

NOVEL MULTIPARTICULATE DRUG DELIVERY APPROACHES FOR COLON TARGETING: A COMPREHENSIVE REVIEW

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ABSTRACT

An intestinal single celled parasite, *Entamoeba histolytica*, is the major cause of morbidity and mortality in the developing countries which are having poor sanitary condition. It is highly pathogenic free-living amoebae, protease of these amoeba are highly virulent influencing the immune system of the host. The parasite exists in two forms i.e. cyst and trophozoites. Trophozoites form of the protozoa invades mucus membrane of colon and destroys the underlying tissue. The treatment approaches against amoebiasis are normally based on conventional approaches. With the understanding of the pathogenesis

of disease and current advancements in the novel drug delivery system, the researcher across the globe are actively participating in the novel treatment approaches against amoebiasis. The unique capability of pH sensitive, mucoadhesive and mucopenetrating delivery system have opened a new avenue against disease. The current review highlights the role and contribution of novel drug delivery system in the delivery of the drugs in the colon.

KEYWORDS: *Entamoeba histolytica*, colon targeting, pH sensitive delivery system, mucoadhesion, mucopenetration, nanoparticles.

INTRODUCTION

Amoebiasis is invasive and non invasive protozoan parasite intestinal infection due to *Entamoeba histolytica*. It is the major reason of infection is poor sanitary condition and unavailability of safe drinking water, which results in high mortality rate in the developing countries. High rates occur in Indian subcontinents, southern and western Africa, Central south and America. It is anticipated that about 10% of the world people are affected but in

tropical the prevalence may approach 50%. *Entamoeba histolytica*, is most potent cytotoxic parasite known till date.^[1] Its species are extremely pathogenic, free-living, protease of these amoeba affecting mucin and extracellular matrix, which in turn are influencing immune response. The parasite exists in two forms i.e in cyst form and in trophozoites form and causes infection of intestinal mucosa which rarely extends to rest of the organs, primarily liver. In addition to this, protease helps in adhesion, encystation and spreading of infection.^[2] The infection initiates when the virulent trophozoites start crossing the mucus and destroy mucoepithelial barrier, after that killing the host cells, starting to cause inflammation and dysentery. When parasite cyst is ingested via contaminated food or water^[3], cyst is resistant to gastric acidity thus stay alive when crossing stomach and the small intestine. It can stay alive for many weeks in wet atmosphere. Motile and invasive trophozoites are formed after excystation of cyst in the bowel lumen. The trophozoites stick to colonic mucins and thus colonize the colon and after that trophozoite penetrate the intestinal mucous layer and causing colitis. The protozoa secrete proteolytic enzymes that interrupting the intestinal mucus and epithelial barrier and thus helping tissue penetration. Subsequently flask shaped ulcers results due to killing the host epithelial by trophozoites. Finally, these protozoa cause extra intestinal infection e.g. amebic liver abscesses as they oppose the host's immune.^[4] The protective mucus barrier is disrupted by cysteine protease which is secreted from the parasite.^[5] The mucus barrier is the host's first line of protection against invasion.^[6] Pathogenesis of Amoebiasis is shown in Fig No. 1

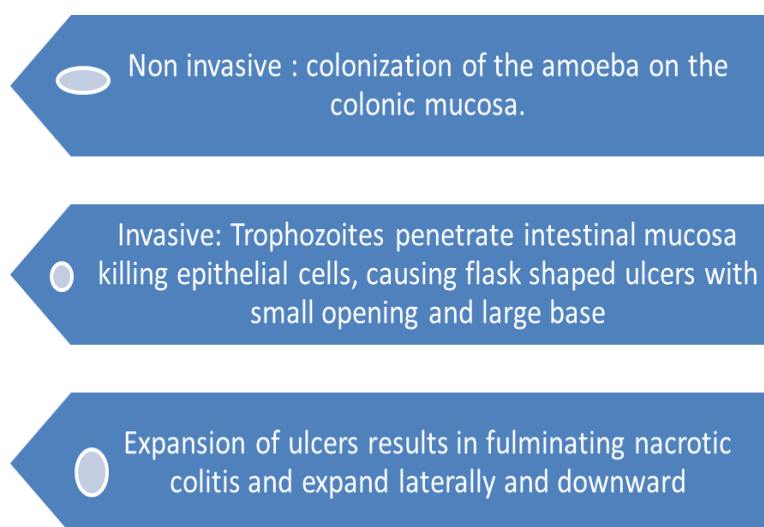


Figure No: 1: Flow diagram showing progress of Amoebiasis.

Clinical symptoms associated with amoebiasis are.

<u>Intestinal disease</u>	<u>Extra intestinal disease</u>
Asymptomatic cyst passer	Liver abscess
Symptomatic non dysenteric disease	Pulmonary amoebiasis
Acute amoebic dysentery	Brain
Fulminant colitis	Cutaneous and genital diseases
Colon perforation	
Amoeboma	
Perianal ulceration	

The nitroimidazole derivatives like Niridazole, Ornidazole, Metronidazole, Secnidazole and Tinidazole are the drugs of choices in the treatment of luminal and tissue amoebiasis but there is no major advancement in the delivery of these drugs.^[7] In these days, pharmaceutical industry has developed curiosity in novel ways of drug delivery rather than discovery of new molecules. Because drug discovery is expensive and time consuming.

Conventional delivery systems used for colonic disorder are not successful as inappropriate drug concentration at target site. So an effective, safe and site specific delivery of drug is desired which could deliver the drug only at target site, with minimum side effects. The parasite resides in the colon so we need a delivery system which prevents or minimize the premature release in stomach and small intestine. Otherwise also there are many advantages of colon as delivery site such as longer transit time, neutral pH and low proteolytic enzyme activity.

Various approach have been used in the literature to target drugs to the inflamed colon after oral administration includes timed release, high pH, Pro-drugs, exploitation colon enzymes, intestinal transit time and colonic pressure.^[8] Time released delivery systems depends upon gastrointestinal transit time. pH-dependent delivery systems releases drugs at higher pH i.e. at the pH of colon pH dependent polymers include methacrylic acid copolymers, S100, which dissolve above pH 6.0. Colonic bacterial enzymes cleave the bonds of prodrugs and thus release drugs. Polysaccharides such as pectin, chondroitin sulphate, guar gum dextran, are broken down to simple saccharides by the colonic microflora. The colon microflora includes *Peptococcus*, *Eubacterium*, *Bacteroides*, *Peptostreptococcus*, *Propionibacterium*, *Ruminococcus*, *Clostridium* and *Bifidobacterium*.^[9] Summary of the different approaches for colon drug delivery is given in Table No 1.

Table No 1: Summary of the different approaches for colon drug delivery.

Sr. No.	Approaches	Polymers	Mechanism/ approaches	Drug candidates	References
1	Pro-drugs	Copolymer of styrene with 2 hydroxy ethyl methacrylate	Azo linkage	Vessopressin , insulin	[10]
		alpha-, beta-, and gamma-cyclodextrins	Amide Linkage	biphenylacetic acid	[11]
		Pectin	Glycosidic linkage	Dexamethsone and prenisolone	[12]
2	pH sensitive	Eudragit L 100, Eudragit S 100 , Eudragit RS 100, Eudragit RSPO, Shallac, cellulose acetate phthalate	pH dependent solubility of coating	Metronidazole, Tinidazole, Indomethacin, Aceclofenac, Mesalamine	[13,14,15,16,17]
3	Colonic microflora	Microcrystalline cellulose, guar gum, Xanthane gum, Sesbania gum, cyclodextrin, pectin, HPMC, Chitosan, sodium alginate, ethyl cellulose,	Microbial degradation of polysaccharides by the colonic microflora	5 FU, Diclofenac sodium, Metronidazole, Tinidazole, , Mesalamine, Sornidazole, Prednisolone	[18]
4	CODES	(Eudragit L(100)	It is combined approach of pH dependent and microbially triggered colon drug delivery system.	Mebeverine Hydrochloride	[19]
5	Osmotic	ethylcellulose and pectin	Water swellable polymers increases the osmotic pressure of the formulation which causes the release of entrapped drug	5-aminosalicylic acid	[20]
6	Pulsetile	cellulose acetate butyrate (CAB), HPMC K4M, guar gum, pectin and Eudragit S100-	In pulsatile systems, decreasing the ratio of the polymer significantly increased the rate and extent of drug release.	Budesonide	[21]

The most suitable drugs for colon targeting include for treating local and systemic colon disorders e.g. amoebiasis, ulcerative colitis, colon cancer and intestinal bowel diseases. The drugs includes Tinidazole, Indomethacin, Aceclofenac, Mesalamine, ornidazole, Prednisolone, 5 FU, Diclofenac sodium, Metronidazole^[22], doxorubicin^[23], budsonide^[24], campritol^[25], BSA^[26], insulin^[27], cisplatin.^[28] Drugs with poor bioavailability (proteins and peptide) from the stomach or intestine are also the most suitable for colonic targeting. These approaches have been summarized in the table no.1.

NOVEL DELIVERY SYSTEM FOR COLON TARGETING

The approaches discussed above deliver the drugs the lumen of the colon and could be effective in the luminal amoeba present locally in the colon. But for complete eradication of the infection drug has to reach at the epithelial layer of the colon and then penetrate the mucus membrane. Thus a complete understanding of the mucus and its protection mechanism has to be understood first along pH sensitive polymer delivery before discussing the concept of mucoadhesion and mucopenetration.



Figure: 2: Schematic representation of muco-penetration via pH sensitive polymer coating and muco-adhesion.

Mucus layer

Mucus is epithelial layers secretion which is transported from base to lumen side and then digested or shed off. It forms a thick gel like structure over the soft tissue and provides a protective barrier between the underlying soft epithelium cells and lumen content. It helps in transportation of toxic material, bacteria and gastric content to the lower portion of GI tract.^[29] Human mucus layer thickness differ in inter and intra anatomical region of the body. The mucus layer thickness decreases in the intestinal region and is mainly depends upon the digestive activity. The diet content also affects the mucus layer thickness. It acts like semi permeable barrier which allow exchange of nutrients, gases, water, hormones, gametes. The main constituent of mucus are water (90-98%), mucin (2-5%), proteins, (0.02%), lipids (1-2%), mineral salts (1%).^[30,31] The viscoelastic of the mucus depends on the composition of

these constituents. The average mucus thickness in stomach and colon is 180 μm and 150 μm respectively.^[31] The more amount of sialic acid and sulfate is responsible for strongly negative surface in mucin.^[32]

The mucus gel able to traps foreign particulates or organism due to presence of negative charge on the mucin proteoglycans. The carboxyl or sulfate groups makes the mucin negatively charge. If foreign particles possess a negative surface charge then they are repelled by negatively charged mucin. The hydration of mucus layer results in decreased viscoelasticity and facilitates the penetration of the bacteria. In such cases, high rate of mucus secretion and replacement create a defense mechanism against colonization and growth of bacteria.

Drug actions for the treatment of amoebic colitis can be made superior by incorporating novelty in delivery system by pH sensitive polymer coating, mucoadhesive and mucopenetrating drug delivery systems. The concepts of mucoadhesion was an idea to provide prolonged contact time to mucosal sites and thus considered the most suitable system for the local drug delivery in mucus layer of stomach and intestine as the diffusion of become easy drug across the mucus layer. For effective mucoadhesion, delivery system should be positively charged so as to interact electrostatically with negatively charged mucin. But nowadays mucopenetrating drug delivery systems are designed for delivering drugs across mucus.^[33]

Nanoparticles have ability to cross biological barriers because of their nanometer size and preserving themselves from their degradation. For transmucosal delivery, the nanoparticles must penetrate quickly through the mucus layer to reach the underlying epithelium.^[34]

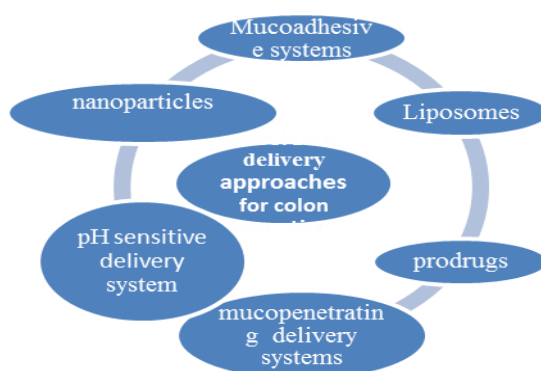


Figure No 3: Novel drug delivery approaches for colon targeting.

Colon Targeting Novel Delivery System consist of coating of delivery system with pH sensitive polymers which could help the delivery system to reach colon unharmed during its way from stomach to small intestine. So that delivery system could release maximum amount of drug in the colon. Poly methyl meth acrylate derivatives i.e. different grades of Eudragit are used in colon targeting of the delivery system and the grade which release at colon site is Eudragit S100.

pH sensitive delivery systems

The pH sensitive delivery system takes the advantage of different pH range of the GIT and these delivery systems are coated with pH sensitive biocompatible polymer which dis-integrate at colonic pH thus also known as “smart carrier”. This type of delivery system is a good indicator of translational applicability. The most commonly used polymer are methacrylic acid copolymer (Eudragits). The Eudragits of varying pH solubility can be manufactured by changing the side chain e.g. Eudragit L-100 dissolve at pH 6, Eudragit S-100 at pH 7, so both can be used in varying composition to get pH solubility 6-7. Eudragit FS-30D dissolve at pH 6.5 so specifically used for colon delivery. In addition, it is having mucoadhesive property.^[35] Hydroxypropyl methylcellulose phthalate (HP-55) have been commercially used as enteric coatings for oral delivery of peptide and protein drugs.^[36] Eudragit coated liposomes showed pH responsive release in In-vitro in simulated conditions, sustained release of 5-FU36. Guo et al., 2015.^[37] prepared a paclitaxel and gemcitabine loaded N-succinyl chitosan nanoparticles to target colon cancer and showed a pH sensitive swelling and release at colonic pH. Local delivery of Curcumin nanoparticle was made possible with PLGA and Eudragit S100^[38] and Budesonide pH-sensitive nanospheres showed strongly pH-dependent drug release followed by a sustained release at pH 7.4 for colon-specific delivery which were prepared using polymeric mixtures of PLGA and a pH-sensitive methacrylate copolymer.^[39] Eudragit S 100 coated prednisolone nanocapsule.^[40] showed pH dependent release in normal physiological condition. Lamprecht et al.^[41] prepared tacrolimus loaded PLGA nanoparticles which were entrapped into pH sensitive microsphere showed colon release.

Mucoadhesive drug delivery systems

Mucoadhesive drug delivery is the most talented approach for the delivering drugs across the mucosal tissue. The nanoparticle formulations got attached to the mucus and penetrate in to the mucus layer and slows the particle transit time through the GI tract to the time scale of

mucus renewal, thereby enhancing drug absorption. These systems are bound to the upper surface of mucus layer via interaction with the mucin fibers.^[42] For oral delivery, the average physiological turnover time for the GI tract mucin is 50 to 270 min.^[43] Hence these systems are eradicated via muco-ciliary clearance within 4-5 h. In the GI tract, for example, nanoparticles delivered orally may adhere to mucin fibers or associated with chyme, followed by mucus clearance and fecal elimination or transport across the mucus mesh for possible entry to the underlying epithelia or prolonged residence time in the unstirred layer of mucus gel.^[44,45] Typically, the majority of administered particles does not adhere or transport through the GI mucus layer, but rather undergo direct transit through the GI tract. The mucoadhesive particulate system improve the oral bioavailability of the drug compared to the plain drug by prolonging the residence time of drug at absorption site by improving the stability of the encapsulated drug in GIT. Gemcitabine -loaded mucoadhesive nanoparticles.^[46] prepared by an ionic gelation method using chitosan and Pluronic F-127 as a carrier showed mucoadhesion behavior of the nanoparticles. The mucoadhesion study results advocated that nanoparticles could be considered as an efficient oral formulation for colon cancer treatment. The nanoparticles are better than micro particles, in mucus penetration ability due to their small size but the strong interaction between the mucin fibers and polymer of the carrier system like ionic interaction between the negatively charged mucin and positively charged nanoparticles immobilize the penetration of particulate system. Hence the mucoadhesive nanoparticles are unsuitable for the delivery of drug and gene to the intracellular or deep gastric mucosal layer close proximate of the epithelial cell.^[47]

Mucoadhesive delivery systems

Mucoadhesive delivery systems are positively charge delivery systems which can interact with negatively charge intestinal mucosa. Colonic mucin is negatively charge due to the presence of sulphate and sialic acid residue. So this adhesion is advantageous for colon targeting of nanoparticles due to reduced clearance in increased motility. There is increased mucus production in Crohn's disease and the mucoadhesive property increases the retention and targeting. The study conducted by Nibel et al., 2012.^[48] on clodronate nanoparticles on colitis model showed mucoadhesion. Clodronate alone was ineffective in colitis therapy but when used with a cationic nanoparticle improves the inflammatory response in colitis model. This type of delivery system is useful for drugs to deliver in the extracellular domain.

The drug transport after mucoadhesion across the epithelial layer is by paracellular transport (size less than 50 nm) or by endocytosis (size smaller than 200 nm).^[49] Hong et al., 2010.^[50] prepared nanoparticles by ionotropic gelation of water-soluble chitosan with sodium tripolyphosphate using Bovine serum albumin as a model drug. The size of nanoparticles range between 100 and 400 nm. In-vitro release studies showed prolong the intestinal absorption of water-soluble chitosan. Amongst various mucoadhesive polymers, chitosan offers a great advantage being polycationic in nature and also has some antibacterial activities, but over hydration converts the chitosan gel into slippery mucilage and adhesion failure may occur.^[51]

Mucopenetrating drug delivery system

In case of Mucoadhesive delivery system, though residence time of particles is increased but polymer swelling delays drug porting in gastric mucus. Also mucoadhesion decrease the mobility and penetrability of the drug in mucus.^[52]

According to the statement given by many researchers, changing the surface chemistry of particles which reduces the interaction with mucus and helping in penetrating mucus membrane. The use of low molecular weight polyethylene glycol (PEG) on the surface of nanoparticles minimizes the interaction and thus helps in mucopenetration. Effective penetration of PEG coated polystyrene nanoparticles in sputum of cystic fibrosis patients, rapid penetration of PEG-PSA (poly sebasic acid) biodegradable nanoparticles in human mucus.^[53] Nasal absorption of Insulin loaded PEG-g-chitosan nanoparticle^[54], PLGA nanoparticle for the gene delivery of DNA in gastric mucus. Particle size less than the mesh size of the mucin fiber are reported good mucus penetration property e.g. chitosan a polymer with cationic charge having mucoadhesion property, the surface chemistry of chitosan can be modified by shielding the cationic charge.^[55] The nanoparticles of 100 nm size range are preferential accumulated in the inflamed colon and it has been confirmed that nanosized particles are taken up more rapidly in experimental colitis.^[56] They can also better attach to mucus layers due to their easier penetration and their relatively small mass. Moreover, there are many pathophysiological changes due to mucosal inflammation which are involved in preferential uptake are disrupted intestinal barrier due to distortions and ulcers, infiltration of immune related cells and more mucus production.^[57]

Lai et al., 2009b^[55] proposed that high density of positive charge and negative charges on the surface facilitate the efficient transport of the virus by reducing the electrostatic adhesive

interaction. Apart from this a hydrophilic surface also minimizes the hydrophobic entrapment between the mucus. So for the efficient mucus penetration of the particle, the surface property of the particle should be designed to diminish the interaction between the particles and mucus. Norris et al., 1997^[56] reported slow transmucosal nanoparticles transport whose surface was tailored with amidine, carboxyl or sulfate groups and the best transport was found with amidine which is having most hydrophilic surface. Lai et al., 2009a hypothesized that coating of an uncharged surface on the particle provides the surface suitably hydrophilic, muco inert and low hydrogen bonding capacity as in viral capsid. For this purpose they tried poly ethylene glycol that satisfy these dual requirements of strong hydrophilicity and neutral charge. In their study, the coating of charged polymeric nanoparticles with a high density of PEG may reduce particle – mucus adhesive interaction. Chondroitin sulfate-chitosan nanoparticles and positively and negatively charged fluorescein isothiocyanate-conjugated bovine serum albumin loaded nanoparticles were prepared and characterized. The uptake of positively charged fluorescein isothiocyanate-conjugated bovine serum albumin loaded nanoparticles across the epithelial membrane was more efficient than that of the negatively charged nanoparticles.

FK506 (tacrolimus) entrapped into nanoparticles (100 nm) was administered either orally or rectally to male Wistar rats suffering from a preexisting experimental colitis. The relative drug penetration into the inflamed tissue is about 3-fold higher compared with healthy tissue when nanoparticles were used as drug carriers. This is due to superior adhesion selectivity and improved drug penetration into the inflamed tissue.^[55] This study suggests surface modification and size both affects muco-penetration. They reported that significant fraction of small size PEG-modified nanoparticles was immobilized or strongly hindered in mucus. They suggested the possible region for slow transport of 100 nm nanoparticles across mucus (Vs 200 and 500 nm) may be inadequate PEGylation of 100nm particles. Larger nanoparticles (200 - 500 nm) PEG modified contains higher drug encapsulation and reduced aggregation when freeze dried, improved drug release kinetics for sustained release formulations and superior therapeutic efficacy.^[57] In contrast, smaller nanoparticles suffer from large burst release typically within hours upon in vitro or in vivo application.^[58,59] Treatment of mucus with mucolytics agents may improve the penetration rates of drug and gene carrier particles. The utilization of mucolytics as an additives to particle transport may be particularly important for diseases where mucus is abnormally viscoelastic, such as CF and COPD.^[60] The summary of novel delivery systems is briefed in Table No. 2.

Table no 2: Summary of novel drug delivery to the colon.

Sr. No.	Delivery system	Method of preparation	Drug candidate	Salient features	References
1	chitosan-alginate polyelectrolyte complex	Ionic gelation	Amoxicillin	Delivery of the drug across the Mucus for the eradication of the infection.	[61]
2	Eudragit S 100 coated microspheres	Emulsification followed by cross linking with CaCl_2	Naproxen	2.5-5% eudragit prevents drug release in upper GIT. SEM images shows rough surface of core and smooth of coated. Core has better surface adhesion than coated microspheres	[62]
3	Asam bora rice starch microspheres	Double emulsification with solvent evaporation	5 FU	Microspheres showed extended release of drug over prolonged period of time with reduced systemic side effects	[63]
4	pH-responsive hydrogel of poly(methacrylic-grafted-ethylene glycol) nanoparticles	Polymerization	doxorubicin	Release of doxorubicin locally in the colon. Loading levels ranged from 49% to 64%. P(MAA-g-EG) containing nanoparticles were less mucoadhesive	[64]
5	poly (lactic-co-glycolic) acid (PLGA) and a pH-sensitive methacrylate copolymer nanospheres	Ionic gelation and solvent evaporation.	Budesonide	quantitative analysis of the fluorescent marker and confocal laser scanning studies showed strong and specific adhesion of the nanospheres to the ulcerated and inflamed mucosal tissue of the rat colon.	[39]
6	Eudragit S100, coated sodium alginate microspheres	Emulsification followed by cross linking	valdecoxib	microspheres applied to the mucosal surface of freshly excised goat colon, showed mucoadhesion.	[28]
7	Novel lipid-polymer composite microspheres	Emulsification	Compritol	the novel pH-sensitive LP-MS has potential for colon-specific drug delivery.	[25]
8	Compritol ATO888 (C) and hydroxypropyl- β -cyclodextrin (HP) solid lipid nanoparticles	emulsification	diclofenac sodium	SLN with a size range of 300-600 nm were formed. In vitro and ex vivo tests demonstrated that dried SLN can be considered as colon delivery systems	[65]
9	Chondroitin sulfate-chitosan (ChS-CS) nanoparticles	Ionic gelation	isothiocyanate-conjugated bovine serum albumin	The uptake of positively charged FITC-BSA-loaded ChS-CS nanoparticles across the epithelial membrane was more efficient than that of the	[66]

				negatively charged nanoparticles.	
10	pH responsive Chitosan nanoparticles	Ionic gelation	Insulin	Chitosan adheres to the mucosal surface and opens the tight junction between the contiguous epithelial cells, therefore delivers the drug in the deeper layers and also infiltrate the mucous layer	[27]
11	Lectin decorated nanoparticles	Covalent bounding conjugation	Lectins	Selective bioadhesion of nanoparticles with the inflamed tissues of the colon	[67]
12	Eudragit S100 coated pectinate/ alginate microbeads	Electrospray method and polyelectrolyte multi layer coating techniques	Cisplatin	The drug release is pH dependent. 25% and 39% drug release at pH 1.2 and 4.5 in 24 hours and quickly rose to 76% when pH rose to 7.4	[68]
13	Eudragit coated Pectin microspheres	Emulsion dehydration method and oil in oil solvent evaporation method	5 FU, aceclofenac	Drug : Polymer Ratio 1:4, core: coat ratio 5:1, drug release showed pH dependent release of drug.	[45,69]
14	HPMC microspheres	Ionic gelation	Atenolol	Microspheres shows good swelling index which confirms good muco-adhesive property	[70]
15	Chitosan and sodium alginate polyelectrolyte complex nanoparticles	Ionic gelation		nanoparticles of 160 nm exhibited pH stable structure	[71]
17	Guar gum nanoparticles	Emulsion crosslinking method	Folic acid charged with methotrexate	Surface modification of methotrexate with folic acid, there is minimum drug release in upper GIT while maximum drug release in the colonic fluid of pH 6.8.	[72]

Future advances needed in colon targeting

Nanoparticles drug delivery system has appreciably advanced for colon targeting, there is requirement for maximum drug exposure for tissue localization with minimum unwanted effects and increased therapeutic efficacy with lower therapeutic doses. The translational use of these carriers in clinics is still pending. Safety of the various nano carriers following uptake is to be explored further. There is limited nano-toxicological studies in the human GIT.

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