SYNTHESIS OF CHITOSAN BASED NANOPARTICLES AND EFFICACY OF CORTICOSTEROIDS AND AMINOSALICYLATES FOR THE TREATMENT OF INFLAMMATORY BOWEL DISEASE

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ABSTRACT

Nanoparticles were considered as the revolutionized drug delivery system besides conventional drug delivery. Nanoparticles prepared with chitosan were found to be biodegradable and biocompatible with low toxicity which made chitosan as a valuable tool to manipulate the molecules and their structures. Chitosan nanoparticles can be delivered through parenteral and per-oral routes that offer a valuable tool for novel targeted drug delivery systems.Chronic inflammation of gastro intestinal tract due to inflammatory bowel disease was frequently occurred in young people. Anti-inflammatory drugs suppress the intestinal inflammatory burden with limited therapeutic efficacy by developing adverse drug reactions. Thus, the drugs have been targeted using novel drug delivery strategies for elevated therapeutic efficacy by diminishing adverse drug reactions. The efficacy of corticosteroids and aminosalicylates were explained using many randomized trials from 4 to 5 decades that continued till today. The review summarizes the structure and properties of chitosan, methods for production of chitosannanoparticles and efficacy of corticosteroids and aminosalicylates in the treatment of Inflammatory Bowel Disease.

Key Words:

Chitosan nanoparticles, Corticosteriods, Aminosalicylates, Ulcerative colitis, Crohn's disease.

1. INTRODUCTION:

1.1 Brief perception on Inflammatory Bowel Disease (IBD):

IBD is collectively called as Crohn's Disease (CD) and Ulcerative Colitis (UC) which was characterized as chronic inflammatory disease of gastro intestinal tract. IBD is very common in western countries with 1.4 million people affected in United States and 2.2

million in Europe [1-3]. IBD is associated with an elusive interaction of environmental factors and genes [4] like smoking[5-7], diet [8,9], intestinal permeability [10], colonic mucus [11,12], genetic factors [13], microbial agents [14] etc. CD and UC are associated with a variety of complaints like fever, bloody diarrhoea, weight loss, abdominal pain and vomiting. Extra intestinal manifestations are common and mostly affect joints, skin, eyes and bile ducts [15-17].

The treatment of IBD depends on the severity of disease subtype, pre-existing illness and patient tolerance to drugs. The most common classes of drugs in the treatment of IBD includes anti-inflammatory (aminosalicylates, corticosteroids etc) and imunosupressive agents (azathioprine, 6-mercaptopurine etc) [18,19]. Considerable challenge in the therapy of IBD is the prevention of drug related side effects including mortality.

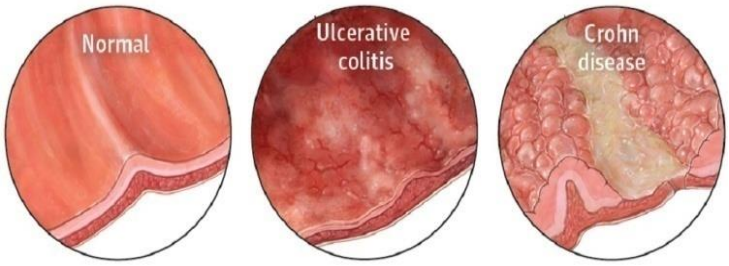


FIG.1: COLON WALL WITH ULCERATIVE COLITIS AND CROHN'S DISEASE

TABLE 1: DRUGS USED IN THE TREATMENT OF IBD AND ITS SIDE EFFECTS

Sl.No	Class		Drugs	Side effects
1	Anti-inflammatory	5-Aminosalicylates (mainly for UC) [20]	Sulfasalazine Mesalazine Olsalazine Balsalazide	Headache, Nausea, Epigastric pain, Diarrhoea [21-23]
		Corticosteroids [24]	Prednisolone Prednisone Budenoside Methylprednisolone	Acne, Moon face, Oedema, dyspepsia, glucose intolerance[25]
2	Immunosuppressant	Thiopurines [26]	Azathioprine, Mercaptopurine	Myalgia, Diarrhoea, Hepatotoxicity [27,28] Tremor, Malaise.
		Cyclic polypeptide	Cyclosporin [29]	Gingival hyperplasia, Hirsutism [30]
3	Anti-neoplastics	Folate derivative	Methotrexate [31]	Nausea, vomiting, Diarrhoea, Stomatitis[32]
4	TNF-α blocker [33]	Monoclonal Antibody	Infliximab	Fever, Myalgia, Joint Pain and stiffness [34,35]

*TNF – Tumor Necrosis Factor.

1.2 Concept of Drug Targeting:

"Magic bullet" was a scientific concept developed by a German physician Paul Ehrlich in 1908 [36]. The concept was evolved to kill a specific microorganism which causes diseases in the body. Just like the bullet hits the target, he found the concept to specifically target invading microbes with maximum therapeutic efficacy and simultaneous reduction of side effects [37].

Currently this concept has become a predominant approach for targeting the drug to a specific organ. Many efforts and attempts were made till today in order to develop an innovative drug delivery system for the treatment of IBD. With considerable advances in the field of pharmacy, innovative drug carriers were developed which can efficiently deliver the drug at inflammed intestinal areas and target the drug to the site of inflammation.

1.3 Targeted Drug Delivery in IBD – Basic requirements:

Main priority of drug targeting in IBD is, availability of concentration of active ingredients at the site of inflammation to improve the therapeutic efficacy with complete biodegradation and biocompatibility without pro-inflammatory properties. Moreover targeted drug delivery systems have to be formulated as peroral dosage forms to induce and maintain compliance.

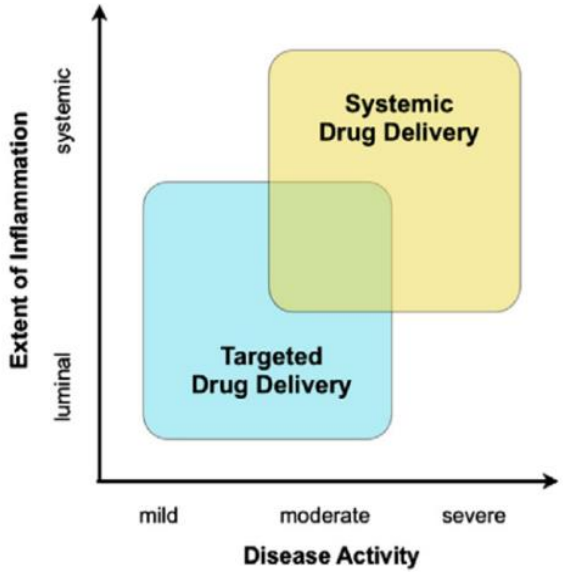


FIG.2:THERAPEUTIC BENEFIT OF TARGETED DRUG DELIVERY STRATEGY [38]

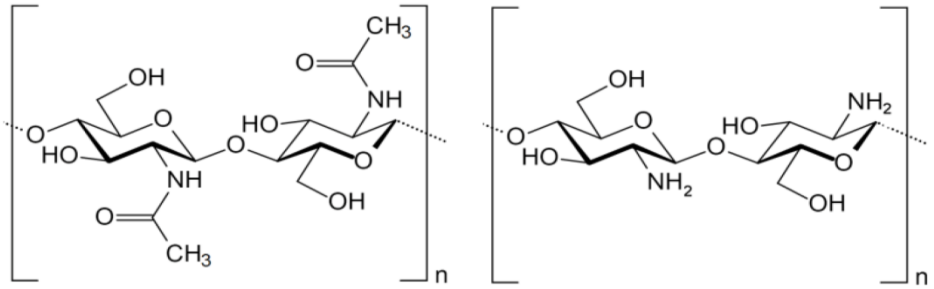
1.4 Chitosan Nanoparticles – Promising system in the treatment of IBD:

Chitosanoffers various advantages over other polymeric carriers in its biodegradability, biocampatibility, easier formulation techniques with low toxicity. Chitosan nanoparticles avoid the use of hazardous organic solvents, since they are soluble in aqueous acidic solutions. Cationic nature of chitosan improves crosslinking with multivalent anions [39]. Microparticulates of various drugs and prodrugs like sulfasalazine [40], prednisolone [41], salazosulfapyridine [42] etc has been successfully formulated for the treatment of IBD.

2. STRUCTURAL FEATURES AND PROPERTIES OF CHITOSAN:

2.1 Chitosan:

Chitosanwas a best polysaccharide which was used as a carrier in various biomedical and pharmaceutical preparations [43-45].Chitosan is a second abundant natural polysaccharide made of randomly distributed β-(1-4) linked D-glucosamine and N-acetyl-D-glucosamine. It is obtained by deacetylation of chitin extracted from the shells of shrimps and crabs [46]. Chitosan is a positively charged, linear, hydrophillic compound with mucoadhesive property for the preparation of nanoparticles [47,48].Chitosan is digested by chitinases, that are secreted by intestinal microorganisms or plant ingredients or by lysozymes [49,50].



Chitin Chitosan

FIG.3: STRUCTURE OF CHITIN AND CHITOSAN[51]

2.2 Structural features:

Chitin and chitosan weredifferentiated based on N-acetyl-D-glucosamine units with more than 50% in chitin and less than 50% in chitosan [49]. The presence of primary amine at C-2 position of glucosamine residue made chitosan as an important polysaccharide for the fabrication of functional drug delivery. Presence of amino groups makes the molecule with high charge density and readily available for chemical reactions and salt formation with acids. It can interact with polyions and forms complexes and gels [52,53].

2.3 Properties:

Physicochemical properties of chitosan mainly depend on degree of deacetylation, molecular weight and its viscosity [49]. Solubility, hydrophobicity, ability to interact with polyanions were mainly affected by degree of deacetylation [54-56]. Greater solubility and fast degradation was exhibited by chitosan having low molecular weights with lower deacetylation and vice versa[56-59]. pKa of chitosan was found to be approximately 6.5 on amine groups with pH less than 6 reflecting polycationic behavior of chitosan [60-62]. Solubility of chitosan can be improved by quarternization that forms trimethyl chitosan derivatives at neutral and basic pH [60]. At higher pH above 6.5 deprotonation of amines takes place that undergo interpolymer associations leading to film and gel formation [52]. Adhesivity of chitosan is mainly due to presence of amino and carboxyl groups that can be combined with glycoprotein in mucus, to form hydrogen bond. The mucoprotein present in the mucus is negatively charged, there by chitosan and mucus was attracted to each other and prolongs the retention time of drug, improving drug bioavailability.

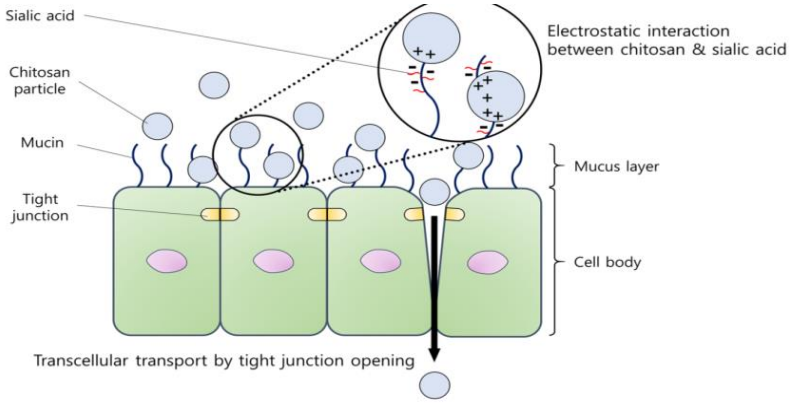


FIG.4:ELECTROSTATIC INTERACTION BETWEEN CHITOSAN AND MUCUS[63]

3. METHODS FOR THE PREPARATION OF CHITOSAN NANOPARTICLES:

3.1 Emulsification and cross linking:

This method involves the preparation of W/O type of emulsion and subsequent addition of cross linking agent to the formed droplets. Glutaraldehyde was used as a cross linking agent to harden the formed droplets. Amino groups of Chitosan undergo covalent crosslinking with the aldehyde groups of glutaraldehyde, thereby production of chitosan nanoparticles [64]. Tedious procedures and application of harsh crosslinking agents (glutaraldehyde) makes this method a major drawback [65]. Glutaraldehyde may induce toxicity and affects drug integrity which leads to a progressive shift towards less tedious procedures.

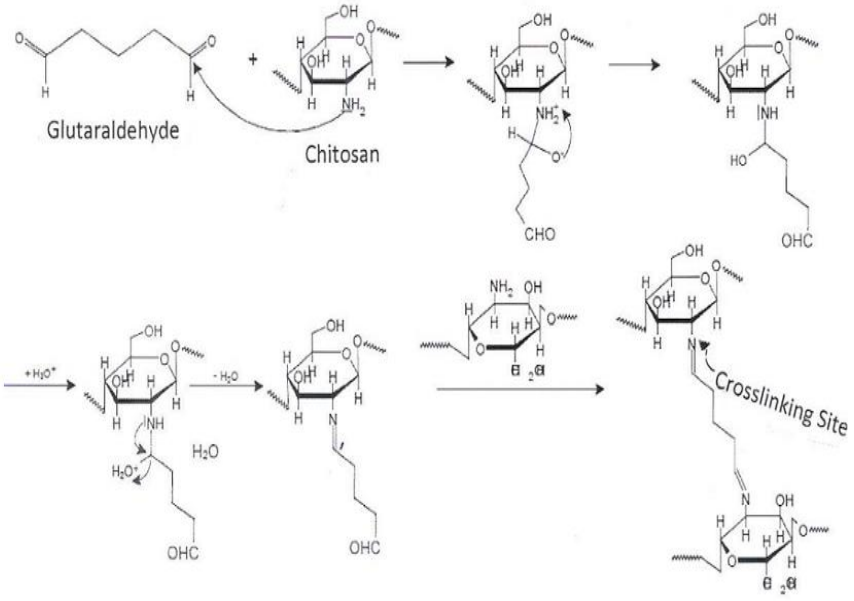


FIG.5:REACTION BETWEEN AMINO GROUPS OF CHITOSAN AND CARBONYL GROUPS OF GLUTARALDEHYDE[66]

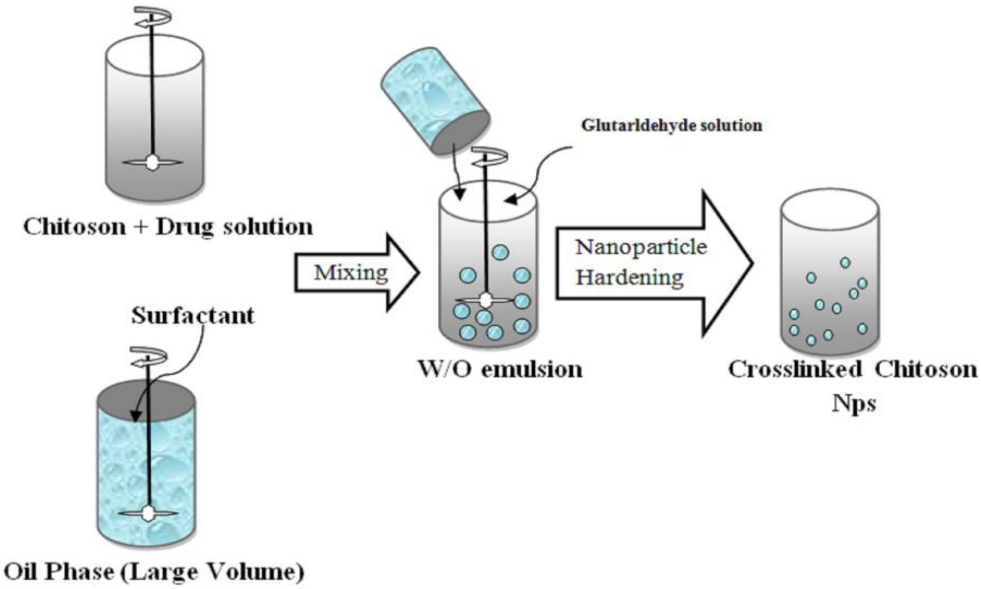


FIG.6:EMULSIFICATION AND CROSS LINKING

3.2 Emulsion droplet coalescence:

Chitosan was dissolved in the aqueous solution of drug and a small aliquot of this solution is added to 10 ml of liquid paraffin containing span 80. W/O type of emulsion was formed using high speed homogenizer. In parallel, another W/O type of emulsion was prepared by adding 1.5 ml sodium hydroxide to 10 ml of same outer phase (10 ml of liquid paraffin containing span 80). Both the emulsions were mixed with high speed homogenizer leading to droplet coalescence. Sodium hydroxide acts as a precipitating agent for the solidification of chitosan particles. Further the particles were centrifuged and washed with the help of water, ethanol or toluene [67].

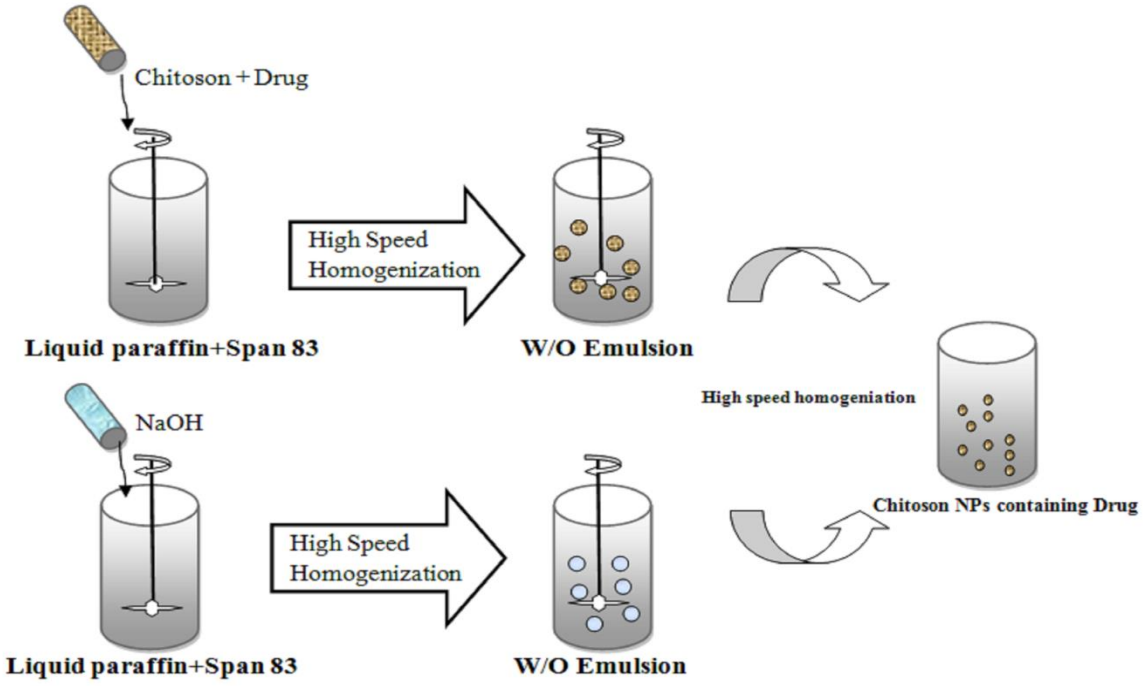


FIG.7:EMULSION DROPLET COALESCENCE

3.3 Ionic gelation and polyelctrolyte complexation:

High degree of protonation due to the presence of amine groups in chitosan has capacity to form hydrogels in the presence of specific polyanions [68,69]. Inter and intra molecular crosslinkages conciliated by anionic molecules has been used to produce chitosan nanoparticles by ionic gelation and polyelectrolyte complexation [70]. Small anionic molecules like citrate, phosphate, sulphate induces ionic gelation while large anionic molecules like tripolyphosphate induces polyelectrolyte complexation, sometimes can be called as complex coacervation or interfacial coacervation [71,72].The mean particle size of nanoparticles at ultrasonication, decreases with increasing in solution temperature [73]. The size and surface charge of nanoparticles can be changed by changing the ratio of chitosan and stabilizer.

Chitosan nanoparticles were prepared in a complete hydrophillic environment with the help of polyanion tripolyphosphate (TPP) reported by Calvo et al (1997). Immediately after the addition of TPP to the solution of chitosan, nanoparticles were formed under mild stirring and room temperature. In order to stabilize the particles stirring should be continued approximately for 10 minuites and obtained suspension was centifuged to separate the nanoparticles from unreacted chitosan and tripolyphosphate. The obtained nanoparticles were washed and dried [74]. Production of chitosan nanoparticles were improved with increasing the concentration of TPP [75]. By analyzing the different ratios of chitosan:TPP, it was found that zeta potential was increased with the increament of chiton concentration which may be due to the increase of protonated groups of chitosan [76-79]. Using complete hydrophilic environment, avoidance of organic solvents and high shear forces makes ionic gelation and polyelectrolyte complexation as a best suitable method for the preparation of chitosan nanoparticles [62].

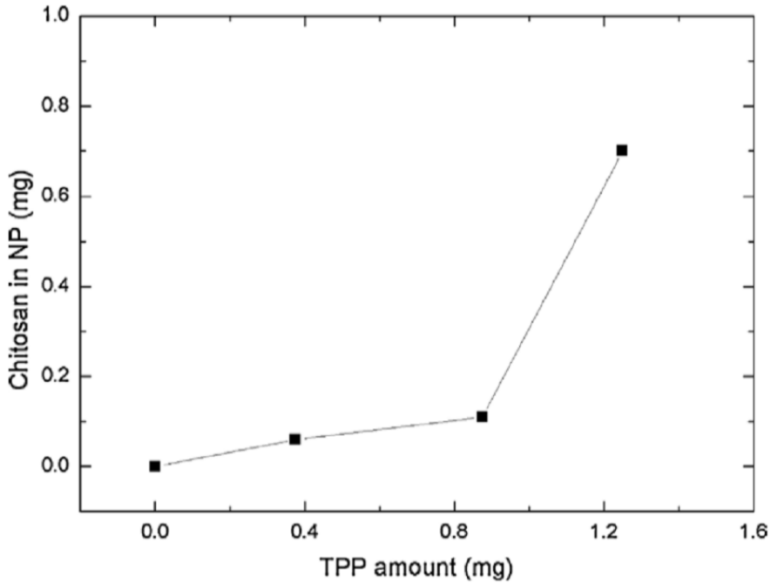


FIG.8: QUANTITY OF RECOVERED NANOPARTICLES AS A FUNCTION OF AMOUNT OF TPP ADDED.[75]

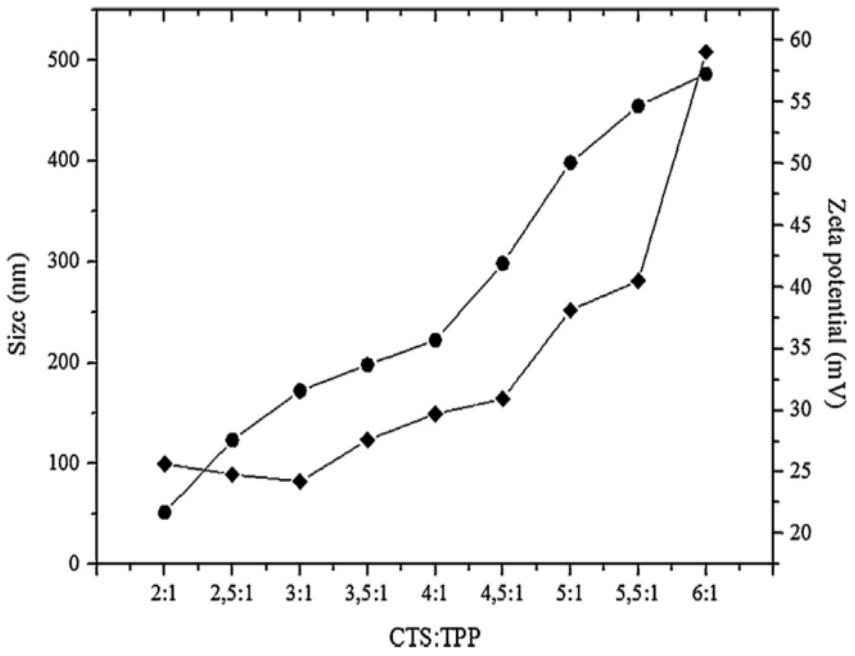


FIG.9: EFFECT ON CHITOSAN:TPP RATIO ON THE SIZE OF NANOPARTICLES AND ZETAPOTENTIAL.[80]

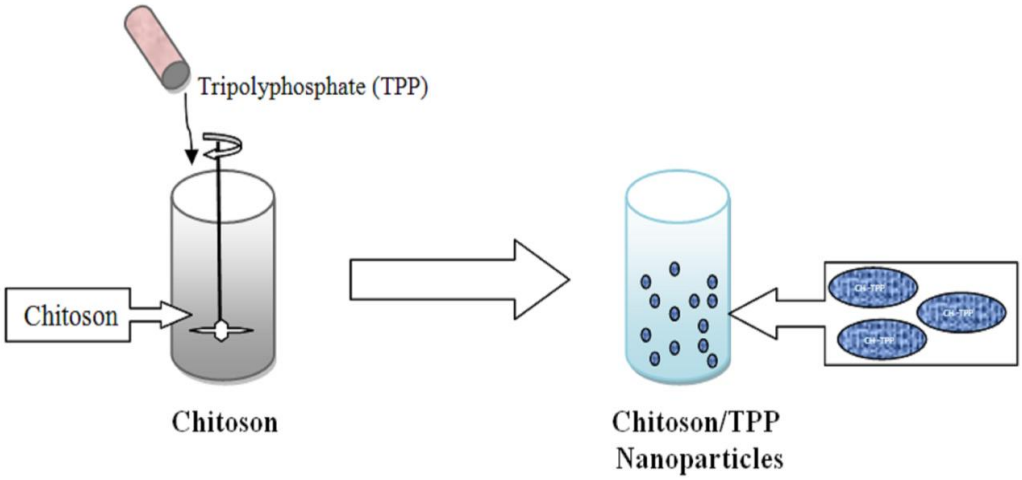


FIG.10:IONIC GELATION AND POLYELCTROLYTE COMPLEXATION

3.4 Modified ionic gelation with radical polymerization:

Modified ionic gelation is derived from ionic gelation with introduction of acrylic acid monomers. The method involves addition of aqueous monomer solution of acrylic or methacrylic acid with aqueous solution of oppositely charged chitosan. Polyethylene glycol or polyether was also added in some cases either separately into monomer solution or following mixing with chitosan. Ionic interactions between the opposite charges of chitosan and acrylic/methacrylic acid occurs and radical polymerization of acrylic/methacrylic acid is initiated by the addition of potassium persulfate. Polymerization reaction lasts for six hours under nitrogen stream with temperature raised from 60-70^{0}C. Formed nanoparticles were allowed to settle overnight, centrifuged and washed thereafter with distilled water [81,82,83].

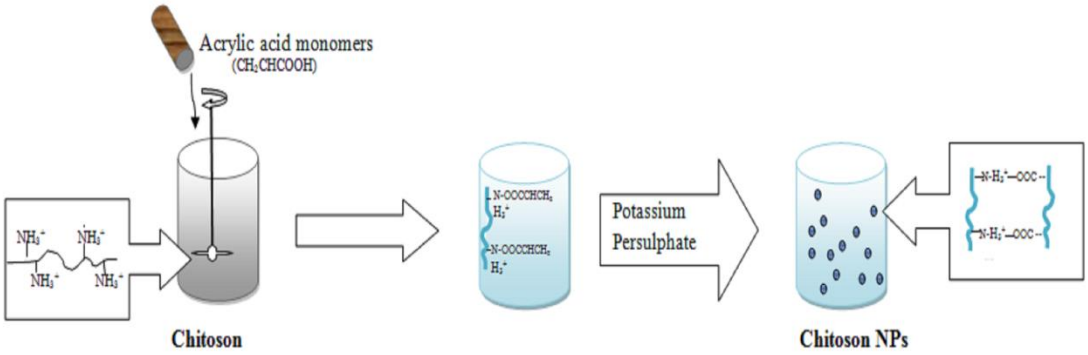


FIG.11:MODIFIED IONIC GELATION WITH RADICAL POLYMERIZATION

3.5 Emulsification and solvent diffusion:

Emulsion solvent diffusion is based on the partial miscibility of organic solvent with water. In this method organic solvents (eg: methylene chloride and acetone) containing a hydrophobic drug was added to an aqueous solution containing chitosan and stabilizer (eg lecithin and poloxamer) under stirring. This leads to the formation of O/W type of emulsion which was then subjected to high pressure homogenization[84]. Under reduced pressure methylene chloride was removed thereby acetone diffuses to the aqueous phase reducing chitosan solubility. Nanoparticles were formed upon polymer precipitation and complete diffusion of acetone was favoured by adding additional amount of water. Finally nanoparticles were centrifuged and isolated. This method is suitable for encapsulating hydrophobic drugs with high encapsulation efficiencies. Limited studies on this method revealed that chitosan molecular weight, homogenization rate were expected to affect final properties of the vehicles [84].

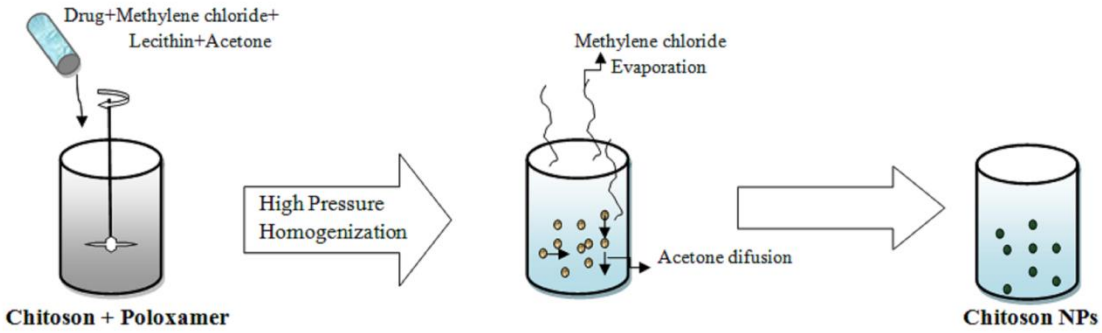


FIG.12:EMULSIFICATION AND SOLVENT DIFFUSION

3.6 Reverse miscellization:

Mitra et al. (2001) reported the production of chitosan nanoparticles in the form of reverse miscelles [85]. Reverse miscelles form W/O type of system in contrast to regular miscelles that form O/W type of system. This method involves the addition of a lipophilic surfactant (cetyl triethyl ammonium bromide or sodium bis(ethylhexyl) sulfosuccinate) to an organic solvent (n-Hexane) to prepare W/O microemulsion.

Chitosan, drug and glutaraldehyde was dissolved in water and added to the organic phase under continuous stirring. At this stage reverse miscelles were produced and nanoparticles were extracted following solvent evaporation [86]. Reverse miscellization has an advantage of producing the nanoparticles with size lessthan 100nm [87].

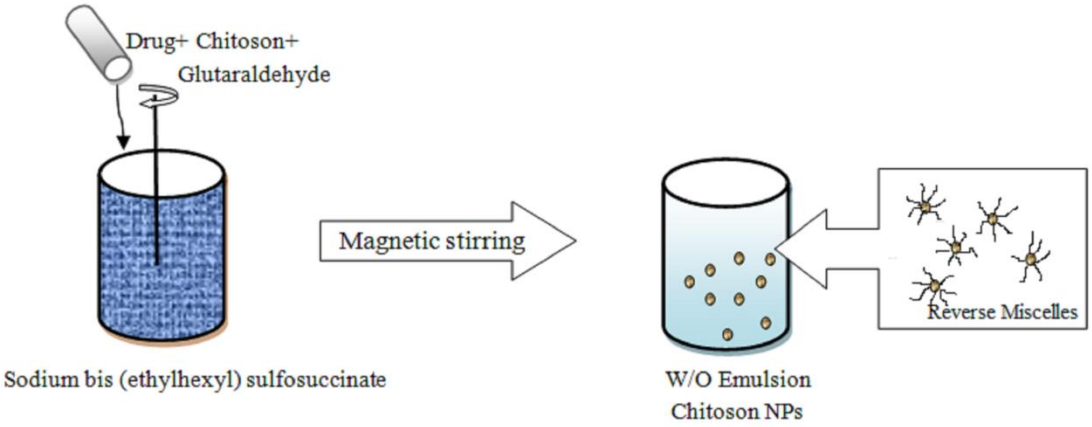


FIG.13:REVERSE MISCELLIZATION

3.7 Precipitation:

This is a quite simple method where chitosan nanoparticles were produced by blowing chitosan solution into an alkaline solution of sodium hydroxide or methanol.Coacervate particles can be formed by blowing the air through compressed air nozzle. Particles were separated by filtration followed by washing with hot air and cold water. Parameters like compressed air pressure, diameter of spray nozzle and the concentration of chitosan affects the particle size and shape [88]. The method may be simple but, particles with weak mechanical strength and irregular morphology may be produced.

3.8 Emulsification and Ultrasonic coalescence:

Ultrasonic W/O emulsion by coalescence and precipitation is a complex process in which two nonstable W/O emusions were prepared. Emulsion with chitosan and emulsion with coagulant was prepared separately by continuous stirring of aqueous phase, oil phase and surfactant at 600RPM for 10 minutes. Both the emulsions were sonicated at 24 kHz amplitude for one minute for the production of stable miniemulsions. W/O miniemulsions of chitosan and coagulant were mixed together while being sonicated with same frequency for 5 minuites. During this process coalescence, precipitation and agglomeration of chitosan nanospheres occurs and centrifuged at 4200 RPM. The chitosans were collected and washed with distilled water [89].

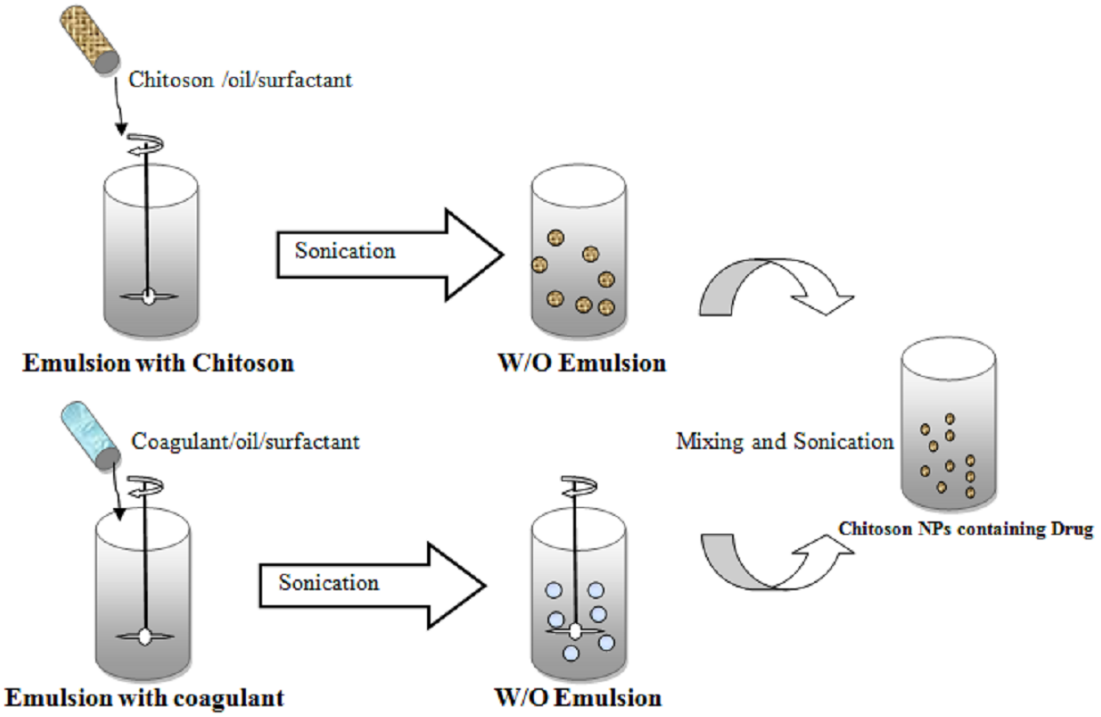


FIG.14:EMULSIFICATION AND ULTRASONIC COALESCENCE

TABLE 2:CHITOSAN NANOPARTICLES PREPARED BY VARIOUS METHODS.

Production Method	Drug	Matrix composition	Therapeutic Use
Ionic gelation	Rifaximin [90]	Chitosan –TPP	IBD
	Resveratrol [91]	Chitosan-Tricarballic acid	IBD
Emulsion droplet coalescence	Gadopentetic acid [67]	Chitosan	Cancer
	5-Fluorouracil [92]	Chitosan, Eudragit S-100	Cancer
Emulsification cross linking	Paclitaxel [93]	Chitosan	Cancer
Ultrasound mediated emulsification	Eugenol [94]	Chitosan	Anti-bacterial
Modified ionic gelation with radical polymerization	Oral Insulin Delivery [95]	Chitosan, Fatty/Amino acid with N-Isopropyl acrylamide and 3-aminopropyltriethoxyxilane	Anti-diabetic
Emulsification and solvent diffusion	Cyclosporin – A [84]	Chitosan – Lecithin, Poloxamer 188.	Immunosupressant
Reverse miscellization	Levofloxacin [96]	Chitosan – Acetic acid, Poloxamer 188.	Antibiotic

Reverse miscellization	Chitosan nanoparticles [97]	Chitosan-glutaraldehyde-sodium bis(ethylhexyl) sulfosuccinate	Antimicrobial/Delivery agent

4. CORTICOSTEROIDS IN THE TREATMENT OF IBD:

Various corticosteroids in the form of prednisone, oral prednisolone, budenoside, methylprednisolone etc were used in the treatment of IBD. These are potent antiinflammatory agents that have no role in maintenance therapy either for ulcerative colitis and crohn's disease. Corticosteroids act through inhibiting the inflammatory pathways like induction of IkB (IkB kinase) that stabilizes NK- kB (Nuclear factor–kappa-light chain enhancer of activated B-cells) complex, supressing interleukin transcription, supression of arachidonic acid metabolism and stimulation of apoptosis of lymphocytes present in the lamina propria of gut [98].

4.1 Efficacy in Ulcerative colitis and Crohn's disease: (Coricosteroids):

Starting at 40 mg daily, oral prednisolone induced remission in 77% (sample size-118) of patients with mild to moderate UC in two weeks.Oral and rectal steroids as combination were found to have better therapeutic efficacy and adverse effects were seen at a dose of 60mg daily. Rapid reduction of prednisolone dose leads to relapse and doses lessthan 15 mg/day was found to be ineffective in the treatment of UC [99-101]. Budenoside was also effective for treating mild to moderate left sided and extensive colitis [102].

Starting at 0.5 to 0.75 mg/kg/day, Prednisone induced remission in 60% (sample size-162) of the patients with CD in the trail conducted by The National Co-operative Crohn's Disease Study for about 17 weeks [104]. Prednisone induced remission in 83% (sample size- 105) of the patients in the trail conducted by European Co-Operative Crohn's Disease Study for about 18 weeks [103]. Budenoside is the best alternative for active ileo- ascending colonic disease [105-107]. Another study conducted for 12 weeks explains that oral budenoside (9 mg/day CR) is effective and remission occurred in over 60% (sample size- 178) of patients when compared to prednisolone (40 mg/day CR) with a remission of 42% [108].

TABLE 3:EFFICACY OF CORTICOSTEROIDS IN IBD

Severity	Type of IBD	Tested Interventions	No of Patients (n)	% of Remission	Period of study
Short Term-(Mild to Moderate)	**CD**	BUD 9 mg Daily	80	48	
		BUD 4.5 mg	79	53	8 Weeks
		Placebo [109]	41	33	
	CD	BUD 9 mg Daily	58	60	
		BUD 4.5 mg BID	61	42	8 Weeks
		PREL 40 mg Daily and tapered [108]	58	60	
	CD	BUD 3 mg TID	34	56	8 Weeks
		PREL 48 mg Daily and tapered [110]	33	72.7	
	CD	BUD 3 mg TID	100	51	8 Weeks
		PREL 40 mg Daily and tapered [111]	101	52.5	

Short Term – (Moderate to Severe)	**UC**	PRED 60 mg	20	65	5 Weeks
		PRED 40 mg	20	65	
		PRED 20 mg TID [112]	20	30	
	CD	Methyl Prednisolone 40 mg/day (8 mg/week tapered)	47	83	
		Sulfasalazine 3 gm/day	54	50	18 Weeks
		Methyl Prednisolone 40 mg/day+ Sulfasalazine 3 gm/day [103]	56	78	
Long Term	**CD**	Methyl Prednisolone 8 mg/day	113	57 and 42	1 and 2 Year
		Sulfasalazine 3 gm/day	117	43 and 33	
		Methyl Prednisolone 8mg/day + Sulfasalazine 3 gm/day	112	50 and 39	
		Placebo [103]	110	30 and 22	
	UC	Cortisone 100 mg/day– 6 weeks	109	48.8	9 months
		Or Cortisone 100 mg/day–3 weeks tapered to 50-75 mg/day for 6 weeks			
		Placebo [113]	101	21.1	

* BUD-Budenoside, PREL – Prednisolone, PRED- Prednisone, BID-Twice Daily, TID-Thrice Daily.

5. AMINOSALICYLATES IN THE TREATMENT OF IBD:

Sulfasalazine, Balsalazide, Mesalamine and Olsalazine are anti-inflammatory compounds that contain 5-aminosalicylic acid (5-ASA). These can be given orally or rectally to decrease inflammation at the wall of intestine. Aminosalicylates can be used to treat ulcerative colitis and maintain remission but may not be effective in treating Crohn's disease. Aminosalicylates works with a mechanism of releasing the lipid mediators, inflammatory cells, cytokines and reactive oxygen species to act on epithelial cells.Asacol®, Salofalk®, Pentasa®, Mezavant XL®, Ipocol®, Mesren®, Salazopyrin®, Dipentum®, Colazide® etc were the marketed oral aminosalicylates that releases the drug by various mechanisms [114-116].

5.1 Efficacy in Ulcerative colitis and Crohn's disease: (Aminosalicylates):

Some of the studies demonstrate that rectal 5-ASA showed maximum percentage of remission in distal ulcerative colitis when compared to rectal steroids [117]. Combination of oral and topical preparations of mesalazine was found to be more effective due to the increased colonic concentrations of 5-ASA, when compared to oral preparation alone [118]. Therapeutic advantage of sulfasalazine for maintaining remission was influenced by tolerability where mesalazine was tolerated by 80% of patients when compared to sulfasalazine [119,120]. One of the evidence suggest that once daily dose is effective when compared to multiple dosing where efficacy depends mainly on prescribed dose than the delivery system [121].

Sulfasalazine at a dose of 4-6 gm/day was found to have some benefit in CD to some extent in an investigation conducted by National Co-operative Crohn's Disease Study [126].

Table 4:Efficacy of Aminosalicylates in IBD

Type of IBD	Tested Interventions	No of Patients (n)	% of Remission Complete/Partial	Period of study
UC	5-ASA 4.8 gm/day 5-ASA 1.6 gm/day Placebo [122]	87	24/50 5/13	6 Weeks
UC	Mesalamine 1.6 gm/day Mesalamine 2.4 gm/day Placebo [123]	158	43 49 23	6 Weeks
UC	Mesalamine 0.8 gm/day Mesalamine 1.6 gm/day Placebo [124]	68 58 63	58.8 65.5 39.7	6 Months
CD	Mesalamine 4 gm/day Placebo [125]	310	43 18	16 Weeks
CD	Sulfasalazine 4.7 gm/day Placebo [126]	569	43 30	17 Weeks

CONCLUSIONS:

Chitosan is an ideal drug carrier for controlled/targeted drug delivery due to its biodegradability, biocompatibility, ability to open tight junctions with greater flexibility and water solubility. Even thoughchitosan nanoparticles can be manufactured using various methods, ionic gelation was found to be the best suitable method according to the literature. Using large quantities of organic solvents in other methods madechitosan nanoparticles more toxic and unstable. Anti-inflammatory efficacy of corticosteroids was effective in short-term and long-term ulcerative colitis and crohn's disease, whereas aminosalicylates were found to be more effective in treating ulcerative colitis when compared to crohn's disease.

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