[6].

B. Surfactants: Polysorbate 20 (Tween 20), Polysorbate 80 (Tween 80), Sorbitan mono oleate (Span 80) and span 20.

C. Co-solvents: Ethanol, Glycerin, Polypropylene glycol, Polyethylene glycol, DCM, Petrolum ether, Ethyl alcohol, Methyl alcohol[6].

Why colon targeting needed

Colon targeted drug delivery would ensures direct treatment at the disease site, lower dosing and less systemic side effects. In addition to restricted therapy, the colon can also be utilized as a portal for the entry of drugs into the systemic circulation. For example, molecules that are degraded/poorly absorbed in the upper gut, such as peptides and proteins, may be better absorbed from the more benign environment of the colon.

Overall, there is less free fluid in the colon than in the small intestine and hence, dissolution could be problematic for poorly water-soluble drugs. In such instances, the drug may need to be delivered in a pre solubilized form, or delivery should be directed to the proximal colon, as a fluid gradient exists in the colon with more free water present in the proximal colon than in the distal colon. Aside from drug solubility, the stability of the drug in the colonic environment is a further factor that warrants attention. The drug could bind in a nonspecific manner to dietary residues, intestinal secretions, mucus or general faecal matter, thereby reducing the concentration of free drug. Moreover, the resident micro-flora could also affect colonic performance via degradation of the drug.

- Targeted drug delivery to the colon would ensure direct treatment at the disease site, lower dosing and fewer systemic side effects[7].

- Site-specific or targeted drug delivery system would allow oral administration of peptide and protein drugs, colon-specific formulation could also be used to prolong the drug delivery[8].
- Colon-specific drug delivery system is considered to be beneficial in the treatment of colon diseases[8].
- The colon is a site where both local or systemic drug delivery could be achieved, topical treatment of inflammatory bowel disease, e.g. ulcerative colitis or Crohn's disease. Such inflammatory conditions are usually treated with glucocorticoids and sulphasalazine (targeted) [9].
- A number of others serious diseases of the colon, e.g. colorectal cancer, might also be capable of being treated more effectively if drugs were targeted to the colon [10].
- Formulations for colonic delivery are also suitable for delivery of drugs which are polar and/or susceptible to chemical and enzymatic degradation in the upper GI tract, highly affected by hepatic metabolism, in particular, therapeutic proteins and peptides [10].

Colon anatomy

- The GI tract is divided into stomach, small intestine and large intestine. The large intestine extending from the ileocecal junction to the anus is divided in to three main parts. These are the colon, the rectum and anal canal[11].
- The entire colon is about 5 feet (150 cm) long, and is divided in to five major segments[11]. Peritoneal folds called as mesentery which is supported by ascending and descending colon. The right colon consists of the cecum, ascending colon, hepatic flexure and the right half of the transverse colon. The left colon contain the left half of the transverse colon, descending colon, splenic flexure and sigmoid. The rectum is the last anatomic segment before the anus. The human intestine and colon were shown in Figure 1 and Figure 2 respectively[14].
- The major function of the colon is the creation of suitable environment for the growth of colonic microorganisms, storage reservoir of faecal contents, expulsion of the contents of the colon at an appropriate time and absorption of potassium and water from the lumen[12]. The absorptive capacity is very high, each about 2000ml of fluid enters the colon through the ileocecal valve from which more than 90% of the fluid is absorbed. On average, it has been estimated that colon contains only about 220 gm of wet material equivalent to just 35 gm of dry matter. The majority of this dry matter is bacteria. The colon tissue containing the villi, lymph, muscle, nerves, and vessels[13].

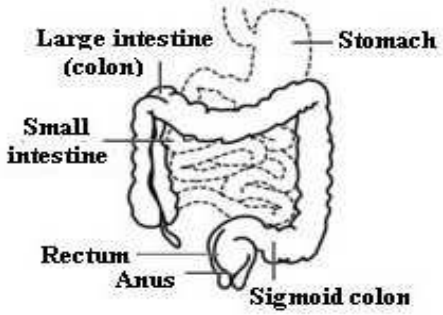

Figure 1: Structure of human intestine

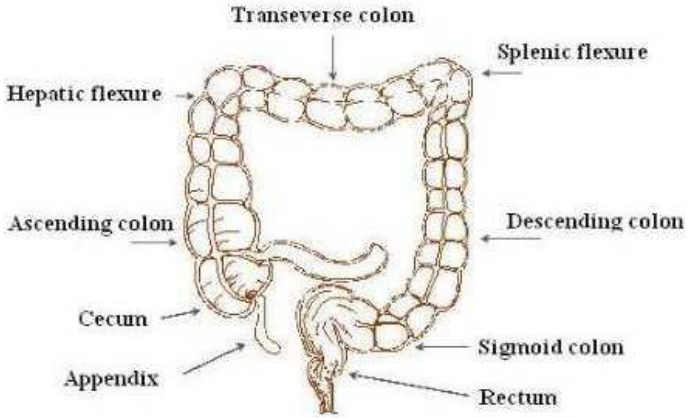

Figure 2: Structure of colon

Colonic microflora

- A large number of anaerobic and aerobic bacteria are present the entire length of the human GI tract. Over 400 distinct bacterial species have been found, 20- 30% of which are of the genus bacteroids. The upper region of the GIT has a very small number of bacteria and predominantly consists of gram positive facultative bacteria. The rate of microbial growth is greatest in the proximal areas because of high concentration of energy source.
- The metabolic activity of microflora can be modified by various factors such as age, GI disease, and intake of drug and fermentation of dietary residues[15].

Drug absorption in the colon

- Drugs are absorbed passively by either Para cellular or Tran cellular route. Tran cellular absorption involves the passage of drugs through cells and this is the route most lipophilic drugs takes, where Para cellular absorption involves the transport of drug through the tight junction between cells and is the route most hydrophilic drug takes.
- The colon may not be the best site for drug absorption since the colonic mucosa lacks well defined villi as found in the small intestine. The slower rate if transit in colon lets the drug stay in contact mucosa for a longer period than in small intestine which compensates much lower surface area.
- The colon contents become more viscous with progressive absorption of water as one travels further through the colon.
- This causes a reduced dissolution rate, slow diffusion of drug through the mucosa. Theoretically, drug absorption can occur along the entire GI tract, while in actuality, most drugs are absorbed in the duodenum and proximal jejunum. Recent studies have shown that some drugs (e.g. Theophyline and Metoprolol) continue to be absorbed in the colon[15].

Polymers used in colon targeting

Natural polysaccharides are extensively used for the development of solid dosage forms. These polymers of monosaccharide (sugars) are inexpensive and available in a variety of structures with a variety of properties. They are highly stable, safe, non-toxic, and hydrophilic and gel forming in nature. Pectin, starch, guar gum, amylase and karaya gum are a few polysaccharides commonly used in dosage forms. Non-starch, linear polysaccharides remain intact in the physiological environment of the stomach and the small intestine, but are degraded by the bacterial inhabitants of the human colon which make them potentially useful in targeted delivery systems to the colon [16].

Pectin

Pectins are nonstarch linear polysaccharides that consist of α-1, 4 D-galacturonic acid and 1, 2 D-rhamnose with D-galactose and D-arabinose side chains having average molecular weights between 50,000 to 150,000. Pectin tends to produce lower viscosities than other plant gums. It is refractory to host gastric and small intestinal enzymes but is almost completely degraded by the colonic bacterial enzymes to produce a series of soluble oligalactorunates [17,18]. Depending on the plant source and preparation; they contain varying degrees of methyl ester substituents [19]. Micro particulate polymeric delivery systems have been suggested as a possible approach to improve

the low bioavailability characteristics shown by standard ophthalmic vehicles (collyria). In this context pectin microspheres of piroxicam were prepared by the spray drying technique. In vivo tests in rabbits with dispersions of piroxicam-loaded microspheres also indicated a significant improvement of piroxicam bioavailability in the aqueous humour (2.5-fold) when compared with commercial piroxicam eyedrops [20,21]. In vivo gamma scintigraphic studies confirmed the in vitro findings. In all the volunteers, the pectin-coated tablets disintegrated in the colon indicating that site-specificity had been achieved and illustrating the potential of a colonic drug delivery system utilizing pectin. This necessitates the development of such derivatives of pectin, which were less water-soluble but were having the capability to be degraded by the colonic microflora[22].

Chitosan

Chitosan is a high molecular weight polycationic polysaccharide derived from naturally occurring chitin by alkaline deacetylation. Chemically, it is a poly (N-glucosamine). Chitosan has favourable biological properties such as nontoxicity, biocompatibility and biodegradability. Similar to other polysaccharides it also undergoes degradation by the action of colonic microflora and hence poses its candidature for colon targeted drug delivery. developed colon-specific insulin delivery with chitosan capsules. Gums are translucent and amorphous substances produced by the plants. Usually pathological products, gums are produced when the plant is growing under unfavorable conditions or when injured. Gums are plant hydrocolloids and may be anionic or non ionic polysaccharides. On hydrolysis gums yield sugar and salts of uronic acid [23].

Guar gums

It is a naturally occurring galactomannan polysaccharide; consists of chiefly high molecular weight hydrocolloidal polysaccharide, composed of galactan and mannan units combined through glycosidic linkages and shows degradation in the large intestine due the presence of microbial enzymes [24-25]. Guar gum is hydrophilic and swells in cold water, forming viscous colloidal dispersions or sols. This gelling property retards release of the drug from the dosage form, making it more likely that degradation will occur in the colon. Guar gum was found to be a colon-specific drug carrier in the form of matrix and compression-coated tablets as well as microspheres [26-27].

Locust Bean Gum

It is also called carob gum, as it is derived from carob (Ceratonia siliqua) seeds. This neutral polymer is only slightly soluble in cold water; it requires heat to achieve full hydration and

maximum viscosity. Cross-linked galactomannan however led to water-insoluble film forming product-showing degradation in colonic microflora [28-29].

Karaya gum

Karaya gum is obtained from Sterculia urens (Family sterculiaceae) is a partially acetylated polymer of galactose, rhamnose, and glucuronic acid. mucoadhesive tablets prepared by karaya gum for buccal delivery, had superior adhesive properties as compared to guar gum and was able to provide zero-order drug release, but concentrations greater than 50 % w/w may be required to provide suitable sustained release [30-31].

Albizia gum

Albizia gum is obtained from the incised trunk of the tree Albizia zygia (DC) J. F. Macbr, family Leguminosae and is shaped like round elongated tears of variable color ranging from yellow to dark brown. It consists of β-1– 3-linked D-galactose units with some ß1-6-linked Dgalactose units. The genus Albizzia containing some twenty-six species is a member of the Mimosacez, a family which also includes the gum-bearing genera Acacia and Prosopis. Only two species of Albizia, A. zygia and A. sassa, are however, known to produce gum. Albizia gum has been investigated as a possible substitute for gum Arabic as a naturalemulsifier for food and pharmaceuticals [32].

Xanthan gum

Xanthan gum is a high molecular weight extra cellular polysaccharide produced by the fermentation of the gram-negative bacterium Xanthomonas campestris. The anionic character of this polymer is due to the presence of both glucuronicacid and pyruvic acid groups in the side chain [33].

Starches

It is the principal form of carbohydrate reserve in green plants and especially present in seeds and underground organs. Starch occurs in the form of granules (starch grains), the shape and size of which are characteristic of the species, as is also the ratio of the content of the principal constituents, amylose and amylopectin. A number of starches are recognized for pharmaceutical use. These include maize (Zea mays), rice (Oryza sativa), wheat (Triticum aestivum), and potato (S olanum tuberosum) [34]. To deliver proteins or peptidic drugs orally, microcapsules containing a protein and a proteinase inhibitor were prepared. Starch/bovine serum albumin mixed-walled microcapsules were prepared using interfacial cross-linking with terephthaloyl chloride. The microcapsules were loaded with native or amino-protected aprotinin by incorporating protease inhibitors in the aqueous phase during the cross-linking process. The protective effect of microcapsules with aprotinin for bovine serum albumin was revealed in vitro [35].

Alginates

Alginates are linear polymers that have 1-4'linked β-D-mannuronic acid and α-L-guluronic acid residue arranged as blocks of either type of unit or as a random distribution of each type. A Eudragit L-30D–coated calcium alginates bead for colonic delivery of 5-aminosalicylic acid has been reported [36]. Different enteric as well as sustained release polymers were applied as coat on calcium alginate beads. A system was prepared by coating calcium alginate beads with Aquacoat® that is a pH-independent polymer followed by 2 % w/w coating of Eudragit L-30D. Being enteric polymer, Eudragit® resisted the release of drug in acidic media and drug release was triggered at alkaline pH and controlled by thickness of Aquacoat®. When drug-loaded calcium alginate beads swell sufficiently (osmotic gradient) to exceed the strength of outer sustained released coat, the film bursts to release the drug. Such a system delivers drug to the distal intestine with minimal initial leak and provides sustained release in the colon [37].

Inulin

Inulin is a naturally occurring storage polysaccharide found in many plants such as onion, garlic, artichoke, and chicory. Most of these fructose chains have a glucose unit as the initial moiety. It is not hydrolyzed by the endogenous secretions of the human digestive tract [38]. However, bacteria harboring in the colon and more specifically Bifido bacteria are able to ferment inulin [39].

Method of preparation of microspheres

Incorporation of solid, liquid or gases into one or more polymeric coatings can be done by micro encapsulation technique[40]. The different methods used for various microspheres preparation depends on particle size, route of administration, duration of drug release and these above characters related to rpm, method of cross linking, drug of cross linking, evaporation time, co-precipitation etc[41]. The various methods of preparations are:

Emulsion solvent evaporation technique

In this technique the drug is dissolved in polymer which was previously dissolved in chloroform and the resulting solution is added to aqueous phase containing 0 .2 % sodium of PVP as emulsifying agent. The above mixture was agitated at 500 rpm then the drug and polymer (eudragit) was transformed into fine droplet which solidified into rigid microspheres by solvent evaporation and then collected by filtration and washed with demineralised water and desiccated at room temperature for 24 hrs. Aceclofenac microspheres were prepared by this technique[42].

Emulsion cross linking method

In this method drug was dissolved in aqueous gelatine solution which was previously heated for 1 hr at 40C. The solution was added drop wise to liquid paraffin while stirring the mixture at 1500 rpm for 10 min at 35□C, results in w/o emulsion then further stirring is done for 10 min at

15 0C. Thus the produced microspheres were washed respectively three times with acetone and isopropyl alcohol which then air dried and dispersed in 5mL of aqueous glutaraldehyde saturated toluene solution at room temperature for 3 hrs for cross linking and then was treated with 100mL of 10mm glyciene solution containing 0.1%w/v of tween 80 at 37 0 C for 10 min to block unreacted glutaraldehyde. Examples for this technique are Gelatin a microspheres[43].

Co-acervation method

Co-acervation thermal change: Performed by weighed amount of ethyl cellulose was dissolved in Cyclohexane with vigorous stirring at 80 0C by heating. Then the drug was finely pulverized and added with vigorous stirring on the above solution and phase separation was done by reducing temperature and using ice bath. Then above product was washed twicely with cyclohexane and air dried then passed through sieve (sieve no. 40) to obtain individual microcapsule. Co-acervation non solvent addition: Developed by weighed amount of ethyl cellulose was dissolved in toluene containing propylisobutylene in closed beaker with magnetic stirring for 6 hr at 500 rpm and the drug is dispersed in it and stirring is continued for 15 mins. Then phase separation is done by petroleum benzoin 5 times with continuous stirring. After that the microcapsules were washed with n-hexane and air dried for 2 hr and then in oven at 50c for 4 hr [41].

Spray drying technique

This was used to prepare polymeric blended microsphere loaded with ketoprofen drug. It involves dispersing the core material into liquefied coating material and then spraying the mixture in the environment for solidification of coating followed by rapid evaporation of solvent. Organic solution of poly (epsilon-caprolactone) (PCL) and cellulose acetate butyrate (CAB), in different weight ratios and ketoprofen were prepared and sprayed in different experimental condition achieving drug loaded microspheres. This is rapid but may loose crystallinity due to fast drying process[44].

Emulsion-solvent diffusion technique

In order to improve the residence time in colon floating microparticles of ketoprofen were prepared using emulsion solvent diffusion technique. The drug polymer mixture was dissolved in a mixture of ethanol and dichloromethane (1:1) and then the mixture was added drop wise to sodium lauryl sulphate (SLS) solution. The solution was stirred with propeller type agitator at room temperature at 150 rpm for 1 hr. Thus the formed floating microspheres were washed and dried in a dessicator at room temperature. The following microperticles were sieved and collected[44].

Multiple emulsion method

Oral controlled release drug delivery of indomethacin was prepared by this technique. In the beginning powder drug was dispersed in solution (methyl cellulose) followed by emulsification in ethyl cellulose solution in ethyl acctate. The the primary emulsion was then re emulsified in aqueous medium. Under optimized condition discrete microspheres were formed during this phase[44].

Application of Microspheres

Medical application[45]

1. Release of proteins, hormones and peptides over extended period of time.
2. Gene therapy with DNA plasmids and also delivery of insulin.
3. Vaccine delivery for treatment of diseases like hepatitis, influenza, pertusis, ricin toxoid, diphtheria, birth control.
4. Passive targeting of leaky tumour vessels, active targeting of tumour cells, antigens, by intraarterial/ intravenous application.
5. Tumour targeting with doxorubicin and also treatments of leishmaniasis.
6. Magnetic microspheres can be used for stem cell extraction and bone marrow purging.
7. Used in isolation of antibodies, cell separation, and toxin extraction by affinity chromatography.
8. Used for various diagnostic tests for infectious diseases like bacterial, viral, and fungal.

Radioactive microsphere's application[46]

1. Can be used for radioembolisation of liver and spleen tumours.
2. Used for radiosynvectomy of arthiritis joint, local radiotherapy, interactivity treatement.
3. Imaging of liver, spleen, bone marrow, lung etc and even imaging of thrombus in deep vein thrombosis can be done.

Other applications[47]

1. Fluorescent microspheres can be used for membrane based technologies for flow cytomettry, cell biology, microbiology, Fluorescent Linked Immuno-Sorbent Assay.
2. Yttrium 90 can be used for primary treatment of hepatocellular carcinoma and also used for pretransplant management of HCC with promising results.

Conclusion

Today the stress is on patient compliance and to achieve this objective there is a spurt in the development of NDDS. As the Natural Polysaccharides are promising biodegradable materials, these can be chemically compatible with the excipients in drug delivery systems. In addition

Natural Polysaccharides are non-toxic, freely available, and less expensive compared to their synthetic counterparts. They have a major role to play in pharmaceutical industry. Therefore, in the years to come, there is going to be continued interest in the natural polysaccharides to have better materials for drug delivery systems.

References

1. N.K.Jain, Controlled and Novel drug delivery, 04 Edition, 236-237, 21.
2. S.P.Vyas and R.K.Khar, Targeted and Controlleddrug delivery, 07 Edition, 418.
3. Kreuter J., Nefzger M., Liehl E., CzokR. AndVoges R. J.Pharm sci.72, 1983, 1146.
4. Margel S. and Wiesel E. J.polym.sci.22, 1984, 145.
5. Wakiyama N., Juni K. and Nakano M. chem...pharm.Bull. 29, 1981, 3363.
6. Sugibayashi K., Akimoto M., Marmot Y., Nadai T. and Kato Y. pharmacobiodyn.23, 1981, 50.
7. Bajpai S K, Bajpai M, Dengree R. J Appl Polym Sci; 89: 2003, 2277–2282.
8. Encyclopedia of controlled drug delivery, John wiley and sons, Inc. Newyork, 2003, 698-726.
9. Sarasija S, Hota A. Indian J Pharmaceutical Sci 2000; 62: 1-8.
10. Chourasia M K, Jain S K. J Pharmaceutical Sci 2003; 6: 33-66.
11. Sarasija S, Hota A. Indian J Pharmaceutical Sci 2000; 62: 1-8.
12. Colonic Delivery Formulations, Recent Patents on Drug Delivery and Formulation, 2007; 12-13.
13. Ardizzone S, Porro G B. Drug Safety 2002; 25: 561-82.
14. Macfarlane GT and Cummings JH. The Colonic Flora, Fermentation and Large Bowel Digestive Function. In Phillips SF, Pemberton JH, Shorter RG. The Large Intenstine: Physiology, Pathophysiology and Disease. New York: Raven press, 1991: 51.
15. Colonic Delivery Formulations, Recent Patents on Drug Delivery and Formulation, 1(1), 2007, 55.
16. Sinha V.R., Rachna K... Polysaccharides in colon specific drug delivery. Int. J. Pharm. 2001; 224:19-38.
17. Englyst HN. Digestion of the polysaccharides of potato in the small intestine of man. Am J Clin Nutr 1987; 45: 423-431.
18. Towle GA, Christensen O. Pectin. In Industrial Gums and Their Derivatives, eds. R. L. Whistler and J. N. BeMiller, New York, Academic Press1973; 429-461.

19. Giunchedi P, Conte U, Chetoni P, Saettone MF. Pectin microspheres as ophthalmic carriers for piroxicam: Evaluation in vitro and in vivo in albino rabbits. Eur J Pharm Sci 1999; 9: 1-7.
20. Rubinstein A, Radai R, Ezra M, Pathak S, Rokem JM. In vitro evaluation of calcium pectinate: A potential colon-specific drug delivery carrier. Pharm Res 1993; 10: 258-263.
21. Musabayane CT, Munjeri O, Matavire TP. Transdermal delivery of chloroquine by amidated pectin hydrogel matrix patch in the rat. Ren Fail 2003; 25: 525-34.
22. Cheng K, Lim LY. Insulin-loaded calcium pectinate nanoparticles: Effects of pectin molecular weight and formulation pH. Drug Develop Ind Pharm 2004; 30: 359-67.
23. Tomolin J, Taylor JS, Read NW. The effect of mixed faecal bacteria on a selection of viscous polysaccharide in vitro. Nutr Rep Int 1989; 39,121–135
24. Macfarlane GT, Hay S, Macfarlane S, Gibson GR. Effect of different carbohydrates on growth polysaccharidases and glycosidase production of Bacteroides ovatus in batch and continuous culture. J. Appl. Bacteriol. 1990; 68: 179–187.
25. Chourasia MK, Jain SK. Potential of guar gum microspheres for target specific drug release to colon. J Drug Target 2004; 12: 435-442.
26. Al-Saidan SM, Krishnaiah YS, Satyanarayana V, Rao GS. In vitro and in vivo evaluation of guar gum-based matrix tablets of rofecoxib for colonic drug delivery. Curr Drug Deliv 2005; 2: 155-63.
27. Krishnaiah YSR, Satyanarayana V, Kumar DB, Karthikeyan RS, Bhaskar P. In Vivo pharmacokinetics in human volunteers: oral administered guar gum-based colon-targeted 5-fluorouracil tablets. Eur J Pharm Sci 2003; 19: 355-362.
28. Amnuaikit C, Ikeuchi I, Ogawara K, Higaki K, Kimura T. Skin permeation of propranolol from polymeric film containing terpene enhancers for transdermal use. Int J Pharm 2005; 289: 167-78.
29. Park CR, Munday DL. Evaluation of selected polysaccharide excipients in buccoadhesive tablets for sustained release of nicotine. Drug Develop Ind Pharm 2004; 30: 609-17.
30. Ashton WA, Jefferies M, Morley RG, Pass G, Phillips GO, Power DMJ. Physical properties and applications of aqueous solutions of Albizia zygia gum. J Sci Food Agric 1975; 26: 697–704.
31. US National Academy of Sciences Tropical legumes. National Academy of Sciences, Washington, DC. 1979.

32. Gohel MC, Amin AF, Patel KV, Panchal MK. Studies in release behavior of diltiazem HCl from matrix tablets containing (hydroxypropyl) methyl cellulose and xanthan gum. Boll Chim Farm 2002; 141: 21-28.
33. Trease GE, Evans WC editors. Text Book of Pharmacognosy, London: Balliere, Tindall; 2002; 15 th ed. 201-31.
34. McIntosh M, Stone BA, Stanisich VA. Curdlan and other bacterial (1-3)- beta- D-glucans. Appl Microbiol Biotechnol 2005; 68: 163-173.
35. Dysseler BS, Hoffen MJ. Inulin, an alternative dietary fiber. Properties and quantitative analysis. Eur J Clin Nutr 1995; 49: S145-S152.
36. Keller C, Modler RC, Metabolism of fructooligosaccharides by Biffidobacterium spp. Appl Microbiol Biotechnol 1989; 31: 537-541.
37. Wang X, Gibson GR. Effects of the in vitro fermentation of oligofructose and inulin by bacteria growing in the human large intestine. J Appl Bacterol 1993; 75: 373-380.
38. Gibson GR, Roberfroid MR. Dietary modulation of the human colonic microflora, introducing the concept of prebiotics. J Nutr 1995; 125: 1401-1414.
39. Vervoort L, Kinget R. In vitro degradation by colonic bacteria of inulin HP incorporated in Eudragit films. Int J Pharm 1996; 129: 185-190.
40. Ghulam M., Mahmood A., Naveed A., Fatima R.A., Comparative study of various microencapsulation techniques. Effect of polymer viscosity on microcapsule charecterestics, Pak.J.Sci.2009; 22 (3):291-300.
41. Li, S.P., Kowalski C.R., Feld K.M., Grim W.M., Recent Advances in Microencapsulation Technology and Equipment, Drug Dev Ind Pharm.1988; 14: 353-376.
42. .Trivedi P., Verma A.M.L., Garud N., Preperation and Characterization of Aceclofenac Microspheres, Asian Journal of pharmaceutics. 2008; 2(2): 110-115.
43. Dandagi MP., Masthiolimath S.V., Gadad P.A., Iliger R.S., Mucoadhesive Microspheres of Propanalol Hcl for Nasal Delivery, Indian Journal of pharmaceutical Sciences. 2007; 69 (3):402-407.
44. Mathew Sam T., Devi Gayathri S., Prasanth V.V., Vinod B; NSAIDs as microspheres, The Internet Journal of Pharmacology .2008;6(1)
45. http://www.springerlink.com /content/r883r91q17576vx6/, 22nd July 2012, DOI: 10.1007/0-306-46891-3_9, Hafeli U, 2002, Physics and Chemistry Basic of Biotechnology. Focus on biotechnology. Review. Radioactive Microspheres for Medical Application, 7:213-248.

46. Shanthi N.C., Dr.Gupta R., Mahato K.A., Traditional and Emerging Applications of Microspheres: A Review, International Journal of Pharm Tech Research. 2010; 2(1):675-681.
47. Fischer S., Foreg C., Merkle P.H., Gander B., Chitosan Coated Plga-Microspheres-A Modular System for Targetting Drug Delivery, European Cells and Materials. 2004; 7:11-12.