

**FORMULATION AND EVALUATION OF DOSAGE FORM  
OF SILVER BHASMA FOR COLON TARGETED DRUG  
DELIVERY**

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## DECLARATION

I declare that the thesis entitled “Formulation and evaluation of dosage form of silver bhasma for colon targeted drug delivery” has been prepared by me under the guidance of Dr. Vidhu Aeri, Associate Professor of Faculty of Pharmacy, Jamia Hamdard and Dr. Yashwant, Associate Professor of School of Pharmaceutical Sciences, Lovely Professional University. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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## ABSTRACT

Cancer is one of the leading cause of death world-wide. The present first line therapy for cancer involves surgery followed by radiation therapy and chemotherapy. The usefulness of therapy is directly associated with the treatment capacity to target and kill cancer cells without affecting healthy cells. Unfortunately this strategy produces severe side effects caused by chemotherapeutic agents. The patient discontinues the treatment either due to intense side effects or very high cost of therapy. This fact triggers researcher to search for chemotherapeutic agents which may be economical with less or no side effects. In last few decades it was reported by scientists that silver nanoparticles possess cytotoxic, antiproliferative and antiangiogenic activity. Moreover it is also reported that it possess activity against ulcerative colitis and colon cancer.

Raupya bhasma is the ancient concept of nanomedicine and used as immunomodulator but no report is available regarding its activity against colon cancer. The safety of raupya bhasma at therapeutic dose is already confirmed by its use since ancient time. The Maximum Tolerated Dose (MTD) of raupya bhasma was reported to be more than 1.5g/kg and lethal dose (LD<sub>50</sub>) was 2.0g/kg. However the therapeutic dose of raupya bhasma for adult human being is only 100mg/ day.

To consider this, it was decided to carry out the screening activity of raupya bhasma against colon cancer and then development of doses form for targeting the colon.

Raupya bhasma was prepared by traditional method and standardised using traditional method as well by modern analytical techniques. Anti-inflammatory activity of raupya bhasma was evaluated using denaturation of protein assay method. Activity of raupya bhasma was also evaluated against colon cancer using HCT116 cell lines. Further, microsphere of raupya bhasma and coated granules of raupya bhasma were prepared and evaluated for colon targeted drug delivery.

Raupya bhasma was found to be of nanorange and crystalline in nature. Anti-inflammatory activity of raupya bhasma was found to be better than diclofenac sodium. Activity of raupya bhasma against colon cancer was found to be better than 5-Fluorouracil (5-FU). Microsphere and coated granules deliver the drug to colon successfully but coated granules shows better release profile of raupya bhasma to colon.

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## LIST OF ABBREVIATIONS

<b>Abbreviation</b>	<b>Description</b>
%	Percentage
°C	Degree celcius
CO <sub>2</sub>	Carbon dioxide
CD	Crohn's disease
CTDD	Colon targeted drug delivery
CTDDS	Colon targeted drug delivery system
etc.	et cetera/and other things
GG	Guar gum
GI	Gastrointestinal
GIT	Gastrointestinal tract
h	Hour
ICP-MS	Inductive coupled plasma mass spectroscopy
min	Minute
mg	Milli gram
ml	Milli litre
nm	Nano meter
np	Nanoparticles
pH	Potential/concentration of hydrogen ion
RB	Raupyra bhasma
rpm	Revolutions per minute
USP	United states pharmacopeia
Vol	Volume
UC	Ulcerative colitis
W/V	Weight by volume
W/W	Weight by weight
XG	Xanthan gum
IBD	Inflammatory bowel disease
PBS	Phosphate buffer solution
CFU	Colony forming units
NDM	Non-fat dry milk
5-FU	5- Fluoro-uracil

## LIST OF PUBLICATIONS

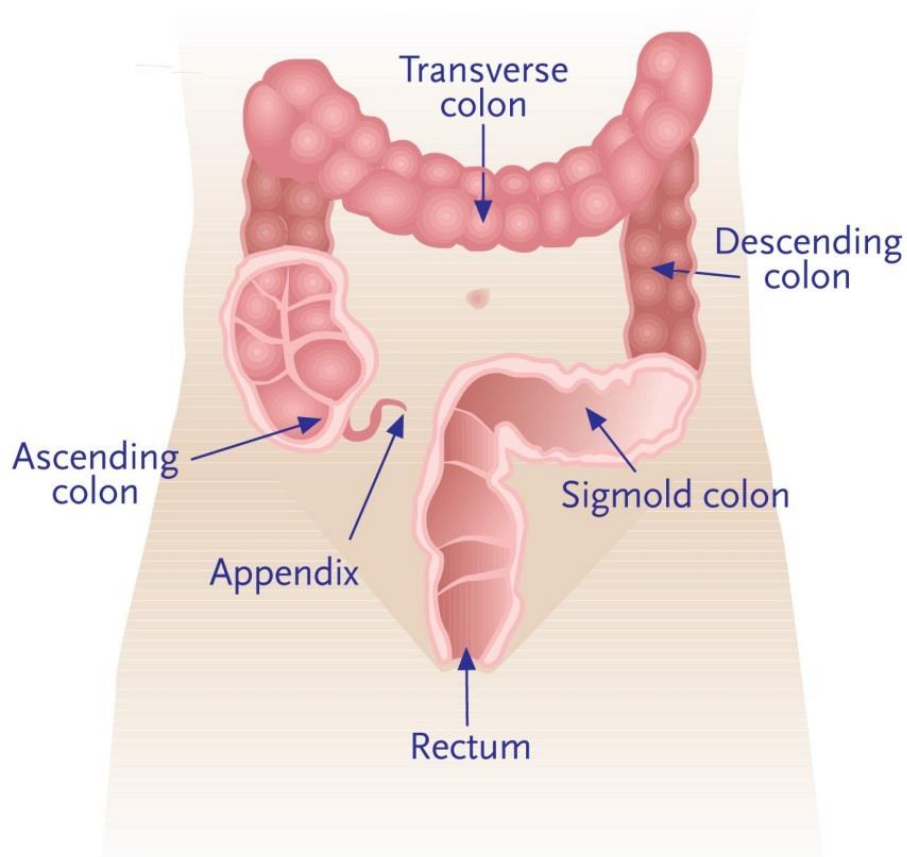
1. Shyam Baboo Prasad, Vidhu Aeri, Yashwant. Current Understanding of Synthesis and Pharmacological Aspects of Silver Nanoparticles, American Journal of Phytomedicine and Clinical Therapeutics 2013; 1 (7) 536-547.
2. Shyam Baboo Prasad, Yashwant, Madhurima Bhargava, Vidhu Aeri. Development of quality standards of ancient silver based nanomedicine: raupya (silver) bhasma. Indo American Journal of Pharm Research 2013;3(10) 8205-8210.
3. Shyam Baboo Prasad, Yashwant, Vidhu Aeri. Formulation and Evaluation of Dosage form of Raupya (Silver) bhasma for colon targeted drug delivery, American Journal of Pharmatech Research 2013; 3 (6) 318-326.
4. Shyam Baboo Prasad, Yashwant, Vidhu Aeri. In vitro anti-inflammatory activity of Raupya (Silver) Bhasma, Journal of chemical and pharmaceutical research 2013; 6 (9) 194-197.
5. Shyam Baboo Prasad, Aeri V, Yashwant. Bhasma: Traditional Concept of Nanomedicine and Their Modern Era Prospective. International Journal of Pharmaceutical and clinical research 2013; 5 (4) 150-154.
6. Shyam Baboo Prasad, Aeri V, Yashwant. Approaches of Colon targeted drug delivery. International journal of drug delivery and technology 2013; 3: 1: 8-11.
7. Shyam Baboo Prasad, Vidhu Aeri, Yashwant, "Role of Traditional and Alternative Medicine in Treatment of Ulcerative Colitis" International Journal of Pharmacognosy and Phytochemical Research 2013; 5(3); 248-253.
8. Shyam Baboo Prasad, Hitesh Verma, Vidhu Aeri, Yashwant, "Probiotics: A Medieval To Modern Era Prospective" International Journal of Toxicological and Pharmacological Research 2013; 5(3): 63-68.



# 1 INTRODUCTION

## 1.1 Colon

The gastro intestinal tract (GIT) consists of stomach, small intestine and large intestine. The large intestine (covering from the ileocecal junction to the anus) is divided in to three main parts i.e. colon, the rectum and anal canal<sup>1</sup>. The length of colon is about 59inch long, and has five major segments (Figure 1.1)<sup>1,2</sup>.



**Figure 1.1: Parts of Colon**

The ascending colon to proximal transverse colon develops embryologically from the midgut and the distal transverse colon to sigmoid colon develops from the hind gut. While observing the plain abdominal radiographs, the colon is seen to be filled with fecal material along with air.

## **1.1.1 Parts of Colon**

### **1.1.1.1 Ascending Colon**

It lies vertically in the utmost lateral right part of the abdominal cavity. The proximal blind end (pouch) of the ascending colon is known as caecum. The ascending colon takes a right-angled turn just below the liver (right colic or hepatic flexure) and converts to transverse colon, which has a horizontal course from right to left<sup>2</sup>.

### **1.1.1.2 Transverse Colon**

Just below the spleen transverse colon turns to right angle and becomes the descending (left) colon, which lies vertically in the most lateral left part of the abdominal cavity. The descending colon lead to the sigmoid colon (inverted V-shaped), which then becomes the rectum<sup>2</sup>.

### **1.1.1.3 Paracolic gutters**

Paracolic gutters lies to lateral to ascending and descending colon are the right and left of the peritoneal cavity, through which fluid/pus in the upper abdomen can trickle down into the pelvic cavity. The ascending and descending colon are related to the kidney, ureter, and gonadal vessels of the corresponding side that lie behind them in the retroperitoneum; the ascending colon is also related to the duodenum<sup>2</sup>.

### **1.1.1.4 Sigmoid Colon**

The transverse colon and the sigmoid colon have a mesentery (ie, transverse mesocolon and sigmoid mesocolon, respectively), but the ascending colon and descending colon are retroperitoneal, while the cecum is intraperitoneal but uses the mesentery of the ileum. The transverse mesocolon base lies horizontally across the duodenum and pancreas. The greater omentum has several parts, including the 4 layered omental apron hanging down off of the transverse colon and the 2-layered gastrocolic ligament connecting the greater curvature of the stomach and the transverse colon.

Three longitudinal teniae coli are present in the cecum, ascending colon, transverse colon, descending colon, and sigmoid colon; they are not present in the rectum. In the ascending and descending colon, they are present anteriorly and on the posterolateral and posteromedial aspects. Appendages of fat, containing small blood vessels, called omental appendages (appendices epiploicae) are attached to colon<sup>2</sup>.

### **1.1.2 Blood Supply**

One of the major function of the colon is to provide environmentally friendly condition for the growth of colonic micro flora, absorption of potassium and water from the lumen as storing reservoir of faecal matter and discharge of its contents. The capacity of absorption of colon is very high. Approximate 2000 ml of fluid pass in the colon via the ileocecal valve among which more than 90% of the fluid gets absorbed. As per an estimate the colon holds only near about 220 g of wet material comparable to 35 g of dry matter. The major component of this dry matter consists of bacteria. The colon tissue contains the villi, lymph, muscle, nerves, and vessels<sup>2</sup>.

### **1.1.3 Common disorders of Colon**

The severity of colorectal diseases may vary from mildly irritating to life threatening and comprise a broad range of conditions and ailments. Due to lack of awareness many patients did not take right medication in early stage. It is proven that screening and treatment of colorectal disease in early stage lead to increase in rate of survival<sup>1,2</sup>.

#### **1.1.3.1 Colorectal Cancer**

Cancer of colon and rectum is called colorectal cancer. More than 90 percent of patients suffering from colorectal cancer are elderly (over 40 years). As an estimate one lakh forty thousand people strikes with colorectal cancer which may lead to death of sixty thousand patients every year. Colorectal cancer can be detected by chemical test of stool and digital rectal examination<sup>2</sup>.

If symptoms like bowel habits and rectal bleeding appear, the patient is advised to visit a colon and rectal surgeon for consultation. It may determine if the patient suffering from bowel disease or is a case of colon cancer. For complete cure, surgery is required in almost all cases of colorectal cancer<sup>2</sup>.

#### **1.1.3.2 Ulcerative Colitis**

Inflammation of colon is called ulcerative colitis. Chronic colitis may lead to colon cancer and affects about five lakh people worldwide, mainly under age of 30.

### **1.1.3.3 Crohn's Disease**

Chronic inflammatory condition of intestinal tract is called Crohn's disease. It affects young adult between 16-40 years of age. Crohn's disease is predominantly reported in developed countries (industrially advanced) mainly prevailed in United States and northern Europe. It is diagnosed by physical examination, barium X-ray of upper and lower intestinal tract and sigmoidoscopy or colonoscopy<sup>2</sup>.

### **1.1.3.4 Irritable Bowel Syndrome (IBS)**

It is a common intestinal muscle functioning disorder which affects more than 30 percent of the American population at some point of time, the data of Indian population is not verified but is expected that same percentage of Indians are also affected by the syndrome. It involves a cumulative symptoms of constipation, diarrhea or combination of both accompanied by pain. Sometimes the situation is life threatening<sup>2</sup>.

### **1.1.3.5 Diverticular Disease**

This is yet another type of colon related disorder and affects mostly the elderly patients. In the conditions there is a formation of some sort of pockets called diverticula on the wall of colon. The patients with age between 60-80 years of age, are at risk of diverticular disease.

With routine colon and rectal examinations, diverticula can be detected and diverticular disease may be prevented.

### **1.1.3.6 Hemorrhoids**

Millions of patients currently suffering from hemorrhoids, which is one of the most common colorectal ailment. It effects more than 50 % the world population and the development of hemorrhoids, usually takes place after the age of 30.

If there is a formation of hard sensitive lumps then it may be a case of external hemorrhoids. These become painful when the blood clot develops in them.

The internal hemorrhoids grow inside the anus, underneath the linings and are well-known by painless bleeding and protrusion during the movements of bowel. It may be due to overuse of enema or laxatives. It also may result with the habit of spending long

period of time on toilet seat. It also may occur during pregnancy, chronic constipation and diarrhea.

#### **1.1.3.7 Anal Fissure**

These are small tears inside layer of the anus caused by hard, dry bowel movements, inflammation or diarrhea of the anorectal area. The diagnosis can be done by checkup following pain, hemorrhage and/or itching of the outer area of anus.

The disease can be cured by use of stool softeners, taking care of constipation and/or soaking in sitz bath (warm water).

#### **1.1.3.8 Bowel Incontinence**

Bowel incontinence is a condition in which the capability to control stool or gas release gets reduced due to weakened anal muscle caused by nerve or muscle injury. This type of problem are generally prominent in old age. Some women also suffers from bowel incontinence due to child birth.

It can be overcome by use of medicament meant for constipation, dietary changes and easier home exercises to toughen muscles. In some of the cases, biofeedback may be used to support patient sense when stool is ready to be evacuated. Weak anal muscles can be repaired with surgery.

#### **1.1.4 Ulcerative colitis**

Inflammation of GIT is known as inflammatory bowel disease (IBD). It is a broad term used for a group of chronic inflammatory disorders involving the gastrointestinal tract. The etiology of IBD is still unclear. There are two major types of the condition, Crohn's disease (CD) and ulcerative colitis (UC). These can be clinically characterized by repeated inflammatory participation of intestinal sections with numerous manifestations often resulting in an unpredictable course<sup>3</sup>. Ulcerative colitis is the inflammatory condition of colonic mucosa of unknown etiology. In its most restricted form, ulcerative colitis may be limited to the distal rectum, however in its most comprehensive form the entire colon is involved. More than 80% of the patients present with disease spreading from the rectum to the splenic flexure, and about 20% have pancolitis<sup>4</sup>. The epidemiology, natural history, diagnosis and treatment contributed significantly in current few years.

#### **1.1.4.1 Symptoms**

Initial symptoms of ulcerative colitis include diarrhoea, blood in stool, pain, weight loss, arthralgia, fever, loss of appetite, ophthalmopathies, nausea, vomiting, abscesses, fistulae and lymph node swelli<sup>5</sup>. Symptoms of mild, moderate and chronic UC is reported in table 1.1.

**Table 1.1: Symptoms of mild, moderate and chronic UC**

Symptoms	Chronic	Moderate	Mild
Stool frequency per day (mostly bloody)	> 6	4–6	< 4
Temperature (° F)	> 100	99–100	Normal
Pulse (beats/min)	> 100	90–100	< 90
Weight loss (%)	> 10	1–10	None
Albumin (g/dL)	< 3	3–3.5	Normal
Haematocrit (%)	< 30	30–40	Normal
Erythrocyte sedimentation rate (ESR) (mm/h)	> 30	20–30	< 20

#### **1.1.4.2 Epidemiology**

Ulcerative colitis is linked with repeated attacks with complete remission of symptoms in the interim. The disorder is more common in certain type of population as compared to others. Caucasians are more effected than Blacks or Orientals. Surprisingly the Jew population has a three to six fold risk of the disease. Both sexes are equally affected. The highest occurrence of both diseases (UC and CD) is among ages 15 and 35, it has been reported in almost every span of life. A hereditary incidence of IBD is under recording of occurrences. In Asia, Africa and South America, cases of UC is reported less as compared to European country. Breast feeding, smoking and appendectomy are associated with reduced rick of UC. Depression, western diet, left-handedness may increase risk of UC<sup>5,6</sup>.

#### **1.1.4.3 Pathophysiology**

The cause of UC still remains unclear. The major pathophysiology involved in UC is inadequate regulation or over stimulation of mucosal immune system. So, the emphasis

should be given to study of immunological reactions or mucosal inflammation. In active form of disease, the mucosa of lamina propria getshighly infiltrated with acute and chronic inflammatory cells. There is a predominant escalation in mucosal Immunoglobulin G (IgG) production, indication of complement activation, and stimulationof macrophages and T cells. The above mentioned immunological activity is connectedthrough the discharge of a vast array of leukotriene, platelet activating factor (PAF), cytokines, kinins and reactive metabolites of oxygen. Discharge of these mediators not only limited to intensify the immune and inflammatory response, but they also play a major role and have direct effects on epithelial function, on endothelial function (which may increase permeability and lead to ischemia), and on repair mechanisms, thus increasing collagen synthesis. On the other hand, many of the cytokines (interleukins 1 and 6, tumour necrosis factor) will trigger an acute phase response which may lead to fever and a rise in serum acute phase proteins<sup>7-9</sup>.

#### **1.1.4.4 Diagnosis**

The diagnosis of UC is made on clinical suspicion. It can be confirmed by stool examinations, biopsy, barium radiographic examination, sigmoidoscopy or colonoscopy<sup>5</sup>.

#### **1.1.4.5 Complication**

Foremostobstacles of UC comprise toxic mega colon, intestinal perforation, and massive bleeding. Toxic mega colon is characterized by a sepsis-like syndrome and extensive distension of the colon (>6 cm). Chronic blood loss lead to microcytic anaemia. Complication of chronic ulcerative colitis may lead to colon cancer. The risk for cancer are generally seen after ten years of colitis.

#### **1.1.4.6 Medication**

There is no an effective medicine to cure the UC but the mainstream treatment depends on reduction of the abnormal inflammation in the colon lining and thereby relieves the symptoms of diarrhoea, rectal bleeding, and abdominal pain. The treatment depends on the severity of the disease; therefore treatment is adjusted for each individual<sup>10</sup>. Most people with mild or moderate ulcerative colitis are treated with corticosteroids (dexamethasone) to reduce inflammation and relieve symptoms<sup>11</sup>. Near about 25% of patients with UC using steroids become steroid-dependent after one year, and virtually

all develop steroid-related adverse events<sup>12</sup>. Other drugs as immune modulators (azathioprine and 6-mercaptopurine) that reduce inflammation by affecting the immune system and amino salicylates are available<sup>13</sup>. However, the side effects associated with amino salicylates is typically accompanied with adverse side effects such as dizziness, nausea, changes in blood chemistry (including anaemia and leukopenia) and skin rashes<sup>14</sup>.

### **1.1.5 Traditional and alternative medicine in treatment of ulcerative colitis**

Proanthocyanidins isolated from grape seed were investigated for their activity in the healing of recurrent ulcerative colitis (UC) in rats<sup>15</sup>. Another study confirmed this fact as, Proanthocyanidins is useful in anti-inflammatory activity in case of the acute phase of 2,4,6-trinitrobenzenesulfonic acid (TNBS) induced colitis in rats<sup>16</sup>.

The administration of alcohol extract of *Garcinia cambogia* (Clusiaceae) in TNBS-induced colitis rats improved significantly the macroscopic damage and caused considerable reductions in myeloperoxidase (MPO) activity and Cyclooxygenase-2 (COX-2) expression. In addition, *Garcinia cambogia* extract was able to decrease prostaglandin E2 (PGE2) and IL-1<sup>17</sup>.

*Zingiber Officinale* (Zingiberaceae) extract was evaluated for anti-ulcerative colitis activity. Activity against UC showed a prominent effect of ginger extract against acetic acid-induced ulcerative colitis. The effect may be possible due to antioxidant and anti-inflammatory properties of extract of *Zingiber Officinale*<sup>18</sup>.

The protective effects of *Angelica sinensis* (Apiaceae) polysaccharides could be explained partially by oxidative stress and glutathione (GSH) depletion<sup>19</sup>.

The effect of polysaccharide obtained from *Rheum tanguticum* (Polygonaceae) on hydrogen peroxide-induced human intestinal epithelial cell injury and it was found that, Pre-treatment of the cells with RTP could significantly elevate cell survival. *Rheum tanguticum* polysaccharide may have cytoprotective and anti-oxidant effects of *Rheum tanguticum* polysaccharide for the treatment of ulcerative colitis in rats<sup>20</sup>.



*Camellia sinensis* (Theaceae) extract was found to be effective in the treatment of ulcerative colitis. Both diarrhoea and loss of body weight can be significantly attenuated by the treatment with *Camellia sinensis* extract<sup>21</sup>.

Aqueous extract of root of *Withania somnifera* (Solanaceae) showed anti-oxidant activity by reducing (Hydrogen peroxide) H<sub>2</sub>O<sub>2</sub> and (Nitric oxide) NO.<sup>22</sup>

Glycoprotein isolated from *Gardenia jasminoides* has reported effective in (Dextran sodium sulphate) DSS induced UC in mice<sup>23</sup>.

The ethanol extracts of *Ficus bengalensis* (Moraceae) may lead to decrease disease activity index and colon mucosal damage index in rats with inflammatory bowel disease<sup>24</sup>.

*Patrinia scabiosaefolia* (Valerianaceae) are commonly used in anti-inflammatory diseases, mainly for colonic inflammations, hepatitis and other virus infections<sup>25</sup>.

*Avicennia marina* (Acanthaceae) decreased the glutathione peroxidase, lipid peroxides of colon, and serum nitric oxide<sup>26</sup>.

Dried seeds aqueous extract of *Benincasa hispida* (Cucurbitaceae) possess prominent antioxidant activity in a dose-dependent manner<sup>27</sup>. The aqueous extracts of dried seed produced noteworthy reduction in ulcer index in Wistar albino rats<sup>28</sup>.

Methanol extract of leaves of *Rhodomyrtus tomentosa* has been investigated by researcher on the production of inflammatory mediator's Nitrous oxide and prostaglandin E<sub>2</sub>. The methanol extract of leaves of *Rhodomyrtus tomentosa* mediated inhibition, as well as target enzymes, were studied with RAW264.7 peritoneal macrophage, and HEK293 cells to determine molecular mechanism. In addition, the in vivo anti-inflammatory activity of this extract was also carried out with mouse gastritis and colitis models. Methanol extract of leaves of *Rhodomyrtus tomentosa* clearly inhibited the generation of NO and PGE<sub>2</sub> in lipopolysaccharide activated RAW264.7 cells and peritoneal macrophages in a dose-dependent manner<sup>29</sup>.

*Berberis vulgaris* fruit extract (BFE) with three different doses (375, 750, and 1500 mg/Kg) was administered orally or rectally prior to ulcer induction. Berberine chloride

(BEC) (10 mg/Kg), prednisolone (5 mg/Kg), hydrocortisone acetate enema (20 mg/Kg) and normal saline (5 mL/ Kg) were considered as respective controls. The tissue was assessed macroscopically for damage scores, area, index and weight/length ratio. They were also examined histopathologically for inflammation extent and severity, crypt damage, invasion involvement and total colitis index. Results indicated that greater doses of oral BFE (750, 1500 mg/Kg) as well as BEC (10 mg/Kg) were effective to protect against colonic damage. By rectal pre-treatment, the extract was only effective to diminish the ulcer index and the efficacy was not significant for mucosal inflammation parameters. In conclusion BFE, which is nearly devoid of berberine, was effective to protect against colitis and this might be attributed to its anthocyanin constituents<sup>30</sup>.

UC has a lesser prevalence in smokers than non-smokers. Studies using a transdermal nicotine patch have shown clinical and histological improvement<sup>31</sup>.

Curcumin possesses marked activity against ulcerative colitis and Crohn's disease<sup>32</sup>.

During clinical studies, it has been proved that *Aloe vera* is effective and safe for the treatment of ulcerative colitis<sup>33</sup>.

*Bromelain* is a proteolytic enzyme and is found effective in UC. It shows improvement of histologic and clinical severity of colonic inflammation for a murine colitis model of IL-10-deficient mice<sup>34</sup>.

During double-blind clinical trials, it has been reported that the *Psyllium* seeds possess marked activity against ulcerative colitis<sup>35</sup>.

*Guggulsterone* is found effective against DSS-induced murine colitis as evaluated by colon length, histology and clinical disease activity score<sup>36</sup>.

Diammonium glycyrrhizinate obtained from *Glycyrrhiza glabra* and found effective against inflammation of intestinal mucosal in rats and, prominently, decreases expression of TNF- $\alpha$  significantly in inflamed colonic mucosa<sup>37</sup>.

In a clinical study 30 patients were administered with 900mg of *Boswellia* gum preparation thrice a day and 10 patients were administered with sulphasalazine, 3gm, thrice a day. The *Boswellia* gum was found to be effective against ulcerative colitis<sup>38</sup>.

Tannins and flavonoids are found to be effective in treatment of ulcerative colitis. Green tea polyphenols was reported to possess marked activity against ulcerative colitis<sup>39</sup>.

Silymarin is a flavonoid component obtained from *Silybum marianum*. It is found to be active against ulcerative colitis<sup>40</sup>.

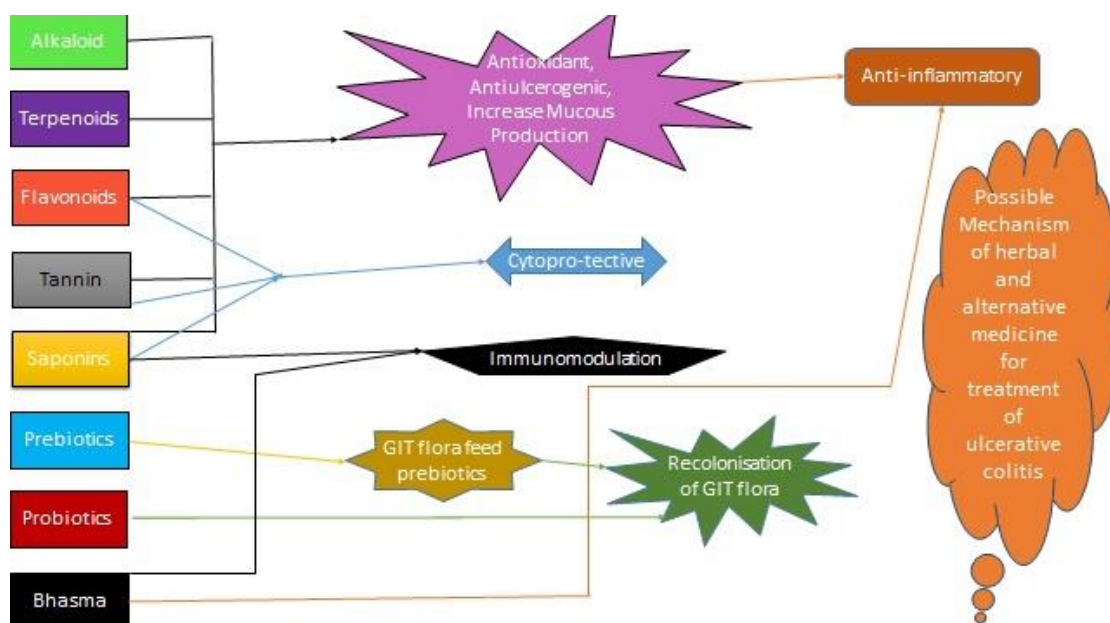
*Terminalia chebula* extract (600 mg/kg) also possess healing activity against acetic acid-induced colonic damage score and weight when administered orally daily for 14 days<sup>41</sup>.

#### **1.1.5.1 Bacterial recolonization**

Alteration in GIT flora may lead to UC. Probiotics as supplement is beneficial in such cases. The available clinical data shows the role of intestinal micro biota in the pathogenesis of IBD and there by provides an evidence that alteration in the intestinal micro biota with the help of probiotics can be helpful in the treatment of disease. E.g. *Bifidobacteria infantis* has been found to reduce the inflammatory response of the gut lining by inhibiting the bacteroides. *Lactobacillus plantarum* has also been reported to be used in IBD. A probiotic formulation containing no of microbes (VSL#3) used in case of ulcerative colitis although its clinical efficacy is not certain. *E. coli* has also been used in case of ulcerative colitis but its clinical efficacy depends upon its dose<sup>42-46</sup>.

#### **1.1.5.2 Iron supplementation**

The gradual loss of blood from GIT often lead to anaemia. Adequate disease control usually improves anaemia of chronic disease, but iron deficiency anaemia should be treated with iron supplements. In Ayurveda lauha bhasma and mandur bhasma is recommended in iron deficiency anaemia<sup>47</sup>.



**Figure 1.2: Proposed hypothesis for mechanism of herbal and alternative medicine for treatment of ulcerative colitis.**

### 1.1.5.3 Conclusion

Herbal and alternative medicine play important role in prophylaxis and cure of UC. As the pathophysiology of UC is still not clear but the possible mechanism of herbal and traditional medicine for treatment of UC is described in Figure 1.2. Alkaloids and terpenoids may be used in Ulcerative colitis because of antioxidant and antiulcerogenic activity. Anti ulcerogenic activity may be due to increase secretion of mucous. Phenolics (flavonoids, tannins) and saponins may act by antioxidant, cytoprotective and antiulcerogenic activity. Saponin and Ayurvedic bhasma may act by immunomodulation and anti-inflammatory activity. Prebiotic and probiotic helps in recolonisation of GIT flora. The proposed hypothesis may trigger the researcher to investigate new medicine which can be used in treatment of ulcerative colitis.

### Traditional and alternative medicine in treatment of ulcerative colitis

The cancer of colon (large intestine) is called as colon cancer. It is a mucosal disease that initiates from caecum and continues throughout up to rectum. Colon cancer is the third most common type of cancer in US. Most of the medicine used for the treatment of cancer have severe side effects. Very few research have been carried out to investigate the role of herbal and traditional medicine especially in colon cancer. Taxol, Etoposide, Vinca alkaloid, Curcumin, Berberine are very few molecule which got attention of scientist but these

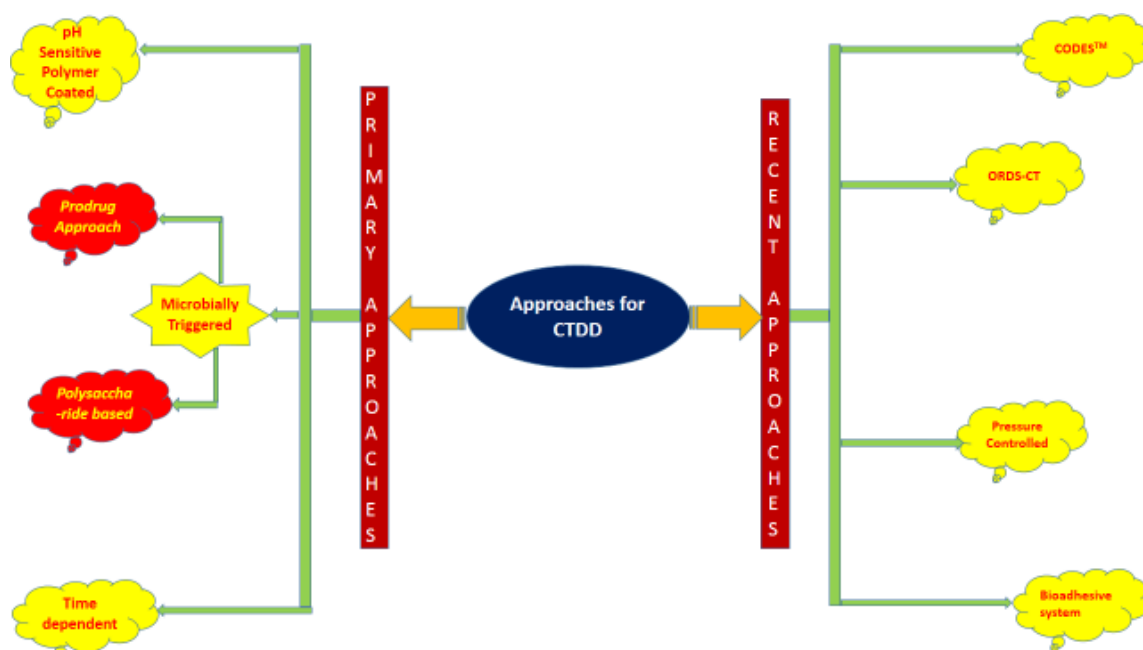
all molecules have major problem of stability and severe toxicity. In last decades it was reported by scientist that bhasma could be good candidate for treatment of cancer.

## **1.2 Approaches for Targeted Drug Delivery to Colon**

The conventional drug delivery system for colonic disease may lead to absorption of drug across biological membrane of gastrointestinal tract (GIT). The absorption of drug throughout GIT may lead to increase in dose and associated side effects. Colon targeted drug delivery (CTDD) is a method of delivering medicament to increases concentration of the medicament in colon relative to other part of GIT. The aim of CTDD is to localize, prolong, target and have protected drug interaction to diseased tissue. The aim of targeted drug delivery (TDD) is effective and selective localization of medicament into the target site at therapeutic dose with restricted or no access to non-target sites. A targeted drug delivery system play important role in drugs having low solubility , instability, short duration of half-life, poor absorption, large volume of distribution, low specificity and narrow therapeutic index<sup>48,49</sup>. Therapeutic efficacy of targeted drug delivery is maximum because it prevents degradation of medicament during transportation to the target site. It can also minimize adverse effects because of inappropriate disposition and minimize toxicity of potent drugs by reducing dose. The colon is a site where both systemic and local delivery of medicament is possible. Local delivery allows topical treatment variety of bowel diseases such as ulcerative colitis, Crohn's disease, amebiasis, colon cancer and local treatment of colonic pathologies<sup>50,51</sup>. The colon targeted drug delivery system can be used for systemic delivery of medicament (protein and peptide drugs)<sup>52</sup>.

### **1.2.1 Primary Approaches for Colon targeted drug delivery**

Primary approaches that are used for colon targeted drug delivery (CTDD) are as follow (Figure 1.3)



**Figure 1.3: Approaches of CTDD**

### 1.2.1.1 pH Sensitive Polymer Coated Drug Delivery

The colon specific drug delivery using pH Sensitive polymer can be achieved as the pH in gastrointestinal tract (GIT) varies. The pH of some colon specific polymer is mention in Table 1.2. This can be accomplished by coating using suitable polymer that are resistant at lower pH of the stomach but that will dissolved/ degrade at neutral pH of the colon. The polymer used for coating should be resistant to the acidic condition of the stomach but gets ionize and get dissolved beyond a definite alkaline pH found in small intestine. Thus by using the same concept it is possible to deliver drugs to the terminal of ileum or colon by use of enteric polymers with a comparatively high threshold pH for dissolution and following drug release. Frequently used enteric polymer for targeting to colon is methacrylic acid and methylmethacrylate that dissolve at pH 6 (Eudragit L) and pH 7 (Eudragit S) have been examined. But the pH of the distal is 6. This colonic delivery system, thus have an inclination to release the drug prior reaching to colon. The problem of premature release can be overcome by using a copolymer of methacrylic acid, methyl methacrylate and ethyl acrylate (Eudragit FS) which gets dissolve at sluggish rate and at higher threshold pH 7 to 7.5 was stated. One must question the impact of gastrointestinal disease on targeting of medicament to colon since patient suffering from ulcerative colitis are known to have distinctly

lower colon pH<sup>53-55</sup>. Polymer used in pH Sensitive Polymer Coated Drug Delivery is shown in Table 1.2.

**Table 1.2: Polymer and their threshold pH for CTDD**

Polymer	Threshold pH
Eudragit® L 100-55	5.5
Eudragit® L-30D	5.6
Eudragit® L 100	6.0
Eudragit® FS 30D	6.8
Eudragit® S 100	7.0
Hydroxypropyl Methylcellulose Phthalate	4.5
Polyvinyl Acetate Phthalate	5.0
Hydroxypropyl Methylcellulose Phthalate 50	5.2
Cellulose Acetate Trimellate	5.0
Hydroxypropyl Methylcellulose Phthalate 55	5.4

### **1.2.1.2 Time dependent drug delivery**

In this approach, drug release takes place after a predetermined lag time. The normal transit time of medicament in the stomach is about 2 hr. which may differ with situations, though in the small intestine it is comparatively constant and may take around 3hr. For targeting of drug to colon, time taken to reach the drug to colon should be similar to lag time (5 h). The lag time of a medicament rely upon GIT motility and the dosage form size. Among all, one of the most primitive methods based on time dependent drug delivery is the Pulsincap device. This Pulsincap device comprises of a non-disintegrating half capsule body which is sealed at the open end with a hydrogel plug, which is enclosed by a water-soluble cap. The complete unit is coated using an enteric polymer to elude the problem of variable gastric emptying. When the capsule passes through the small intestine, the enteric coating gets dissolved and the hydrogel plug begins to swell. The quantity of hydrogel is adjusted so that it pops out only after the stated period of time to release the medicament to colon.<sup>56-59</sup>.

### **1.2.1.3 Microbially Triggered Drug Delivery**

The microflora found in the intestine varies from the range of  $10^{11}$ - $10^{12}$  CFU/mL, which may contain mostly the anaerobic bacteria, e.g. eubacteria, ruminococcus, clostridia, bifidobacteria, bacteroides, enterococci and enterobacteria. Fermentation of these substrates (disaccharides, trisaccharides and polysaccharides) is the energy source of this huge microflora with numerous types of substrates which have been remains undigested in the small intestine. For the purpose of fermentation of undigested food, the microflora produces a huge number of enzymes like aabinosidase, deaminase, azareducatase, xylosidase, galactosidase, nitroreductase glucoronidase and urea dehydroxylase. As the biodegradable enzymes present in the colon, use of biodegradable polymers for colon targeted drug delivery is recognised as a more site precisemethod as compared to other methods. Prodrug approach is another choice of microbially triggered drug delivery as bacteria present in colon produces numerous enzymes which help in biotransformation of prodrugs. These diversity of enzymes, chiefly of which are from bacterial origin present in the colon, are essential<sup>60,61</sup>.

Microbially triggered drug delivery involve prodrug approaches of drug delivery and polysaccharide based drug delivery.

### **1.2.1.4 Prodrug Approach for Drug Delivery**

Prodrug is defined as pharmacologically inert derivative of a parent medicament that needs spontaneous or enzymatic transformation in vivo to release the active drug. Various prodrug have been investigated which are susceptible to bacterial hydrolysis especially in the colon. In prodrug approach drug is attached to hydrophobic moieties like azo linkage, amino acids, glucuronic acids, glucose, glactose, cellulose etc<sup>62</sup>.

Metabolism of azo compounds (Prodrug) by intestinal bacteria is one of the mostcomprehensively studied bacterial metabolic process<sup>63-66</sup>.

Drawbacks of the prodrug approach is that it is not applicable to all types of drug.It depends upon the functional group present on drug moiety for chemical linkage.



### **1.2.1.5 Polysaccharide based drug delivery**

The polymers used in polysaccharide based drug delivery protect the medicament from the surroundings of stomach and small intestine, and are capable to target the drug to the colon. The micro-organism present in colon causes assimilation of polysaccharide based polymer. Microflora of colon produces enzyme that break down of the polymer backbone leading to a consequent decrease in their molecular weight of polymer and thereby loss of mechanical strength. Once the mechanical strength of polymer reduces medicament liberated in colon<sup>67</sup>.

## **1.2.2 Recent approaches for CTDD**

Primary as well as recent approaches of CTDD is shown in Figure 1.3. The recent approaches of CTDD is as follows

### **1.2.2.1 CODES™ technology**

CODES™ is a recent and distinctive colon targeted drug delivery approach which was made to elude or overcome the intrinsic difficulties associated with pH dependent or time dependent drug delivery. CODES™ is a collective tactic of microbially triggered and pH dependent drug delivery system. In this system lactulose play an important role and acts as a trigger for site specific drug release that is in colon. One of the example of configuration of CODES™ comprises of a core tablet which is coated with three layers of polymer using suitable coating technique. The outer layer of unit is composed of a Eudragit® L. Once the unit (CODES™) passes through the pyloric and into the duodenum, Eudragit® L coating dissolves and exposes to second layer of coating. Second layer of coating is made up of Eudragit® E. Eudragit® E coating is resistant in the environment of the small as well as large intestine. The undercoating allows lactulose to get release into the environment adjacent to the tablet. Metabolism of lactulose produces short chain fatty acids which may lead to decrease in pH. Once the pH low to certain level Eudragit E gets dissolves and drug release in colon. In this way CODES™ techniques deliver the drug to colon safely without releasing to non target site<sup>68,69</sup>.

### **1.2.2.2 Osmotic controlled drug delivery (ORDS-CT)**

If targeting of drug to colon is not achieved by other techniques then osmotic controlled drug delivery is the choice. ORDS-CT can be as simple as single osmotic unit or may

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be a combination of as many as 5-6 push-pull units, each one of which may have diameter of 4 mm and encapsulated with in a hard gelatine capsule. OROS-CT units can release medicament with uniform rate up to 24 h in the colon<sup>70,71</sup>.

### **1.2.2.3 Pressure Controlled Drug-Delivery Systems**

The robust peristaltic waves in the colon that lead to a momentarily increased luminal pressure is the basis of pressure controlled drug delivery in colon. Due to pressure in the lumen of colon release of medicament takes place following disintegration of water soluble polymer<sup>71</sup>.

### **1.2.2.4 Bio adhesive system**

This method has been developed upon principle of adhesion between drug and the biological membrane by the virtue of which the medicament remains in contact with particular organ for longer duration. It lead to extended residence time of the drug molecule it tends to high local concentration. This approach can be applied to colon target delivery system. Various polymers employed for bio adhesive system are polycarbophils, polyurethanes, polyethylene oxide and polypropylene oxide<sup>72</sup>.

## **1.3 References**

1. Tortora GJ and Derrickson BH (2008) Principles of anatomy and physiology. Wiley International 12: 922-23
2. <http://www.cancercare.ns.ca/en/home/preventionscreening/coloncancerprevention/faq.aspx>
3. Drisko JA, Giles CK and Bischoff BJ (2003) Probiotics in health maintenance and disease prevention. *Alt Med Rev* 8: 143-155.
4. Packey CD and Sartor RB (2008) Interplay of commensal and pathogenic bacteria, genetic mutations, and immunoregulatory defects in the pathogenesis of inflammatory bowel diseases. *J Int Med* 263: 597-606.
5. Kathleen AH and Julie SJ (2003) Inflammatory bowel disease Part I : Ulcerative colitis- pathophysiology and conventional and alternative treatment option. *Alt med rev* 8: 247-283.
6. Lindberg E, Tysk C, Andersson K and Jarnerot G (1988) Smoking and inflammatory bowel disease. A case control study. *Gut* 39: 352-357.

7. Jewell DP (1989) Aetiology and pathogenesis of ulcerative colitis and Crohn's disease *Postgraduate Medical Journal* 65: 718-719.
8. Tysk C, Lindberg E, Jarnerot G and Floderus MB (1988) Ulcerative colitis and Crohn's disease in an unselected population of monozygotic and dizygotic twins. A study of heritability and the influence of smoking. *Gut* 29: 990-996.
9. Biemond I, Burnham WR, D'Amaro J and Langman, MJS (1986) HLA-A and B antigens in inflammatory bowel disease. *Gut* 27: 934-941.
10. Botoman VA, Bonner GF and Botoman DA (1998) Management of inflammatory bowel disease. *Am. Fam. Phys* 57: 57-68.
11. Hanauer SB, Korelitz BI, Rutgeerts P, Peppercorn MA, Thisted RA, Cohen RD and Present DH (2004) Postoperative maintenance of Crohn's disease remission with 6-mercaptopurine, *Gastroenterol* 127: 723-729.
12. Faubion WA, Loftus EV and Harmsen WS (2001) The natural history of corticosteroid therapy for inflammatory bowel disease: a population-based study. *Gastroenterol* 121: 255-260.
13. Bresci G, Parisi G, Gambardella L, Banti S, Bertoni M, Rindi G and Capria A (1997) Evaluation of clinical patterns in ulcerative colitis: along-term follow-up. *Int J Clin Pharmacol Res* 17:17-22.
14. Rachmilewitz D (1989) Coated mesalazine (5-aminosalicylic acid) versus sulphasalazine in the treatment of active ulcerative colitis: a randomised trial. *BMJ* 298: 82-86.
15. Wang YH, Yang XL, Wang L, Cui MX, Cai YQ, Li XL and Wu YJ (2010) Effects of proanthocyanidins from grape seed on treatment of recurrent ulcerative colitis in rats. *Can J Physiol Pharmacol* 88: 888-898.
16. Li XL, Cai YQ, Qin H and Wu YJ (2008) Therapeutic effect and mechanism of proanthocyanidins from grape seeds in rats with TNBS-induced ulcerative colitis. *Can. J Physiol Pharmacol* 86:841-849.
17. Dos RSB, de OCC, Acedo SC, Miranda DD, Ribeiro ML, Pedrazzoli JJ and Gambero, A (2009) Attenuation of colitis injury in rats using *Garcinia cambogia* extract. *Phytother Res* 23: 324-329.
18. El-Abhar HS, Hammad, LN and Gawad HS (2008) Modulating effect of ginger extract on rats with ulcerative colitis. *J Ethnopharmacol* 118: 367-372.

19. Wong VK, Yu L and Cho CH (2008) Protective effect of polysaccharides from *Angelica sinensis* on ulcerative colitis in rats. *Inflammopharmacology* 16: 162–167.
20. Liu LN, Mei QB, Liu L, Zhang F, Liu ZG, Wang ZP and Wang RT (2005) Protective effects of *Rheum tanguticum* polysaccharide against hydrogen peroxide-induced intestinal epithelial cell injury. *World J. Gastroenterol* 11: 1503–1507.
21. Mazzon E, Muia C, Paola RD, Genovese T, Menegazzi M, De Sarro A, Suzuki H and Cuzzocrea S (2005) Green tea polyphenol extract attenuates colon injury induced by experimental colitis. *Free Rad Res* 39: 1017–1025.
22. Pawar P, Gilda S, Sharma S, Jagtap S, Paradkar A, Mahadik K, Ranjekar P and Harsulkar A (2011) Rectal gel application of *Withania somnifera* root extract expounds anti-inflammatory and muco-estorative activity in TNBS-induced inflammatory bowel disease. *BMC Complement Altern Med* 28: 11–34.
23. Oh PS and Lim KT (2006) Plant originated glycoprotein has anti-oxidative and anti-inflammatory effects on dextran sulfate sodium-induced colitis in mouse. *J Biomed Sci* 13: 549–560.
24. Patel MA, Patel P K and Patel MB (2010) Effects of ethanol extract of *Ficus bengalensis* (bark) on inflammatory bowel disease. *Indian J Pharmacol* 42: 214–218.
25. Cho EJ, Shin JS, Noh YS, Cho YW, Hong SJ, Park JH, Lee JY, Lee JY and Lee KT (2011) Anti-inflammatory effects of methanol extract of *Patrinia scabiosaefolia* in mice with ulcerative colitis. *J Ethnopharmacol* 136: 428–435.
26. Rise CL, Prabhu VV and Guruvayoorappan C (2012) Effect of marine mangrove *Avicennia marina* (Forssk.) Vierh against acetic acid-induced ulcerative colitis in experimental mice. *J Environ Pathol Toxicol Oncol* 31: 179–192.
27. Samad NB, Debnath T, Jin HL, Lee BR, Park PJ, Lee SY, Lim BO (2013) Antioxidant activity of *Benincasa hispida* seeds. *J Food Biochem* 37: 388–395.
28. Rachchh MA and Jain SM (2008) Gastroprotective effect of *Benincasa hispida* fruit extract. *Indian J Pharmacol* 40: 271–275.

29. Deok J, Woo SY, Yanyan Y, Gyeongsug N, Ji HK, Deok HY, Hyung JN, Skchan L, Tae WK, Gi HS and Jae Y ((2013) In vitro and in vivo anti-inflammatory effect of *Rhodomyrtus tomentosa* methanol extract. *J Ethnopharmacol* 146: 205–213.
30. Mohsen M, Alireza G, Parvin M, and Elham JS (2011) Comparative Study of *Berberis vulgaris* Fruit Extract and Berberine Chloride Effects on Acetic Acid-Induced Colitis in Rats. *Iranian J Pharm Res* 10: 97-110
31. Guslandi M (1999) Nicotine treatment for ulcerative colitis. *J Clin Pharmacol* 48: 481-484.
32. Peter R, Holt MD, Seymour K and Robert K (2005) Curcumin Therapy in Inflammatory Bowel Disease: A Pilot Study. *Digestive Diseases and Sciences* 50: 2191-2193
33. Langmead L, Feakins RM, Goldthorpe S, Holt H, Tsironi E, De Silva A, Jewell DP and Rampton DS (2004) Randomized, double-blind, placebo-controlled trial of oral aloe vera gel for active ulcerative colitis. *Aliment Pharmacol Ther* 19: 739-47.
34. Kane S and Goldberg M (2000) Use of bromelain for mild ulcerative colitis. *JAnn Intern Med* 18:680-682
35. Fernandez BF, Hinojosa J, Sanchez L JL, Navarro E, Martinez SJF, Garcia PA, Gonzalez HF, Riera J, Gonzalez LV, Dominguez AF, Gine JJ, Moles J and Gomollon F (1999) Randomized clinical trial of *Plantago ovata* seeds (dietary fiber) as compared with mesalamine in maintaining remission in ulcerative colitis. Spanish Group for the Study of Crohn's Disease and Ulcerative Colitis. *Gassull MA* *Am J Gastroenterol* 94:427-33.
36. Cheon JH, Kim JS, Kim JM, Kim N and Jung HC (2006) Plant sterol guggulsterone inhibits nuclear factor-kappaB signaling in intestinal epithelial cells by blocking IkappaB kinase and ameliorates acute murine colitis. *Bowel Dis* 12:1152-61.
37. Yuan H, Ji WS, Wu KX, Jiao JX, Sun LH, Feng YT (2006) Anti-inflammatory effect of Diammonium Glycyrrhizinate in a rat model of ulcerative colitis. *World J Gastroenterol* 12:4578-81.

38. Gupta I, Parihar A, Malhotra P, Gupta S, Ludtke R, Safayhi H, Ammon HP(2011) Effects of gum resin of *Boswellia serrata* in patients with chronic colitis. *Planta Med* 67:391-395.
39. Catherine C (2009) Plant tannins: A novel approach to the treatment of ulcerative colitis. *Natural Medi J* 1: 1-4
40. Vahid HJ, Farshid K and Habibollah J (2012) The investigation of silymarin effect on colon ulcer induced acetic acid in mice. *Annals Biol Res* 3:3691-369.
41. Gautam MK, Goel S, Ghatule R, Singh A, Nath G and Goel RK (2012) Curative effect of *Terminalia chebula* extract on acetic acid-induced experimental colitis: role of antioxidants, free radicals and acute inflammatory marker *Inflammopharmacol* 32: 1-7.
42. Ishikawa H, Akedo I, Umesaki Y, Tanaka R, Imaoka A and Otani T (2003) Randomized controlled trial of the effect of bifidobacteria-fermented milk on ulcerative colitis. *J American College of Nutrition* 22: 56-63.
43. Kruis W, Schutz E, Fric P, Fixa B, Judmaier G and Stolte M (1997) Double-blind comparison of an oral *Escherichia coli* preparation and mesalazine in maintaining remission of ulcerative colitis. *Aliment Pharmacol Therapy* 11: 853-858
44. Shiba T, Aiba Y, Ishikawa H (2003) The suppressive effect of Bifidobacteria on *Bacteroides vulgatus*, a putative pathogenic microbe in inflammatory bowel disease. *Microbiol Immunol* 47: 371-378.
45. Niedzielin K, Kordecki H and Birkenfeld BA (2001) Controlled, double blind, randomized study on the efficacy of *Lactobacillus plantarum* 299V in patients with irritable bowel syndrome. *European J Gastroenterol Hepatol* 13: 1143–1147.
46. Steidler L, Hans W and Schotte L (2000) Treatment of murine colitis by *Lactococcus lactis* secreting interleukin-10. *Scientifica* 289: 1352-1355.
47. Sarkar PK, Prajapati PK, Chaudhary AK, Shukla VJ and Ravishankar B (2010) Haematinic evaluation of lauha bhasma and mandur bhasma on Hgcl<sub>2</sub> induced anaemia in rats. *Indian j pharmacol* 59: 791-795.

48. Ahrabi SF, Madseh G, Dyrstad K, Sande SA and Graffner C (2000) Development of pectin matrix tablets for colonic delivery of model drug ropivacaine. *European J Pharm Sci* 10: 43-52.
49. Hita V, Singh R and Jain SK (1992) Colonic targeting of metronidazole using azo aromatic polymers, development and characterization. *Drug deliv* 4: 19-22.
50. Mrsny RJ (1992) The colon as a site for drug delivery. *J Contr Rel* 22: 5-34
51. Prasad SB, Yashwant and Aeri V (2013) Role of Traditional and Alternative Medicine in Treatment of Ulcerative Colitis. *Int J Pharmacog Phytochem Res* 5, 248-253
52. Davis S., (1990), Overcoming barriers to the oral administration of peptide drugs. *Trends Pharm Sci* 11: 353-355.
53. Rubinstein A (1995) Approaches and opportunities in colon-specific drug delivery. *Crit Rev Ther Drug carrier Syst* 12:101-149.
54. Evans DF, Pye G, Bramley R, Clark AG, Dyson TJ and Hardcastle JD (1988) Measurement of gastrointestinal pH profiles in normal ambulant human subjects. *Gut* 29:1035-1041.
55. Ashord M, Fell JT, Attwood D, Sharma H and Woodhead P (1993) An evaluation of pectin as a carrier for drug targeting to the colon. *J Control Rel* 26:213- 220.
56. Gazzaniga A, Iamartino P, Maffino G and Sangal ME (1994) Oral delayed release system for colonic specific drug delivery. *Int J Pharm* 108:77-83.
57. Hergenrother RW, Wabewr HD and Cooper SL (1992) The effect of chain extenders and stabilizers on the in vivo stability of polyurethanes. *J App Biomat* 3:17-22.
58. Kinget R, Kalala W, Vervoort L, and Mooter G (1998) Colonic drug delivery. *J Drug Target* 6:129-149.
59. Bussemer T, Otto I and Bodmeier R. Pulsatile drug-delivery systems. *Crit Rev Ther Drug Carr Sys* 18:433-458.
60. Prasad SB, Verma H, Aeri V and Yashwant (2013) Probiotics: A Medieval To Modern Era Prospective. *Int J Toxicol Pharmacol Res* 5: 63-68

61. Cole E, Scott R, Connor A, Wilding I, Petereit HU, Schminke C, Beckert T and Cade D (2002) Enteric coated HPMC capsules designed to achieve intestinal targeting. *Int J Pharm* 231: 83-95.
62. Friend DR and Chang GW (1985) Drug Glycosides: Potential prodrugs for colon specific drug delivery. *J Med Chem* 28:51-57.
63. Chavan MS, Sant VP, Nagarsenker MS (2001) Azo-containing urethane analogues for colonic drug delivery: synthesis, characterization and in vitro evaluation. *J Pharm Pharmacol* 53:895-900.
64. Chourasia MK and Jain SK (2003) Pharmaceutical approaches to colon targeted drug delivery systems. *J Pharm Pharm sci* 6: 33-66.
65. Simpkins JW, Smulkowski M, Dixon R and Tuttle R (1988) Evidence for the delivery of narcotic antagonists to the colon as their glucuro-nide conjugates. *J Pharmacol Exp Thera* 244:195-205.
66. Jung YJ, Lee JS, Kim HH, Kim YK and Han SK (1998) Synthesis and evaluation of 5-aminosalicylicylglycine as a potential colon specific prodrug of 5-aminosalicylic acid. *Arch Pharmacol Res* 21:174-178.
67. Ashord M, Fell JT, Attwood D, Sharma H and Woodhead P (1993) An evaluation of pectin as a carrier for drug targeting to the colon. *J Control Rel* 26:213- 220.
68. Hata T, Shimazaki Y, Kagayama A, Tamura S and Ueda S (1994) Development of a novel drug delivery system, time-controlled explosion system (TES). *Int J Pharm* 110: 1-7.
69. Watanabe S, Kawai H, Katsuma M, Fukui M (1998) Colon specific drug release system. US patent application. 09; 183,339.
70. Swanson D, Barclay B, Wong P, Theeuwes F (1987) Nifedipine gastrointestinal therapeutic system. *Am J Med* 83: 3-7.
71. Hu Z, Kimura G, Mawatari S, Shimokawa T, Yoshikawa Y and Takada K (1998) New preparation method of intestinal pressure-controlled colon delivery capsules by coating machine and evaluation in beagle dogs. *J Control Release* 56: 293-302.
72. Philip AK and Philip B (2003) Colon Targeted Drug Delivery Systems: A Review on Primary and Novel Approaches. *Oman Med J* 25: 70-78.



## 2 LITERATURE REVIEW

### 2.1 Bhasma

Nanomedicine are gaining popularity day by day owing to their various therapeutic applications with more efficacies and lesser side effects<sup>1</sup>. The popularity is due to their specific and selective pharmacological action. Bhasma the ancient concept of nano medicine is used treatment of various chronic ailments since 7<sup>th</sup> century BC. It is very clear from the history of civilization traditional medicines were used to cure human ailments in every possible condition. In modern era we can use them over the synthetic molecules because they have fewer side effects<sup>2-4</sup>. *Bhasma* is the calcination product of inorganic and organic substances. Bhasma as a medicine is a mystery due to severe side effect associated with metal when administered internally<sup>5</sup>. As per ayurvedic physician bhasma is nontoxic if metal is processed according to ancient ayurvedic literature. The rational pharmaceutical and therapeutically approach of Ayurveda in general and Rasa shastra in particular has transformed metal into medicinal form. The processes of shodhana (purification/potentiation) and marana (calcinations/detoxification – treatment with that quantum of energy which is needed for physico-chemical conversion of raw materials to Bhasma: a therapeutic form) which are very individualized in terms of material, media, method and absolute medicinal form<sup>6</sup>. The bhasmas are taken along with honey, milk, butter or ghee which makes these elements easily assimilable, eradicating their adverse effects and improving their biocompatibility<sup>3</sup>. Our ancient literature describes various method to ensure the quality of bhasma. In current few year tremendous work has been carried out to ensure the quality of bhasma. The present review deals with ancient as well as modern method of preparation of bhasma, therapeutic application of almost all bhasma and their method of characterisation by traditional method ( as per ancient literature) and using modern analytical techniques.

## 2.2 Preparation of Bhasma

Bhasma can be prepared by putapaka method and kupipakwa method<sup>3,7</sup>. Summarised method of preparation is shown in Figure-2.1

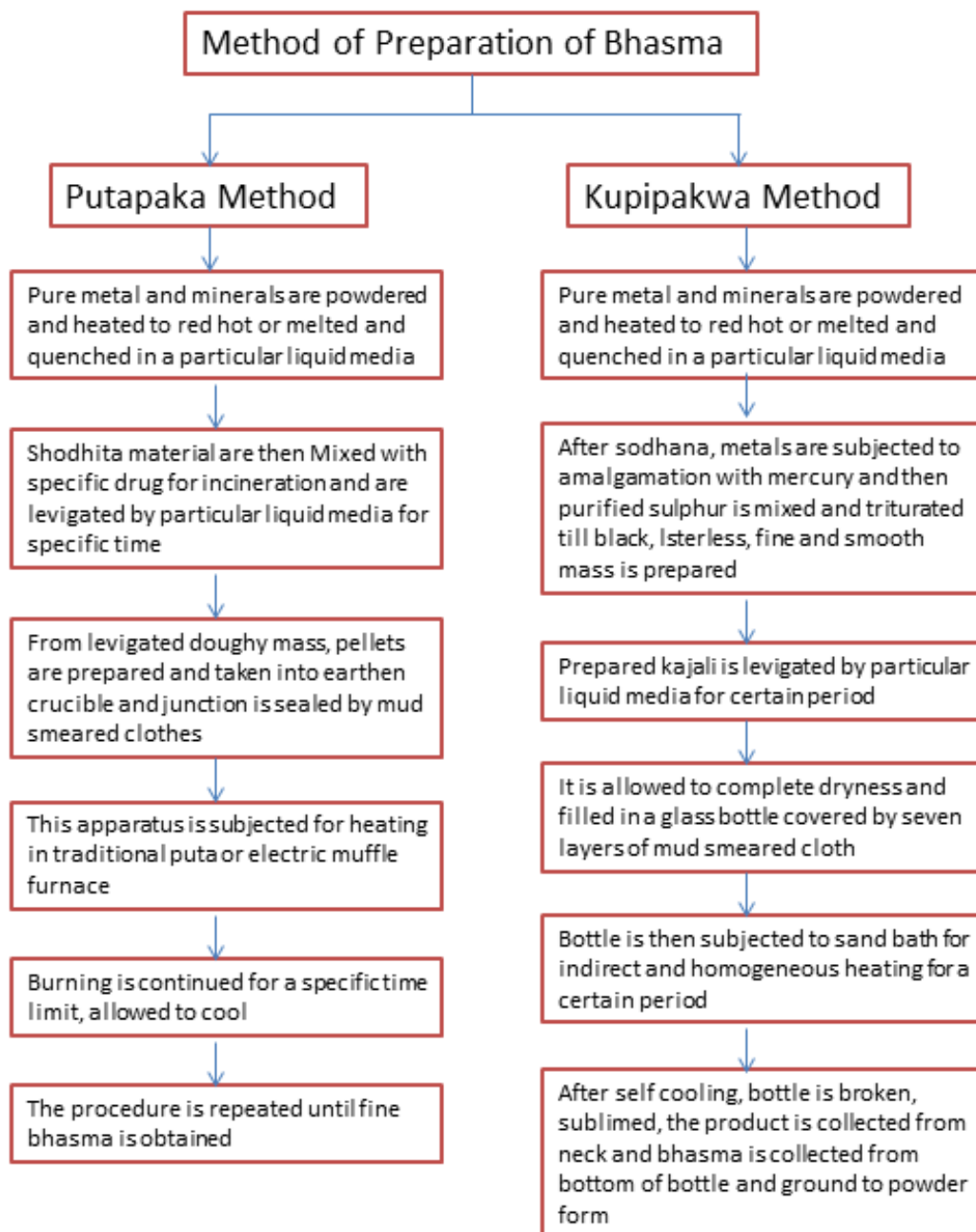


Figure 2.1: Methods of preparation of bhasma

In recent time burning (Calcination) process is done in crucible at specific temperature as per nature of metal and the remaining procedure is kept same for preparation of bhasma.

### **2.3 Bhasma as nano medicine**

Bhasma is considered as biologically produced nanomedicine as the size of individual particle is found in nano range. Heating of metal during sodhana may lead to increase in tension causing expansion of metal foil followed by cooling in liquid media lead to decrease in tension and increase in compression force. Repeated heating and cooling process may lead to brittleness, reduction in hardness and finally reduction in particle size. It is confirmed by various research carried out for characterisation of bhasma. The size of swarna bhasma and silver bhasma were found to be of 56 and 16 nm respectively<sup>7</sup>.

### **2.4 Chemical nature of bhasma**

Bhasma is produced by the process of calcination of metal and minerals. Calcination of metal may lead to conversion of metal into its metallic oxide<sup>8,9</sup>. Major chemical composition of bhasma is reported in Table-2.1.

### **2.5 Evaluation of bhasma**

The quality of bhasma can be evaluated by traditional method of evaluation (Bhasma pariksha). Evaluation can be done by physical and chemical test<sup>10,11</sup>.

#### **2.5.1 Physical test**

##### **2.5.1.1 Nishachandratva**

Bhasma can be observed under bright sunlight to detect the presence and absence of lustre. A good quality of bhasma should be free from metallic lustre indicating metal is completely converted in ash.

##### **2.5.1.2 Rekha Purita**

Small amount of bhasma is taken between the thumb and index finger and spread, it should be so fine as to get easily into the furrows of finger lines indicating that the size of bhasma is reduced to very fine size.

##### **2.5.1.3 Varitara**

When a small quantity of bhasma is spread on cold and distilled water, it should float on the surface. Properly calcined bhasma will float over surface of water. This test is performed to evaluate lightness and fineness of bhasma.

#### **2.5.1.4 Unama**

It is further assessment of varitara test. In this test a rice grain is kept over floating bhasma and observed either bhasma float or sinks. If grain float over bhasma, then it can be concluded that the bhasma is excellently prepared.

#### **2.5.1.5 Anjana sadrusha sukshmatva**

When bhasma is applied on eye lid as *kajal* should not cause any irritation indicating prepared bhasma is of micro fine size.

#### **2.5.1.6 Gatarastva**

Very small quantity of bhasma is placed over tongue for any specific taste. The good quality bhasma should be tasteless.

#### **2.5.1.7 Mridutva and slakshnatva**

This test is performed to detect softness and fineness of bhasma. A good quality of bhasma should be very fine and soft in touch.

#### **2.5.1.8 Avami**

The bhasma on oral administration should not produce nausea.

### **2.5.2 Chemical test**

#### **2.5.2.1 Nirdhumatva**

Small amount of bhasma is taken in spatula and subjected to heat treatment on flame directly. If no fumes are produced it indicates that bhasma is free from organic impurity.

#### **2.5.2.2 Apunarbhava**

This test is applied to metallic bhasma only. In this test bhasma is mixed with equal quantity of mitra panchaka (seed of *Abrus pectorius*, honey, ghee, borax and jagery) and sealed in earthen pot then similar grade of heat used for preparation of particular bhasma is applied and on self-cooling product is observed. If any particle with lusture is found indicating bhasma is not properly calcined.

#### **2.5.2.3 Niruttha**

In this test bhasma is mixed with fixed weight of silver leaf and sealed in earthen pot then similar grade of heat used for preparation of particular bhasma is applied and on

self-cooling, weight of silver is taken. Increase in weight of bhasma indicate improper preparation of bhasma.

#### **2.5.2.4 Amla pariksha**

Small amount of prepared bhasma was mixed with a little quantity of curds in a petri dish and a little amount of lemon in a neat and clean test tube for any colour change. No colour change of curd and lemon was observed indicating metal is completely converted into ash.

### **2.6 Evaluation of bhasma by Modern analytical technique**

Traditional method of evaluation of bhasma is self-satisfactory but not accepted by modern scientist. To consider this and to enhance the acceptance of bhasma as nano medicine it is necessary to characterise it using modern analytical technique.

#### **2.6.1 Loss on drying**

When bhasma gets exposed to atmosphere absorb moisture and particles of bhasma associates each other may lead to fail of varitara and unama test even the quality of bhasma is good. It can be understood by particle size analysis report by zeta sizer and SEM the particle size of copper oxide was found bigger in tamra bhasma when analysed with SEM obviously due to agglomeration of particles<sup>9</sup>.

Weigh accurate sample of bhasma. Transfer in tared weighed petriplate. Transfer the petri plate containing bhasma in hot air oven maintained at 105°C. Takeout the petri plate from hot air oven at certain interval and weigh on analytical balance. Repeat the procedure until constant weight.

$$\text{Moisture content} = \frac{\text{Initial weight}-\text{Final weight}}{\text{Initial weight}} \times 100$$

#### **2.6.2 Particle Size**

As per literature the size of bhasma lies in nanorange so particle size was determined using a laser diffraction particle size analyzer (Zetasizer Nano ZS90). Bhasma was suspended in the chamber of the particle size analyzer containing distilled water, and subjected to dynamic light scattering (DLS) to analyse the particle size<sup>9</sup>.

### 2.6.3 Morphological characterisation and size distribution:

For morphological characterisation and size distribution of bhasma, scanning electron microscopy and transmission emission microscopy is generally used<sup>9</sup>.

### 2.6.4 Structural analysis

Fourier-transformed infrared spectroscopy (FT-IR) spectroscopy can be used for structural analysis using potassium bromide disc. The bhasma can be analysed by recording their spectra in the wavelength range 4000-400  $\text{cm}^{-1}$ . The bhasma should be free from any organic impurity. Presence of organic impurity indicates that bhasma is not processed in proper way or process of calcination is not complete<sup>9</sup>.

The prepared bhasma can be analysed for their crystallinity or amorphous behaviour using X-ray diffraction (XRD). Generally metal oxide are crystalline in nature<sup>9</sup>.

Chemical composition of bhasma:

The chemical composition of bhasma is analysed using energy dispersive x-ray analysis (EDX) attached to SEM<sup>9</sup>.

### Reported preclinical activity of Bhasma

Preclinical activity about different bhasma is reported in Table-2.1.

**Table 2.1: Preclinical activity about different bhasma**

Bhasma	Main chemical composition	Traditional Use	Reported Preclinical activity	Reference
Lauha Bhasma	Ferric oxide ( $\text{Fe}_2\text{O}_3$ )	In treatment of Anaemia, Diabetes, tuberculosis	Antianaemic	12,13
Naga Bhasma	Lead oxide ( $\text{Pb}_3\text{O}_4$ )	Appetizer, Immunomodulator		
Swarna Bhasma	Aurous oxide ( $\text{Au}_2\text{O}$ ) and Auric oxide ( $\text{Au}_2\text{O}_3$ )	Immunomodulator, Aphrodisiac, Cardiac stimulant	Analgesic, Arthritis, free-radical scavenging activity, Anti-cataleptic, anti-anxiety and anti-depressant activity, Immunomodulator, Antioxidant	14, 15,16,17,18,19,20

<b>Bhasma</b>	<b>Main chemical composition</b>	<b>Traditional Use</b>	<b>Reported Preclinical activity</b>	<b>Reference</b>
<b>Raupya Bhasma</b>	Argentous and Argentic oxide (Ago, Ag <sub>2</sub> O)	Aphrodisiac, Immunomodulator, Anti-ageing	Hypolipidemic, Anticataleptic, Analgesic	21,22,23,24
<b>Tamra Bhasma</b>	Cupric oxide (CuO)	Wound healer, purgative, wound healer	Hepatoprotective	24
<b>Yasada bhasma</b>	Zinc oxide (ZnO)	Ophthalmic nourisher, Immunomodulator	In arrest of myopia	25
<b>Praval Bhasma</b>	Calcium carbonate	Antacid	Treatment of bone metabolic disorder (Osteoporosis)	26,27
<b>Mukta shouktic bhasma</b>	Calcium oxide	Antacid	anti-inflammatory	28
<b>Mandur bhasma</b>	Ferric oxide	Immunomodulator, Anaemia	Hepatopocurative, Anti anaemic	29

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## **2.7 Toxicity of Bhasma**

Accumulated toxicity report on the hazardous effects of heavy metals have made health scientists apprehensive to use metal as medicine. However, Ayurvedic metal based medicine (bhasma) is used in treatment of various disease. Other than Ayurveda, the traditional system of medicine of China and Egypt have described about ample use of metals. Heavy metals are toxic, but their oxides are usually not. FDA has approved Arsenic trioxide to be used in acute leukemia. If the bhasma is not prepared in correct manner, it would be toxic to human. Preparation of bhasma involves several calcination cycles, which lead to conversion of a metal into mixed oxides. Transformations of metal in to oxide may lead to conversion of the zero valent metal state into a form with higher oxidation state and the most important aspect of this synthesis is that the toxic nature (i.e. systemic toxicity causing nausea, vomiting, stomach pain, etc.) of the resulting metal oxide is completely destroyed while inducing the medicinal properties into it<sup>8,9</sup>.

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## **2.8 Current Understanding of Synthesis and Pharmacological Aspects of Silver Nanoparticles**

Metallic nanoparticles, including zinc, silver, iron, gold and metal oxide nanoparticles, have made knowncountlesspotential in biomedical application. Due to their large surface area to volume ratio such properties are observed<sup>30,31</sup>. SNPs or nanosilver (NS) are emerging as one of the fastest growing product in the field of nanotechnology. In daily life NS is used in room spray, wall paints, water purifier and laundry detergent. SNPs are also incorporated in textiles for manufacturing of cloth, vests, underwear and socks. It is estimated that all nano materials in medical and healthcare sector, NS application has higher degree of commercialization. A wide category of product is already available in market. In medical sector they are used in wound dressing, contraceptive devices, surgical instrument and bone prostheses. SNPs or NS are being used increasingly in catheters, wound dressingsand various household products due to their antimicrobial activity<sup>31</sup>.The antibacterial property of silver has been known for thousands of years with the ancient Greeks cooking from silver pot. The antimicrobial activities of silver were in practice to keep water safe as early as 1000 BC. In recent year it is proved that antimicrobial properties of silver is due toreleased Ag<sup>+</sup> ions. The first documentedtherapeutic use of silver goes back to 8th century<sup>32</sup>. Treatment of ulcers with the use of Silver nitrate was a common practice during 17th and 18th century. Now a days, the silver is in practice in medical sector as a biocide to prevent traumatic wounds infection in burns, and diabetic ulcers<sup>33</sup>. Interaction of silver with structural proteins and preferentially binding with DNA bases causes inhibitionof replication. Additionally, bactericidal properties of silver has also been recognised to inactivation of the enzyme (phosphomannose isomerase)<sup>34</sup>. Presently silver is considered a non-essential accumulative element. Silver is widely distributed in human body fluid and tissues including heart, lungs, aorta, blood, erythrocytes, plasma, bones, brain, breast, caecum, oesophagus, colon, diaphragm, duodenum, hair, ileum, larynx, kidney, urinary bladder, urine, liver, pancreas, adrenal gland, thyroid gland, lymph nodes, muscles, nails ovary, prostate gland, rectum, serum, skin, spleen, testes, teeth (dentine and enamel), trachea, uterus etc. Such wide distribution in the human body suggests that this metal could have some specific functions which are not clear at present <sup>35</sup>.



There has been a resurgence of promotion of colloidal silver as an alternative medicine since 1990's. It was claimed with colloidal silver that it can treat various diseases being an essential mineral supplement<sup>36,37</sup>. Even though the products of colloidal silver are legally accessible as health supplements, still it is illegal in USA to make claim of medical effectiveness of silver in colloidal state. The marketable product stated to as colloidal silver comprises solutions that contain various concentrations of compound of ionic silver. Unlike other modern medicine, the manufacturing of colloidal silver is not subjected to quality control and colloidal silver of various concentrations and particle sizes are available in market. In recent time, there are no fact-based medical uses for colloidal silver through oral route. The national center of USA for complementary and alternative medicine has issued an advisory showing that the therapeutic claims made about colloidal silver are not supported scientifically<sup>36</sup>.

## **2.9 Method of preparation of SNPs**

SNPs can be prepared by traditional Ayurvedic literature, physico-chemical method and biological method.

### **2.9.1 Traditional Ayurvedic method**

*Bhasma* is the calcination product of inorganic or organic substances and claimed to be biologically produced nanoparticles. Silver nanomedicine of ancient Ayurveda is known as *raupya bhasma*. It is prepared by methods described in Ayurvedic text in *Rasendrasara Samagraha*. Equal amount of pure silver and sulphur (by weight) are mixed together with half amount of arsenic trisulphite, then it is soaked in lemon juice and subjected to calcination. The process is repeated 14 times to obtain *raupya bhasma*<sup>38</sup>.

### **2.9.2 Physicochemical syntheses of SNPs**

Physical and chemical methods are mainly used for preparation of nanoparticles. SNPs can be prepared by “top down” and “bottom-up” methods. Mechanical grinding methods are mainly used as a top down method and the reduction of metal by electrochemical method is used in bottom up method<sup>39,40</sup>. SNPs synthesized by different physicochemical method is shown in Table 2.2.

**Table 2.2: SNPs synthesized by different physicochemical method**

S.no	Methods	Size	Reference
1	Chemical method of reduction of the metal salt AgBF <sub>4</sub> by NaBH <sub>4</sub> in water.	3-40 nm	12
2	Electrochemical method which involves the electro reduction of AgNO <sub>3</sub> in aqueous solution in the presence of polyethylene glycol	10 nm	13
3	Sonodecomposition of an aqueous silver nitrate solution in an atmosphere of argon-hydrogen	20	14
4	Electrostatically complexing silver ions with an anionic surfactant aerosol in extremely stable liquid foam. The foam is then drained off and reduced by introducing sodium borohydride. These silver nanoparticles are very stable in solution, suggesting that the aerosol stabilizes them.	5-40nm	15
5	Reduction of silver nanoparticles using variable frequency microwave radiation.	15-25	16

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### 2.9.3 Biological synthesis of SNPs

It is also possible to synthesise SNPs by biological methods. Different methods are reported using bacteria, fungi and plant extracts by different researchers<sup>46,47</sup>.

#### 2.9.3.1 Synthesis of SNPs using bacteria

The mechanism behind synthesis of SNPs is the existence of the nitrate reductase enzyme which causes conversion of nitrate into nitrite<sup>48</sup>. The bacteria involve in synthesis of silver nanoparticles is shown in Table 2.3.

**Table 2.3: Different bacteria for synthesis of SNPs**

Organism	Size (nm)	Reference
<i>B. licheniformis</i>	50	49
<i>Bacillus megaterium</i>	46.9	50
<i>Bravibacterium casei</i>	50	51

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Organism	Size (nm)	Reference
<i>Escherichia coli</i>	5-25	52
<i>Enterobacter cloacae</i>	50-100	53
<i>Klebsiella pneumonia</i>	50	54
<i>Lactobacillus fermentum</i>	11.2	55
<i>Proteus mirabilis</i>	10-20	56
<i>Plectonema boryanum</i>	1 to 200	57
<i>P. stutzeri</i> AG259	200	58

### 2.9.3.2 Synthesis of SNPs using Fungi

It is believed that the mechanism involve in synthesis of SNPs by fungi (Table 2.4) is trapping of Silver ion (Ag<sup>+</sup>) in the exterior of the fungal cells and the succeeding reduction of the silver ions by the enzymes present in the fungal system<sup>59-61</sup>.

**Table 2.4: Different fungi for synthesis of SNPs**

Organism	Size (nm)	Reference
<i>Aspergillus clavatus</i>	10 to 25	62
<i>Aspergillus flavus</i>	7 to 10	63
<i>Aspergillus fumigatus</i>	5 to 25	64
<i>Coriolus versicolor</i>	25	65
<i>F. oxysporum</i>	20 to 50	66
<i>Fusarium solani</i>	5 to 35	67
<i>Phanerochaete chrysosporium</i>	100	68
<i>Phoma sp.</i> 3.2883	70	69

### 2.9.3.3 Synthesis of SNPs using Plant

The major mechanism for synthesis of SNPs using plant (table 2.5) involves the reduction of the ions. The main phytochemicals which participates are flavones, amides, terpenoids, ketones, aldehydes, and carboxylic acids. Flavones, organic acids, and quinones are water-soluble phytochemicals that are responsible for the instant reduction of the ions<sup>70</sup>.

**Table 2.5: Different Plant used in synthesis of SNPs**

<b>Plant</b>	<b>Size (nm)</b>	<b>Reference</b>
<i>Aloe vera</i>	15 to 20	71
<i>Azadirachta indica</i>	50	72
<i>Carica papaya</i>	15	73
<i>Cinnamomum camphora leaf</i>	55 to 80	74
<i>Cinnamomum zeylanicum bark</i>	50 to 100	75
<i>Coriandrum sativum leaf</i>	26	76
<i>Desmodium triflorum</i>	5 to 20	77
<i>Jatropha curcas</i>	10 to 20	78
<i>Medicago sativa</i>	2 to 20	79
<i>Piper betle leaf</i>	3 to 37	80

## 2.10 Pharmacological Aspects

Different researches independently reported several pharmacological activities of Silver Nano Particles. The activities reported includes antibacterial, antifungal, antiplatelet, antiproliferative, antiangiogenic, anti-inflammatory, analgesic, cytotoxic, genotoxic. Recently reported activity of Silver NPs include against ulcerative colitis, colon cancer and HIV-I. <sup>35,81-93</sup>.

## 2.11 Toxicity

In minute concentration, silver is considered to be non-toxic in normal use. One of the most important side effect reported for silver product is argyria. Argyria is irreversible grey to black colouration of skin due to deposition of silver in sub dermal layer. Argyria is just a cosmetic problem it do not cause any physical harm<sup>31</sup>.

On the other hand raupya bhasma (Ancient silver nanomedicine) was consider as safe at due to their use from ancient time therapeutic doses.

Recently it is proved scientifically by Inder D et al (2011) who had carried out toxicity of raupya bhasma on mice and concluded that minimum toxic dose of raupya bhasma was 1.5 g/kg and and LD<sub>50</sub> was 2.0 g/kg. However the therapeutic dose for human being is 125 mg only.

## **2.12 Probiotics**

Pharmaceuticals have not been able to completely control the global morbidity and mortality in case of both acute and chronic diseases. Hence, search for the other alternatives has always been there<sup>94</sup>. The old age quote of Hippocrates becomes most pertinent in the current health scenario i.e. “let food be thy medicine and medicine be thy food”<sup>95</sup>. In the late 90’s, microbiologists identified the difference between the micro flora of the diseased human beings and those of normal human beings. The beneficial micro flora were termed as “probiotics”<sup>96</sup>. There are billions of bacteria present in human Gastro Intestinal Tract (GIT) forming about 1 kg of the human weight, which includes both harmful as well as beneficial bacteria. Together they are called as gut flora. Delicate balance between the harmful and the beneficial bacteria is responsible for maintenance of health. When this balance is disturbed, the person becomes diseased. One of the ways to regain this balance is the external administration of probiotics (beneficial bacteria) into the body of the diseased person. Probiotics include a large number of different types of bacteria that are normal inhabitants of human GIT. The most common among them are various species of *Lactobacilli* and *Bifidobacteria*. They reside in small intestine and colon. Probiotics have been able to attract the maximum attention among several food supplements as they have additional benefits beyond their nutritional value<sup>97</sup>. In 1965, Stillwell and Lilly introduced the term “probiotics”<sup>98</sup>. The term is made up of two words Latin preposition pro means "for" and the Greek adjective βιωτικός means “biotic”. Hence, it means “for favour of life”. According to World health organisation (WHO) and Food and Agriculture Organization (FAO) it is defined as “living microorganism intended for administration into the host body in adequate amount so as to confer health benefits”<sup>99</sup>.

### **2.12.1 History**

It has been known since long that there are benefits of using fermented milk products and poultices of bread moulds. But Ellie Metchnikoff started the probiotic therapy via fermented milk products in 1907. In 1915, the therapy was used for the treatment of urogenital infections. However, in the intertwining period of 7-8 decades less study is reported on probiotics due to an increased interest in antibiotics. These were labelled as “alternative medicines”. Recently there has been a resurgence of probiotics due to

demand of consumers for better treatment. This resurgence can also be attributed to development of resistance against antibiotics<sup>99</sup>.

### **2.12.2 Probiotic criteria**

An organism must fulfil the following criteria in order to be considered as probiotics: There should be high cell viability, and should be able to survive in low pH. Even if strain cannot colonize in gut, it should have the ability to persist. They should have the ability to adhere to the epithelium of GIT so as to overcome the flushing effect due to peristalsis. They should have the ability to interact or to send signals to the immune cells associated with GIT, capable of being isolated from humans, processing resistance, non-pathogenic and positive influence on local metabolic activities.

A dose of five billion colony forming units are generally recommended for adequate health benefits. Probiotics should be Generally Recognized as Safe (GRAS). Probiotics preparations involve the use of both single as well as mixture of microorganisms<sup>99</sup>.

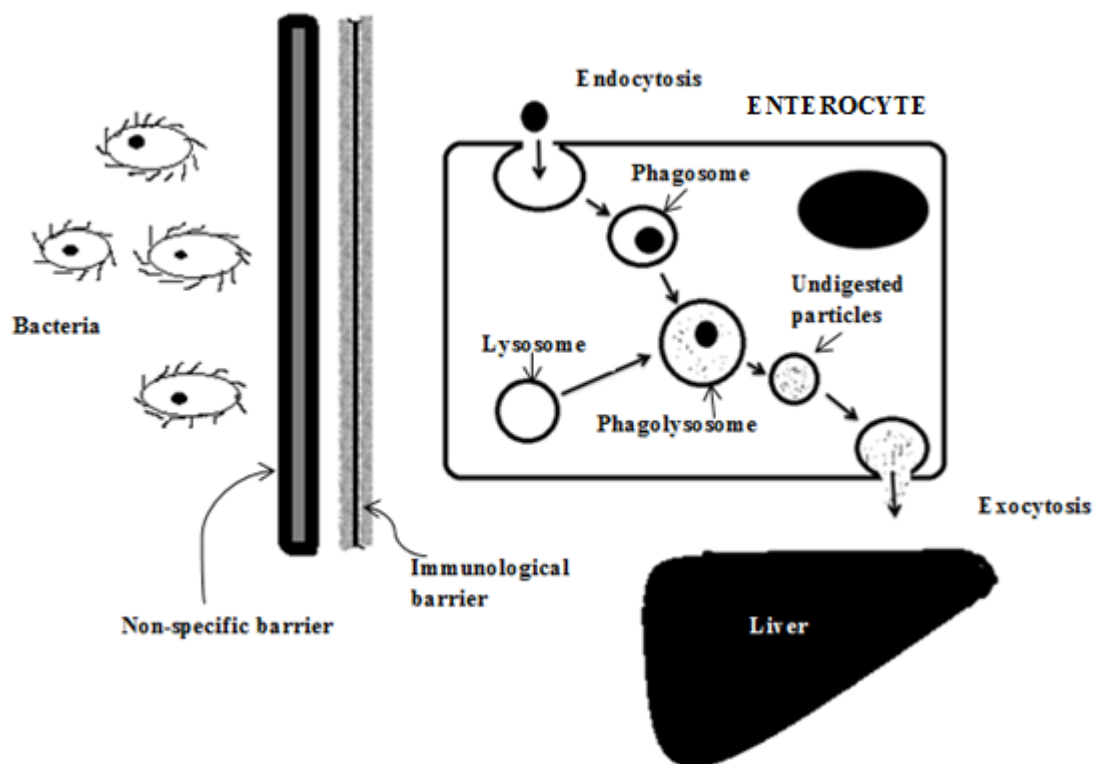
### **2.12.3 Mechanism of action of probiotics**

To explain the effects of probiotics several mechanisms have been proposed. The effects can be attributed to a number of activities and their action is proposed to be multipronged. Probiotics stimulate the intestinal lactase activity. They partially digest the lactose and can be used in the case of lactose intolerance and in certain types of diarrhoea<sup>100</sup>. Various fermented milk industries use lactobacilli in order to decrease the lactose concentration in the dairy products which ultimately affects the severity of osmotic diarrhoea<sup>100</sup>. Lactic acid bacteria inhibit the growth of various pathogenic microorganisms present in dairy products by producing various metabolites such as free fatty acids, bacteriocins and hydrogen peroxide etc<sup>101</sup>.

Probiotics also cause modification in the toxin receptors and thus hinder the toxin receptor mediated pathology of disease<sup>102</sup>. They also offer competitive inhibition during colonization to the pathogenic bacteria<sup>103</sup>. The other mechanisms involve lowering of pH, releasing the gut protective metabolites, production of mucous and regulation of gut motility<sup>104</sup>. Gastrointestinal mucosa acts as an interface between the body's immune system and the external environment. Whenever there is decrease in gut

flora the antigen transportation increases. This clearly depicts that gut flora maintain the gut defences (Figure 2.2)<sup>104, 105</sup>.

The interaction between the gut epithelial and immune cells with non-pathogenic probiotic micro-organisms may lead to generation of immunological signals. This interaction occurs in the Peyer's patches<sup>106</sup>. Probiotics also modulate the immunoglobulin (Ig) production. They increase the production of IgA, a secretory immunoglobulin which plays an important role in mucosal immunity and thus act as a barrier against the various pathogenic microorganisms and viruses<sup>106, 107</sup>. It has also been demonstrated that probiotics also causes induction of T independent IgA<sup>108</sup>. Probiotics also increase the production of certain types of cytokines tumour necrosis factor-  $\alpha$  (TNF-  $\alpha$ ), interleukins-10 (IL-10). The up and down regulation of immune response is also affected by probiotics so as to maintain the intestinal homeostasis<sup>109</sup>.



**Figure 2.2: Barrier to antigen absorption in intestine.**

### **2.12.4 Probiotics in health**

The overall health of the person depends upon his/her eating habits and life style. In ancient time humans used to take enormous live bacteria but as the concept of hygiene developed, there has been a decrease in intake of live bacteria along with the food. The dietary habits in the western world are a cause of development of certain diseases like ulcerative colitis. Their diet lacks fruits, vegetables and omega-3 fatty acids. Due to which they have more chances of development of diseases such as heart diseases and cancer. The increase in allergic and inflammatory conditions, obesity, heart diseases and cancer has been found to be proportional to the decrease in probiotic content in the diet<sup>110</sup>.

### **2.12.5 Probiotics in specific diseases**

#### **2.12.5.1 Allergies /Eczema**

Probiotics are very effective in treatment of food allergy especially in case of infants suffering from atopic eczema or cow's milk allergy. With the use of *Lactobacillus* GG, there occurs a significant clinical improvement among the people suffering from atopic dermatitis. The clinical improvement is accompanied by reduction in inflammatory marker<sup>111, 112</sup>.

#### **2.12.5.2 Antioxidant activity**

*Bacillus coagulans* RK-02 has been reported to produce extracellular polysaccharide having four heteromonosaccharides as its constituents. This has shown a significant antioxidant and free radical scavenging activity<sup>113</sup>. The powerful antioxidant activity is also shown by *Streptococcus thermophilus*. It protects the body from many dangerous free radicals that develop in the body due to aging, sugar, stress, antibiotics other toxins and chemicals<sup>114</sup>. Significant antioxidant activity is also shown by *Bifidobacterium bifidum* due to which it has been reported to produce protection to the intestinal lining from the lipid peroxidation in iron over loaded mice<sup>115</sup>.

#### **2.12.5.3 Antibacterial activities**

Multiple probiotics via *in vitro* studies have been found to be effective against many of the pathogenic microorganisms including *Listeria monocytogenes*, *Salmonella typhimurium*, *E.coli* and *H.pylori*. Therefore, prototypic antimicrobial substances can



be obtained from probiotic agents. It may prove to be useful for the pharmaceutical companies to develop new antibiotics<sup>116,117</sup>.

#### **2.12.5.4 Diarrhoea**

Probiotics are used for prevention as well as for the cure of various types of diarrhoea. The activity of dietary probiotics against various types of diarrhoea successfully investigated. e.g.

*Lactobacillus rhamnosus* GG, strains of *L. Casei*, strains of *L. Acidophilus*, *L. Reuteri*, *Escherichia coli* strain, Bifidobacteria and Enterococci, and Probiotic yeast *Saccharomyces boulardii*<sup>118</sup>.

#### **2.12.5.5 Rota virus diarrhoea**

Both preventive as well as curative probiotic treatment is available which has been proven with the help of randomized, double blind and placebo studies. *Bifidobacterium lactis* BB-12 and *Lactobacillus rhamnosus* GG are used for prevention whereas *Lactobacillus reuteri* SD 2222 is used for the treatment in acute cases<sup>119-121</sup>.

#### **2.12.5.6 Antibiotic associated diarrhoea**

Although broad spectrum newer antibiotics have been developed with fewer side effects but they are liable to cause antibiotic associated diarrhoea (AAD). The chances of incidence ranges from 3.2 to 29/100 patients admitted to the hospital. The complications of AAD involve: electrolyte imbalance, dehydration, pseudo membrane colitis. Antibiotics which are used against anaerobic bacteria are supposed to cause more AAD. *Saccharomyces boulardii* can be used in the treatment of AAD<sup>122</sup>.

#### **2.12.5.7 Radiation induced diarrhoea**

The patients who are receiving radiation therapy during cancer usually develop diarrhoea. A study of high potency probiotics preparation was done on such patients (double blind and placebo) taking VSL#3 as a preparation. It has been shown that probiotic preparation decreases the bowel movements and daily incidences of diarrhoea. From the study, a conclusion was withdrawn that lactic acid bacteria can be a safe, efficient and easy approach to treat radiation associated diarrhoea in cancer patients<sup>123</sup>.

#### **2.12.5.8 Traveller's diarrhoea**

It is the diarrhoea associated with the travellers. The chance of incidence ranges from 5 % to 15% depending on destination<sup>31</sup>. A mixture of *Saccharomyces boulardii*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* is found to have high efficacy in this regard<sup>124</sup>.

#### **2.12.5.9 Hyperlipidemia**

Probiotic strains can be used to lower the body cholesterol level (especially the lactic acid bacteria). Two strains found in yogurt have been found to have significant cholesterol lowering effect. These include *Lactobacillus acidophilus* and *Bifidobacterium lactis*. When *Lactobacillus sporogenes* was given to hyperlipidemic patients over a period of three months a reduction of 32% total cholesterol level and 35% reduction in low density lipoprotein (LDL). The mechanism behind this effect is the inhibition of production of HMG CoA reductase. *L. plantarum* has also been shown to possess cholesterol lowering activities<sup>125,126</sup>.

#### **2.12.5.10 Hepatic diseases**

A report was published which demonstrates the role of multicultural probiotics in the treatment of liver cirrhosis. The study included first one month probiotic treatment followed by 1 month wash out period, followed again by the second probiotic treatment. During the study, blood pressure of hepatic portal vein was measured, which usually was found to lower in case of liver cirrhosis. During the first probiotic therapy period there was an increase in portal vein pressure followed by decrease in that at the end of wash out period which again rise during second period of probiotic therapy. The microorganisms present in formulation included *Streptococcus thermophilus*, *Bifidobacteria*, *Lactobacillus plantarum*, *L. acidophilus*, *L. casei*, *L. debrueckii bulgaricus* and *Streptococcus faecum*<sup>127</sup>.

#### **2.12.5.11 Hypertension**

Blood pressure (BP) decrease with the consumption of fermented milk products with species of *Lactobacilli*. The antihypertensive effect of probiotic is attributed to the bacterial cell wall components. Moreover the bacteria are known to generate peptides which have angiotensin converting enzyme inhibiting property<sup>128</sup>.

#### **2.12.5.12 Hemolytic uremic syndrome**

This syndrome usually develops in children taking antibiotic therapy for *E. coli*<sup>129</sup>. In this syndrome, epithelial injury occurs due to drop in transepithelial electrical resistance. Such injury can be prevented by pre-treatment of intestinal (T84) cells with lactic acid producing bacteria. *E. coli* produces verotoxin which causes haemorrhagic colitis and haemolytic uremic syndrome in humans. This can be inhibited by the use of probiotic containing *Bifidobacterium longum*, which produces substances that can bind to verotoxins<sup>130</sup>. *Bifidobacterium longum* also offers protection against *Salmonella typhimurium*<sup>131</sup>.

#### **2.12.5.13 Inflammation /Arthritis**

Probiotics produce both direct as well as indirect effects. The direct effects produce locally within the GIT includes vitamin production etc. The indirect effects which are produced outside the GIT occur in joints, skin and lungs. Amongst the indirect effects it exerts its influence on immunity and alters the level of inflammatory mediators. Modulation of inflammatory response can be localized within the GIT or it may be systemic. It is postulated that inflammation associated with rheumatoid arthritis can be modulated with the help of probiotics. In chronic juvenile arthritis, there is a disturbance in the gut defence mechanism and an alteration in the permeability of GIT which may account for the inflammation associated with arthritis. The effects of *Lactobacillus GG* administration to the patients for two weeks shows remarkable improvement<sup>132</sup>.

#### **2.12.5.14 Inflammatory Bowel Disease (IBD)**

IBD involves two chronic diseases: Ulcerative colitis and Crohn's disease

The available clinical data shows the role of intestinal micro biota in the pathogenesis of IBD and thereby provides an evidence that alteration in the intestinal micro biota with the help of probiotics can be helpful in the treatment of disease. E.g. *Bifidobacteria infantis* has been found to reduce the inflammatory response of the gut lining by inhibiting the bacteroides. *Lactobacillus plantarum* has also been reported to be used in IBD. A probiotic formulation containing no of microbes (VSL#3) used in case of ulcerative colitis although its clinical efficacy is not certain. *E. coli* has also been used in case of ulcerative colitis but its clinical efficacy depends upon its dose<sup>133-137</sup>.

**2.12.5.15 Kidney stones**

Kidney stones develop as the result of increased concentration of oxalate in the urine. *Oxalobacter formigenes* is responsible for degrade oxalate with the help of enzyme oxalyl-CoA. People with renal stone are found to have less *Oxalobacter formigenes* which ultimately leading to increased oxalate in the urine (hyperoxaluria). *Bifidobacterium breve* has also been reported to be beneficial in case of kidney stones as it also exhibits oxalate degrading property<sup>138,139</sup>.

**2.12.5.16 Neonatal enterocolitis**

Caplan and Jilling reported that supplementation with probiotics can be helpful in preventing the neonatal enterocolitis. They developed murine model explaining different characters of neonatal enterocolitis that are clinically and pathologically important. *Bifidobacterium infantis* was found to be effective in this case<sup>140</sup>.

**2.12.5.17 Cancer**

There are several hypotheses which explain the mechanism of action of probiotics in treatment of various types of cancers. These include: Detoxification of ingested carcinogens. Inhibition of bacterial growth and/or production of their metabolites those are carcinogenic in nature. Inhibition of tumour cells growth via production of specific compounds. Immune system stimulation against tumour cells. Metabolite production (e.g. butyrate) which improve apoptosis<sup>141</sup>. *Lactobacilli* have been reported to prevent establishment and growth of tumour cell and their metastasis<sup>142</sup>. With increase in consumption of products containing *Lactobacilli* or *Bifidobacterium* the chances of breast and colon cancer reduces<sup>143,144</sup>. The recurrence of superficial bladder cancer has been found to be significantly reduced with the consumption of *Lactobacillus casei*<sup>145</sup>.

**2.12.5.18 Peptic ulcers**

According to a report *Lactobacillus salivarius* has been found to be useful in *H.pylori* induced peptic ulcers as it produces lactic acid which can completely inhibit *H.pylori* growth, this in turn, inhibits the inflammatory response associated with it. The first bacteriocin (natural antibiotic substance) was isolated from *Lactobacillus salivarius*. *L. casei* (strain Shirota) can also be used in case of *H.pylori* induced peptic ulcer<sup>146,147</sup>.

### **2.12.5.19 Lactose intolerance**

Probiotics relieve the symptoms associated with intolerance as well as they decrease oro-cecal transit. Those individuals who have such intolerance can tolerate 12 to 15g of lactose when probiotic therapy is given. Yogurt is used in case of such patients as it contains less lactose as compare to milk. Moreover, it contain lactase enzyme and delay gastric emptying time<sup>148-151</sup>.

### **2.13 References**

1. Bhattacharya R and Mukherjee P (2008) Biological properties of naked metal nanoparticles, *Adv drug deliv rev.* 60: 1289-1306.
2. Sarkar PK and Chaudhary AK (2010) Ayurvedic bhasma: The most ancient concept of nanomedicine. *J Sci Ind Res* 69: 901-905.
3. H Verma, SB Prasad, Yashwant and H Singh (2013) Herbal Drug Delivery System: A Modern Era Prospective. *International Journal of Current Pharmceutical Review Research* 4: 88-101.
4. Kumar A, Nair AGC, Reddy AVR and Garg AN (2006) Unique Ayurvedic Metallic–Herbal Preparations, Chemical Characterization. *Biol Trace Elem Res*109: 231-254.
5. Prasad SB, Aeri V and Yashwant (2013) Role of Traditional and Alternative Medicine in Treatment of Ulcerative Colitis. *International Journal of Pharmacognosy and Phytochemical Research* 5: 248-253
6. Sarkar PK, Das S and Parajapati PK (2010) Ancient concept of metal pharmacology based on Ayurvedic literature. *Ancient sci life* 29: 1-6.
7. Raisuddin S, Chapter 6 Ayurvedic bhasmas. In: Mishra LC, editor. *Scientific basis for ayurvedic therapies*. 1<sup>st</sup> ed. CRC press, New Delhi (India), 2004, 83-95.
8. Kapoor RC (2010) Some observations on the metal-based preparation in the Indian system of medicine. *Indian j traditional knowledge* 9: 562-575.
9. Wadekar MP, Rode CV, Bendale YN, Patil KR and Prabhune AA (2005) Preparation and characterization of a copper based Indian traditional drug: Tamra bhasma. *J Pharm Biomed Anal* 39: 951–955.
10. Mishra G, Upadhyay M. *Ayurveda*. Chaukhamba bharti academy New Delhi (India), 1994, 289-305.

11. Kulkarni DA, Vagbhatacharyas R. Ratnasamuchay. Meharchand lakshaman Das Publication, New Delhi (India) 1998, 198-199.
12. Pandit S, Biswas TK, Debnath PK, Saha AV, Chowdhury U, Shaw BP, Sen S and Mukherjee B (1999) Chemical and pharmacological evaluation of different Ayurvedic preparations of iron, J Ethnopharmacol 65: 149.
13. Sarkar PK, Prajapati PK, Chaudhary AK, Sukla VJ and Ravishankar B (2007) Haematinic evaluation of lauha bhasma and mandura bhasma on Hgcl2 induced Anaemia in rat. Indian j pharm Sci 69: 791-795.
14. Bajaj S and Vohora SB (1998) Analgesic activity of gold preparations used in Ayurveda and Unani-Tibb. Indian j of med res 108: 104-111.
15. Brown CL, Bushell G, Michael W, Agrawal DS, Tupe SG, Paknikar KM, Tiekink, ERT (2007) Nanogold-pharmaceutics: (i) The use of colloidal gold to treat experimentally-induced arthritis in rat models; (ii) Characterization of the gold in Swarna bhasma, a microparticulate used in traditional Indian medicine From Gold Bulletin 40: 245-250.
16. Mitra A, Chakraborty S, Auddy B, Tripathi P, Sen S, Saha AV and Mukherjee B (2002) Evaluation of chemical constituents and free-radical scavenging activity of Swarnabhasma (gold ash), an ayurvedic drug. J Ethnopharmacol 80:147-153.
17. Bajaj S and Vohora SB (2000) Anti-cataleptic, anti-anxiety and anti-depressant activity of gold preparations used in Indian systems of medicine. Indian J Pharmacol 32: 339-346.
18. Bajaj S, Ahmad I, Fatima M, Raisuddin S, and Vohora SB (1999) Immunomodulatory activity of a Unani gold preparations used in Indian system of medicine, Immunopharmacol Immunotoxicol 21: 151-155.
19. Bajaj S, Ahmad I, Raisuddin S, and Vohora SB (2001) Augmentation of non-specific immunity in mice by gold preparations used in traditional systems of medicine. Indian J Med Res 113: 192-196.
20. Shah ZA and Vohora SB (2002) Antioxidant/restorative effects calcined gold preparations used in Indian systems of medicine against global and focal models of ischaemia. Pharmacol Toxicol 90: 254-257.

21. Sharma DC, Budania R, Shah M, Jain P and Gaur BL (2004) Hypolipidemic activity of silver preparations in chicks, *Gallus serregineus*. *Indian J Exp Bio* 42: 504-507.
22. Nadeem A, Khanna T and Vohora SB (1999) Silver preparations used in Indian systems of medicine: neuropsychobehavioural effects. *Indian J Pharmacol* 31: 214-221.
23. Khanna AT, Sivaraman R and Vohora SB (1997) Analgesic activity of silver preparations used in Indian systems of medicine. *Indian J Pharmacol* 29: 393-398.
24. Inder D, Rehan HS, Bajaj VK, Kumar P, Gupta N and Singh J (2012) Analgesic activity and safety of Ash of silver used in Indian system of medicine in mice: A reverse pharmacological study. *Indian j of pharmacol* 44: 46-50.
25. Tripathi YB and Singh VP (1996) Role of Tamra bhasma, an Ayurvedic preparation, in the management of lipid peroxidation in liver of albino rats. *Indian j exp bio* 34: 66-70.
26. Puri RN, Thakur V and Nema HV (1983) Role of zinc (Yashad Bhasma) in arrest of myopia. *Indian J ophthalmol* 31: 816-822.
27. Prabhakara NR, Lakshmanab U and Venkatesh U (2003) Effect of Praval bhasma (Coral calx), a natural source of rich calcium on bone mineralization in rats *Pharmacol Res* 48: 593–599.
28. Chauhan O, Godhwani JL, Khanna NK and Pendse VK (1998) Antiinflammatory activity of muktashukti bhasma. *Indian J Exp Biol* 36: 985-988.
29. Kanase A, Patil S and Thorat B (1997) Curative effects of mandur bhasma on liver and kidney of albino rats after induction of acute hepatitis by CCl<sub>4</sub>. *Indian J Exp Bio* 35: 754-764.
30. Albrecht MA, Evans CW, Raston CL (2006) Green chemistry and the health implication of nanoparticles. *Green Chem* 8: 417-432.
31. Bhattacharya R and Mukherjee P (2008) Biological properties of “naked” metal nanoparticles. *Adv drug deliv rev* 60: 1289-1306.
32. Wadhera A and Fung M (2005) Systemic argyria associated with ingestion of colloidal Silver. *Dermatol Online J* 11: 12.

33. Klasen HJ (2000) Historical review of the use of silver in the treatment of burns. I. Early uses. *Burns* 26: 117-130.
34. Atiyeh BS, Costagliola SN and Hayek SA (2007) Effect of silver on burn wound infection control and healing: review of the literature. *Burns* 33 139–148.
35. Nadeem A, Khanna T and Vohora SB (1999) Silver preparations used in Indian systems of medicine neuropsychobehavioural effects. *Ind Journal of Pharmacol.* 31: 214-221.
36. [http://nccam.nih.gov/sites/nccam.nih.gov/files/Colloidal\\_Silver.pdf](http://nccam.nih.gov/sites/nccam.nih.gov/files/Colloidal_Silver.pdf) (Cited on 07/07/2013)
37. Fung MC, Weintraub M and Bowen DL (1995) Colloidal silver medicine marketed as health supplement. *J Am Med Assoc* 274: 1196-1197.
38. Krishna G. Rasendrasara Samagrah (Sanskrit) 1<sup>st</sup> ed. Varanasi, Chaukhambha Prakashan; 1967:74-77.
39. Gaffet E, Tachikart M, El Kedim O and Rahouadj R (1996) Nanostructural materials formation by mechanical alloying: morphologic analysis based on transmission and scanning electron microscopic observations. *Mater Charact* 36: 185-190.
40. Amulyavichus A, Daugvila A, Davidonis R and Sipavichus C (1998) Study of chemical composition of nanostructural materials prepared by laser cutting of metals. *Fizika Met Met* 85: 111–117.
41. Thirumalai AV, Prabhu D and Soniya M (2010) Stable silver nanoparticle synthesizing methods and its applications. *J Bio Sci Res* 1: 259-270.
42. Zhu J, Liao X and Chen HY (2001) Electro-chemical preparation of silver dendrites in the presence of DNA. *Mate Res Bull* 36: 1687-1692.
43. Salkar RA, Jeevanandam P, Aruna ST, Koltypin Y and Gedanken A (1999) The sonochemical preparation of amorphous silver nanoparticles. *J Mater Chem* 9: 1333–1335.
44. Mandal S, Arumugam S, Pasricha R and Sastry M (2011) Silver nanoparticles of variable morphology synthesized in aqueous foams as novel templates. *Bull Mater Sci* 28: 503–510.



45. Jiang H, Moon K, Zhang Z, Pothukuchi S and Wong CP (2006) Variable frequency microwave synthesis of silver nanoparticles. *J Nanoparticle Res* 8: 117-124.
46. Kalishwaralal K, Deepak V, Ramkumarpandian S, Nellaiah H and Sangiliyandi G (2008) Extracellular biosynthesis of silver nanoparticles by the culture supernatant of *Bacillus licheniformis*. *Mater Lett* 62: 4411-4413.
47. Parashar UK, Saxena SP and Srivastava A (2009) Bioinspired synthesis of silver nanoparticles. *Dig J Nanomat Biostruct* 4: 159-166.
48. Vaidyanathan R, Gopalram S, Kalishwaralal K, Deepak V, Pandian SR and Gurunathan S (2010) Enhanced silver nanoparticle synthesis by optimization of nitrate reductase activity. *Colloids Surf B Biointerfaces* 75: 335-341.
49. Kalimuthu K, Babu RS, Venkataraman D, Bilal M and Gurunathan S (2008) Biosynthesis of silver nanocrystals by *Bacillus licheniformis*. *Colloids Surf B Biointerfaces* 65: 150-153.
50. Fu JK, Zhang WD, Liu YY, Lin ZY, Yao BX, Weng SZ and Zeng JL (1999) Characterization of adsorption and reduction of noble metal ions by bacteria. *Chem J Chin Univ* 20: 1452-1454.
51. Kalishwaralal K, Deepak V, Pandiana SBRK, Kottaisamy M, BarathMKS, Kartikeyan B and Gurunathan S (2010) Biosynthesis of silver and gold nanoparticles using *Brevibacterium casei*. *Colloids Surf B Biointerfaces* 77: 257-262.
52. El-Shanshoury AER, ElSilk SE and Ebeid ME (2011) Extracellular biosynthesis of silver nanoparticles using *Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 6633, and *Streptococcus thermophilus* ESh1 and their antimicrobial activities. *ISRN Nanotechnology*. 1-7.
53. Minaeian S, Shahverdi RA, Nohi SA and Shahverdi RH (2008) Extracellular biosynthesis of silver nanoparticles by some bacteria. *J Sci IAU*. 17: 1-4.
54. Mokhtari M, Deneshpojoh S, Seyedbagheri S, Atashdehghan R, Abdi K, Sarkar S, Minaian S, Shahverdi RH and Shahverdi RA (2009) Biological synthesis of very small silver nanoparticles by culture supernatant of *Klebsiella pneumoniae*: the effects of visible-light irradiation and the liquid mixing process. *Mater Res Bull* 44: 1415-1421.

55. Sintubin L, De Windt W, Dick J, Mast J, van der Ha D, Verstraete W and Boon N (2009) Lactic acid bacteria as reducing and capping agent for the fast and efficient production of silver nanoparticles. *Appl Microbiol Biotechnol.* 84: 741-749.
56. Samadi N, Golkaran D, Eslamifar A, Jamalifar H, Fazeli MR and Mohseni FA (2009) Intra/extracellular biosynthesis of silver nanoparticles by an autochthonous strain of *Proteus mirabilis* isolated from photographic waste. *J Biomed Nanotechnol* 5: 247-253.
57. Lengke FM, Fleet EM and Southam G (2007) Biosynthesis of silver nanoparticles by filamentous cyanobacteria from a silver (I) nitrate complex. *Langmuir* 23: 2694-2699.
58. Tanja K, Ralph J, Eva O and Claes-Goran G (1999) Silver-based crystalline nanoparticles, microbially fabricated. *PNAS.* 96: 13611–13614.
59. Mohanpuria P, Rana KN and Yadav SK (2008) Biosynthesis of nanoparticles: technological concepts and future applications. *J Nanopart Res* 10: 507-517.
60. Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, Parischa R, Ajaykumar PV, Alam M, Kumar R and Sastry M (2001) Fungus mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: a novel biological approach to nanoparticle synthesis. *Nano Lett* 1; 515-519.
61. Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, Kumar R and Sastry M (2003) Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. *Colloids Surf B Biointerfaces* 28: 313-318.
62. Verma VC, Kharwar RN and Gange AC (2010) Biosynthesis of antimicrobial silver nanoparticles by the endophytic fungus *Aspergillus clavatus*. *Nanomed.* 5: 33-40.
63. Vigneshwaran N, Ashtaputre NM, Varadarajan PV, Nachane RP, Paralikal KM and Balasubramanya RH (2006) Biological synthesis of silver nanoparticles using the fungus *Aspergillus flavus*. *Mater Lett* 66: 1413-1418.
64. Bhainsa CK and Dsouza FS (2006) Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus funigatus*. *Colloids Surf B Biointerfaces* 47: 160-164.

65. Basavaraja S, Balaji SD, Lagashetty A, Rajasabd AH and Venkataraman A (2008) Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium semitectum*. *Mater Res Bull* 43: 1164-1170.
66. Duran N, Marcato DP, Alves LO, De Souza G, Esposito E (2005) Mechanical aspect of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains. *J Nanobiotechnol* 3: 8-15.
67. Gade A, Ingle A, Bawaskar M and Rai M (2009) *Fusarium solani*: a novel biological agent for extracellular synthesis of nanoparticles. *J Nanopart Res* 11: 2079-2085.
68. Vigneshwaran N, Kathe AA, Varadarajan PV, Nachane RP and Balasubramanya RH (2006) Biomimetics of silver nanoparticles by white rot fungus, *Phaenerochaete chrysosporium*. *Colloids Surf B Biointerfaces* 53: 55-59.
69. Chen JC, Lin ZH and Ma XX (2003) Evidence of the production of silver nanoparticles via pretreatment of *Phoma* sp. 3.2883 with silver nitrate. *Lett Appl Microbiol* 37: 105-108.
70. Jha AK, Prasad K, Prasad K and Kulkarni AR (2009) Plant system: nature's nanofactory. *Colloids Surf B Biointerfaces* 73: 219-223.
71. Chandran SP, Chaudhary M, Pasricha R, Ahmad A and Sastry M (2006) Synthesis of gold nanotriangles and silver nanoparticles using *Aloe vera* plant extract. *Biotechnol Prog* 22: 577-583.
72. Shankar SS, Rai A, Ahmad A and Sastry M (2004) Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using *Neem* (*Azadirachta indica*) leaf broth. *J Colloid Interface Sci* 275: 496-502.
73. Jain D, Kumar DH, Kachhwaha S and Kothari SL (2009) Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their anti microbial activities. *Digest J Nanomaterials Biostructures* 4: 557-563.
74. Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X, Wang H, Wang Y, Shao W and He N (2007) Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf. *Nanotechnol* 18: 1-11.
75. Sathishkumar M, Sneha K, Won SW, Cho CW, Kim S and Yun YS (2009) *Cinnamon zeylanicum* bark extract and powder mediated green synthesis of

- nano- crystalline silver particles and its bactericidal activity. *Colloids Surf B Biointerfaces* 73: 332-338.
76. Sathyavathi R, Krishna MB, Rao SV, Saritha R and Rao DN (2010) Biosynthesis of silver nanoparticles using *Coriandrum sativum* leaf extract and their application in nonlinear optics. *Adv Sci Lett* 3: 138-143.
77. Ahmad N, Sharma S, Singh VN, Shamsi SF, Fatma A and Mehta BR (2011) Biosynthesis of silver nanoparticles from *Desmodium triflorum*: a novel approach towards weed utilization. *Biotechnol Res Int* 2011: 1-8.
78. Bar H, Bhui KD, Sahoo PG, Sarkar P, De PS and Misra A (2009) Green synthesis of silver nanoparticles using latex of *Jatropha curcas*. *Colloids Surf. A Physicochem Eng Asp* 339: 134-139.
79. Gardea-Torresdey JL, Gomez E, Peralta-Videa JR, Parsons JG, Troiani H and Jose-Yacaman M (2003) Alfalfa sprouts: a natural source for the synthesis of silver nanoparticles. *Langmuir* 19: 1357-1361.
80. Mallikarjuna K, Dillip GR, Narasimha G, John SN and Deva PRB (2012) Phytofabrication and characterization of silver nanoparticles from *Piper betle* broth. *Res J Nanosci Nanotechnol* 2: 17-23.
81. Forster MJ, Mulloy B and Nermut MV (2000) Molecular modelling study of HIV p17gag (MA) protein shell utilising data from electron microscopy and X-ray crystallography. *J Mol Biol* 298: 841-857.
82. Leonard CK, Spellman MW, Riddle L, Harris RJ, Thomas JN and Gregory TJ (1990) Assignment of intrachain disulfide bonds and characterization of potential glycosylation sites of the type 1 recombinant human immunodeficiency virus envelope glycoprotein (gp120) expressed in Chinese hamster ovary cells. *J Biol Chem* 265: 10373–10382.
83. Keuk-Jun1 K, Sung WS, Moon SK, Choi JS, Kim JG and Lee1 DG (2008) Antifungal Effect of Silver NPs on Dermatophytes. *J Microbio Biotech* 18: 1482-1484
84. Rastogi SK, Hendricks VJ, Gibson C, Newcombe DA, Branen JR and Branen AL (2010) Ag colloids and Ag Clusters over EDAPTMS-Coated Silica NPs: Synthesis, Characterization, and Antibacterial activity against *Escherichia coli*. *Nanomed* 7: 305-314.
-

85. Shrivastava S, Bera T, Singh SK, Singh G, Ramachandrarao P and Dash D (2009) Characterization of novel anti-platelet properties of silver nanoparticles. ACS Nano 3: 1357-1364.
86. Asha Rani PV, Hande MP and Valiyaveetil S (2009) Anti-proliferative activity of silver NPs. BMC Cell Biology 10: 65.
87. Asha RPV, Mun, GLK and Hande MP (2009) Cytotoxicity and genotoxicity of silver NPs in human cells ACS nano. 3: 279-290.
88. Kemp MM, Kumar A, Mousa S, Dyskin E, Yalcin M, Ajayan P, Linhardt RJ and Mousa SA (2009) Gold and silver NPs conjugated with heparin derivative possess anti-angiogenesis properties. Nanotech 20: 45.
89. Nadeem A, Khanna T and Vohora SB (1997) Analgesic activity of silver preparations used in Indian systems of medicine. Ind Journal of Pharmacol 29: 393-398.
90. Sharma DC, Budania R, Shah M, Jain P and Gaur BL (2004) Hypolipidemic activity of silver preparations in chicks, *Gallus serregineus*. Ind j of exp boil 42: 504-507.
91. Patricia LN, JianFei W, Edward E, Tredget MD and Robert EB (2008) Anti-inflammatory activity of nanocrystalline silver in a porcine contact dermatitis model. Nanomed 4: 241-251.
92. Kailash CB and Paul JS (2007) Effects of Nanocrystalline Silver (NPI 32101) in a Rat Model of Ulcerative Colitis. Dig Dis Sci 52: 2732-2742.
93. Satapathy SR, Mohapatra P, Preet R, Das D, Sarkar B, Choudhuri T, Wyatt MD, and Kundu CN (2013) Silver-based nanoparticles induce apoptosis in human colon cancer cells mediated through p53. Nanomedicine (Lond) 8: 1307-1322.
94. Butler RN (2008) Non-invasive tests in animal models and humans: A new paradigm for assessing efficacy of biologics including prebiotics and probiotics. Curr Pharma Des 14: 1341-1350.
95. Bengmark S (2000) Colonic food: Pre and Probiotics. American J Gastroenterol 95: 5-7.
96. McFarland LV (2000) Beneficial microbes: health or hazard. European J Gastroenterol Hepatol 12:1069-1071.

97. Kaur IP, Chopra K (2002) Saini A. Probiotics: potential pharmaceutical applications. *European J of Pharm Sci* 15: 1-9.
98. Lilly DM, Stillwell RH (1965) Probiotics: Growth promoting factors produced by micro-organisms. *Scientifica* 147: 747-748.
99. Food and Agriculture Organization of the United Nations and World Health Organization Report. [http:// www.fao.org/es/ESN/probio/ probio.html](http://www.fao.org/es/ESN/probio/probio.html) (accessed on 16/6/2013)
100. Vimala Y, Dileep P (2006) Some aspects of probiotics. *Indian J Microbiol* 46: 1-7.
101. Vandenberg PA (1993) Lactic acid bacteria, their metabolic products and interference with microbial growth. *FEMS Microbiol Rev* 12: 221-238.
102. Mack DR, Michail S, Wei S, McDougall L (1999) Hollingsworth MA. Probiotics inhibit enteropathogenic *E. coli* adherence in vitro by inducing intestinal mucin gene expression. *American J Physiol* 276: 941-950.
103. Walker WA (2002) Role of nutrients and bacterial colonization in the development of intestinal host defense. *J Paediatrics Gastroenterol Nutrition* 30: 2-6.
104. Madsen K, Cornish A, Soper P, McKaigney C, Jijon H, Yachimec C (2001) Probiotic bacteria enhance murine and human intestinal epithelial barrier function. *Gastroenterol* 121: 580-591.
105. Isolauri E, Kaila M, Mykkanen H, Ling WH, Salminen S (1994) Oral bacteriotherapy for viral gastroenteritis. *Digestive Diseases and Sciences* 39: 2595-2600.
106. Szajewska H, Kotowska M, Murkowicz JZ, Armanska M, Mikolajczyk W (2001) Efficacy of *Lactobacillus GG* in prevention of nosocomial diarrhoea in infants. *J Paediatrics* 138: 361-365.
107. Mao Y, Nobaek S, Kasravi B, Adawi D, Stenram U, Molin G (1996) The effects of *Lactobacillus* strains and oat fibre on methotrexate-induced enterocolitis in rats. *Gastroenterology* 111: 334-344.
108. Arvola T, Laiho K, Torkkeli S, Mykkänen H, Salminen S, Maunula L (1999) Prophylactic *Lactobacillus GG* reduces antibiotic-associated diarrhoea in

- children with respiratory infections: A randomized study. *Paediatrics* 104: 64-65.
109. Begmark S (2000) Bacteria for optimal health. *Nutrition* 16: 611-615.
110. Isolauri E, Arvola T, Sutas Y, Moilanen E, Salminen S (2000) Probiotics in the management of atopic eczema. *Clinical and Experimental Allergy* 30: 1604-1610.
111. Rosenfeldt V, Benfeldt E, Neilson SD, Michelsen KF, Jappesen DL, Valerius NH (2003) Effects of probiotics Lactobacilli strains in children with atopic dermatitis. *Journal of Allergy and Clinical Immunology* 111: 390-395.
112. Von der Weid T, Billiard C, Schiffrin E (2001) Induction by a Lactic acid bacterium of a population of CD4 T cells with low proliferative capacity that produces transforming growth factor and IL 10. *Clinical Diagnostics and Laboratory Immunology* 8: 695-701.
113. Saulnier DMA, Hutt P, Mikelsaar M, Bosscher D, Gibson G, Kolida S (2007) Effects of a symbiotic on biomarkers of oxidative stress and faecal microbiota in healthy adults: results of a cross-over double-blind placebo-controlled trial. *Proceedings of Nutrition Society* 66: 101.
114. Ito M, Sawada H, Ohishi K (2001) Suppressive effects of Bifidobacteria on lipid peroxidation in the colonic mucosa of iron-overloaded mice. *J Dairy Sci* 84: 1583-1589.
115. He X, Kim SS, Park SJ, Seong DH, Yoon WB, Lee HY (2010) Combined effects of probiotic fermentation and high pressure extraction on the antioxidant, antimicrobial, and antimutagenic activities of deodeok (*Codonopsis lanceolata*). *J Agriculture Food Chem* 58: 1719-1725.
116. Mack DR, Michail S, Wei S, McDougall L, Hollingsworth MA (1999) Probiotics inhibits enteropathogens *E. coli* adherence in vitro by inducing intestinal mucin gene expression. *American Journal of Physiology* 276: 941-950.
117. Kabir AM, Aiba Y, Takagi A, Kamiya S, Miwa T, Koga Y (1997) Prevention of *Helicobacter pylori* infection by lactobacilli in agnotobiotic murine model. *Gut* 41: 49-55.

118. Szajewska H, Kotowska M, Murkowicz JZ, Armanska M, Mikolajczyk W (2001) Efficacy of Lactobacillus GG in prevention of nosocomial diarrhoea in infants. *J Paediatrics* 138: 361-365.
119. Shornikova AV, Isolauri E, Burnakova L, Lukovnikova S, Vesikari T (1997) A trial in the Karelian republic of oral rehydration and Lactobacillus GG for treatment of acute diarrhoea. *Acta Paediatrica* 86: 460-465.
120. Pant AR, Graham SM, Allen SJ, Harikul S, Sabchareon A, Cuevas L (1996) Lactobacillus GG and acute diarrhoea in young children in the tropics. *Tropical J Paediatrics* 42: 162-165.
121. Bartlett JG (1992) Antibiotics associated diarrhea. *Clinical Infections and Diseases* 15: 573-581.
122. Delia P, Sansotta G, Donato V, Frosina P, Messina G, DeRenzi C (2007) Use of probiotics for prevention of radiation induced diarrhea. *World J Gastroenterol* 13: 912-915.
123. McFarland LV (2007) Meta-analysis of probiotics for the prevention of traveller's diarrhea. *Traveller Medical and Infectious Diseases* 5: 97-105.
124. Muthukumarasamy P, Holley RA (2006) Microbial and sensory quality of dry fermented sausages containing alginate microencapsulated Lactobacillus reuteri. *Int J Food Microbiol* 111: 164-166.
125. Ataie-Jafari A, Larijani B, Alavi MH, Tahbaz F (2009) Cholesterol-lowering effect of probiotic yogurt in comparison with ordinary yogurt in mildly to moderately hypercholesterolemic subjects. *Annals of Nutrition and Metabolism* 54: 22-27.
126. Jones ML, Chen H, Ouyang W, Metz T, Prakash S (2004) Microencapsulated genetically engineered Lactobacillus plantarum 80 (pCBH1) for bile acid deconjugation and its implication in lowering cholesterol. *J Biomed Biotechnol* 1: 61-69.
127. Armuzzi A, Cremonini F, Ojetti V, Bartolozzi F, Canducci F, Candelli M (2001) Effect of Lactobacilli GG supplementation on Antibiotic-Associated Gastrointestinal side effects during H. pylori eradication therapy: A pilot study. *Digestion* 63: 1-7.



128. Parvez S, Malik KA, Kang S, Kim HY (2006) Probiotics and their fermented foodproducts are beneficial for health. *J Applied Microbiol* 100: 1171-1185.
129. Kim SH, Yang SJ, Koo HC (2001) Inhibitory activity of *Bifidobacterium longum* HY8001 against Vero cytotoxin of *Escherichia coli* O157:H7. *J Food Protection* 64: 1667-1673.
130. Silva AM, Barbosa FH, Duarte R, Vieira LQ, Arantes RM, Nicoli JR (2004) Effect of *Bifidobacterium longum* ingestion on experimental salmonellosis in mice. *Applied Microbiology* 97: 29-37.
131. Vanderhoof JA, Young RJ (1998) Use of probiotics in childhood gastrointestinal disorders. *JPediatricGastroenterol Nutrition* 27: 323-332.
132. Gorbach SL, Goldin BR (1992) Nutrition and the gastrointestinal micro flora. *Nutrition Reviews* 50: 378-381.
133. Ishikawa H, Akedo I, Umesaki Y, Tanaka R, Imaoka A, Otani T (2003) Randomized controlled trial of the effect of bifidobacteria-fermentedmilk on ulcerative colitis. *J American College of Nutrition* 22: 56-63.
134. Kruis W, Schutz E, Frick P, Fixa B, Judmaier G, Stolte M (1997) Double-blindcomparison of an oral *Escherichia coli* preparation and mesalazine inmaintaining remission of ulcerative colitis. *Aliment Pharmacology and Therapy* 11: 853-858.
135. Shiba T, Aiba Y, Ishikawa H (2003) The suppressive effect of *Bifidobacteria* on *Bacteroides vulgatus*, a putative pathogenic microbe in inflammatory bowel disease. *Microbiology and Immunology* 47: 371-378.
136. Niedzielin K, Kordecki H, Birkenfeld BA (2001) Controlled, double blind, randomized study on the efficacy of *Lactobacillusplantarum* 299V in patients with irritable bowel syndrome. *European Journal of Gastroenterology and Hepatology* 13: 1143–1147.
137. Steidler L, Hans W, Schotte L (2000) Treatment of murine colitis by *Lactococcus lactis* secreting interleukin-10. *Scientifica* 289: 1352-1355.
138. Kampman E, Goldbohm RA, Vanden Brandt PA, Van't Veer P (1994) Fermented dairyproducts, calcium and colorectal cancer in the Netherland cohort study. *Cancer Res.* 54: 363-365.

139. Caplan MS; Jilling T (2000) Neonatal necrotizing enterocolitis: possible role of probiotic supplementation. *J Pediatr Gastroenterol Nutr* 30: 18-22.
140. Pothoulakis C, Kelly CP, Joshi MA, Gao N, O'Keane CJ, Castagliuolo I (1993) *Saccharomyces boulardii* inhibits *Clostridium difficile* toxin A binding and enterotoxicity in rat ileum. *Gastroenterol* 104: 1108-1115.
141. Aso Y, Akaza H, Kotake T, Tsukamoto T, Imai K, Naito S (1995) Preventive effects of a *Lactobacilli casei* preparation on the recurrence of superficial bladder cancer in a double-blind trial. *European J Urol* 27: 104-109.
142. Saran S, Gopalan S, Krishna TP (2002) Use of fermented foods to combat stunting and failure to thrive. *Nutrition* 18: 393-396.
143. Jose MS, Adel AH, Nancy M, Robert HY (2004) Long term consumption of infant formulas containing live probiotic bacteria: tolerance and safety. *American Journal of Clinical Nutrition* 79: 261-267.
144. Vanderhoof JA (2001) Probiotics: future directions. *American J Clinical Nutrition* 73: 1152-1155.
145. Baron MA (2009) Patented strain of *Bacillus coagulans* increased immune response to viral challenge. *Postgraduate Med J* 121: 114-118.
146. Canducci F, Cremonini F, Armuzzi A (2002) Probiotics and *Helicobacter pylori* eradication. *Digestive and Liver Disease* 34: 81-83.
147. Scrimshaw NS, Murray AB (1988) The acceptability of milk and milk products in populations with a high prevalence of lactose intolerance. *American J Clinical Nutrition* 48: 1083-1084.
148. Sanders ME (1993) Summary of the conclusions from a consensus panel of experts on health attributes on lactic cultures: significance to fluid milk products containing cultures. *Journal of Dairy Science* 76: 1819-1828.
149. Shaukat A, Levitt MD, Taylor BC, MacDonald R, Shamliyan TA, Kane RL (2010) Systematic review: Effective management strategies for lactose intolerance. *Annals of Internal Medicine* 152: 797-803.
150. Sanders ME (1993) Summary of the conclusions from a consensus panel of experts on health attributes on lactic cultures: significance to fluid milk products containing cultures. *J Dairy Science* 76: 1819-1828.

151. Yakabe T, Moore EL, Yokota S (2009) Safety as Assessment of *Lactobacillus brevis* KB290 as a probiotic strain. *Food Chem Toxicol* 47: 2450-2453.

## **3 RATIONALE AND OBJECTIVES OF THE STUDY**

### **3.1 Rationale**

The inflammation of large intestine known as Ulcerative Colitis (UC) affects about five lakh people annually, predominantly under the age of 30, and can ultimately enhance the chances of growing large bowel cancer. Foremost complications of UC include toxic mega colon, intestinal perforation, and massive bleeding. Toxic mega colon is characterized by extensive distension of the colon (>6 cm) and sepsis-like syndrome. Chronic blood loss leads to microcytic anaemia. Complication of chronic ulcerative colitis may lead to colon cancer. At present, first-line cancer therapy involves invasive processes such as catheters to permit chemotherapy to shrink any tumour present and surgical removal of the tumour followed by a regimen of chemotherapy and/or radiation therapy. The main goal of chemotherapeutic agent and radiation therapy is to destroy the cells of cancer. In this, the usefulness of the therapy is directly associated to the treatment's capacity to target and kill the tumour cells and not affecting healthy cells as much as possible. This, in turn, is directly related to patient for their quality of life and life expectancy. Unfortunately, this strategy often fails because of recurrent or metastatic disease. In some cases, the patients must discontinue the chemotherapy before the drug has a chance to eradicate the tumour because of its intense side effects. Another dilemma associated with chemotherapy is the inherent insolubility of most of the anticancer drugs in water, which necessitates the use of pharmaceutical solvents for their clinical administration. The use of these solvents may have life-threatening effects. Antitumor activity of certain metallic compound is well proven. Anticancer property of cis-platin was exposed in 1969 that encouraged the exploration of other metal having anticancer activity. Biocompatibility of silver NPs has been confirmed by various in vitro and in vivo research studies and their use from the ancient time. The antiangiogenic activity of silver NPs is proved<sup>38</sup>. Research carried out for antiangiogenic activity indicates that most of the molecules possessing antiangiogenic activity are organic in nature and have substantial toxicities like perforations of gastrointestinal tract. Hence, opportunities exist to search the potentials of

antiangiogenic molecule which may be inorganic compound with minute or no severe side effects. Silver bhasma is a perfect model to search such potentials because biocompatibility of silver NPs has been already confirmed by their use since ancient time. Recently it is proved scientifically by Inder D et al (2011) who had carried out toxicity of raupya bhasma on mice and concluded that minimum toxic dose of raupya bhasma was 1.5 g/kg and LD<sub>50</sub> was 2.0 g/kg. However, the therapeutic dose for human being is 125 mg only.

Silver bhasma is selected for treatment of colon cancer because, bhasmas are claimed to be biologically produced nanoparticles. When metal is converted into metal bhasma the toxicity of metal gets reduced while therapeutic activity gets increased. The cytotoxicity, antiproliferative and anti angiogenic activity of silver NPs has been reported. Recently it is reported that SNPs possess activity against inflammation, ulcerative colitis and colon cancer.

### **3.2 Objectives**

The necessity of present investigation is to develop formulation of Raupya (Silver) bhasma for colon targeted drug delivery with the following objectives.

- Preparation of raupya (Silver) bhasma
- Evaluation of raupya bhasma by ancient Ayurveda literature
- Characterisation of raupya bhasma using modern analytical technique
- Evaluation of anti-inflammatory activity of raupya bhasma
- Screening of raupya bhasma for anticancer (colon cancer) activity
- Preparation and evaluation of microsphere of raupya bhasma
- Preparation and evaluation of coated granule of raupya bhasma

Development of animal sparing dissolution media for testing the drug release of polysaccharide based formulations used for colon specific drug delivery.

### **3.3 Plan of work**

- PREPARATION AND CHARACTERISATION OF ROUPYA BHASMA
  - Preparation of bhasma*
- Samanya sodhana (general purification)

- Vishesha sodhana (special purification)

- Marana

*Evaluation of RB by traditional method*

*Characterisation of RB by modern analytical technique*

- Standard plot of RB
- Determination of silver content
- Sample preparation
- Flame test
- SEM
- Particle size analysis
- XRD (X-ray diffraction analysis)
- TGA
- Anti-inflammatory activity of silver bhasma
- DRUG EXCIPIENT EXTRACTION

*On the basis of visual observation*

*On the basis of FTIR spectroscopy*

- FORMULATION AND EVALUATION OF MICROSPHERE FOR CTDD

*Formulation of microsphere*

*Characterisation of microsphere*

- Percentage yield

*In vitro release of study of microsphere*

- Preparation of Dissolution Media
- In vitro drug release using human fecal slurries
- In vitro drug release using goat caecal content
- In vitro drug release using probiotic culture

- FORMULATION OF COATED GRANULES OF RB AND THEIR EVALUATION FOR CTDD

*Development of formulation (Guar gum and eudragit FS 30D coated RB loaded granule*

- Preparation of RB loaded granule core
- Coating of the Prepared Granules

*Characterisation of coated granules*

### ***Chapter 3: Rationale and Objectives of the Study***

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#### *In vitro release of coated granules*

- Preparation of Dissolution Media
- In vitro drug release using goat caecal content
- In vitro drug release of formulation using probiotic culture

## 4 EXPERIMENTAL

### 4.1 MATERIALS AND METHODS

Chemicals/ Materials required in study are given below in Table 4.1

**Table 4.1: List of chemicals/ Materials required in study**

Chemicals/ Materials	Manufacturer
Acetic acid	Sd Fine Chemicals Limited, India
Castor oil	Central Drug House, India
Dry milk power	Everyday milk products, New Delhi, India
Eudragit FS 30 D	Evonik Industries, Germany
Glutaraldehyde	Central Drug House, India
Guar gum	Molychem Manufacturers (P) Ltd., Mumbai, India
Honey	Dabur India Ltd., New Delhi, India
Hydrochloric acid	Sd Fine Chemicals Limited, India
Iso propyl alcohol	Loba Chemie Pvt. Ltd., Mumbai, India
Nitric acid	Sd Fine Chemicals Limited, India
Probiotic capsules-Velgut	Eris life sciences Pvt.Ltd.
Potassium dihydrogen phosphate	Loba Chemie Pvt. Ltd., Mumbai, India
Sodium chloride	Loba Chemie Pvt. Ltd., Mumbai, India
Sodium Dihydrogen Phosphate	Sd Fine Chemicals Limited, India
Sodium Hydroxide pellets	Loba Chemie Pvt. Ltd., Mumbai, India
Sulphuric acid	Sd Fine Chemicals Limited, India
Twin 80	Sd Fine Chemicals Limited, India
Xanthan gum	Molychem Manufacturers (P) Ltd., Mumbai, India



## **Instruments**

The sources/ model of the instruments used during the studies are given in the following Table 4.2:

**Table 4.2: List of instrument**

Instrument Name	Model/source
Centrifuge	REMI, India
Coating Pan	Raj Analytical service., Mohali, India
Dissolution Test Apparatus	Lab India DS 800, India
Electronic balance (BL-220H)	Shimadzu Co. Ltd, Japan
FTIR Spectrophotometer	Shimadzu 8400 S, Singapore
Hot Air Oven	Navyug, India
Hot plate	Popular, India
Humidity Control Cabinet	Navyug Q-5247, Ambala Cantt., India
Laser diffraction particle size analyzer	Mastersizer 2000 Ver. 5.22, Malvern Instruments Ltd., Malvern, UK
Magnetic Stirrer	Remi, India
Mechanical stirrer	Remi, India
pH Meter	Systronics, India
Photomicroscope	KYOWA Getner 10390
Ultra Sonicator (Bath)	Raj analytical services, India

## **4.2 Standard plot of raupya bhasma**

Solution of raupya bhasma was prepared in 10-50 ppb and subjected to ICP-MS. The value of intensity obtained for each concentration was noted.

### **4.3 Preparation and characterisation of RB bhasma**

#### **4.3.1 Preparation of bhasma**

Raupya bhasma was prepared as per Ayurvedic literature which involved sodhana (purification) and marana (detoxification).

##### **4.3.1.1 Samanya sodhana (General purification)**

Silver foil was heated till red hot and dipped subsequently in *kanji, takra, kulatha, kwatha, gomutra and tila taila* three times each.

##### **4.3.1.2 Vishesha sodhana (special purification)**

Sodhit silver was again heated till red hot and dipped in *nimbu swarasa* (lemon juice). The process was repeated seven times using fresh juice in each dip.

##### **4.3.1.3 Marana**

Sodhit silver leaves were soaked in lemon juice and triturated for making pellets. Pellets were kept in electric furnace and temperature was gradually increased to 550° C. The process was repeated 14 times to obtain desired quality of bhasma<sup>1,2</sup>. In each step lemon juice was added.

#### **4.3.2 Evaluation of RB by traditional method**

The quality of bhasma can be evaluated by traditional method for Nishchandravam, Rekhapurnata, Varitara test and Unama test<sup>1</sup>.

#### **Characterisation of RB by modern analytical technique**

##### **4.3.2.1 Determination of silver content**

Silver content in raupya bhasma was estimated using ICP-MS [Elan-DRCe (Perkin Elmer)]. Instrumental conditions were as follows:

Power	1100Watt
Nebulizer gas flow	0.93 litre/min
Auxiliary gas flow	1.5litre/min
Plasma gas flow	15 litre/min

#### **4.3.2.2 Sample preparation**

Silver bhasma (0.1g) was weighed accurately and transferred to a 100 ml volumetric flask, followed by 5 ml AR grade nitric acid. Flask containing silver bhasma and nitric acid was heated for 10 min on hot plate or until the sample was digested. After digestion the flask was allowed to cool and volume was made up to 100 ml. The sample was filtered through 0.45  $\mu$  filter paper. The final solution was subjected to ICP-MS.

Flame test

Sample of bhasma was taken in spatula and heated directly over flame.

#### **4.3.2.3 SEM**

To determine shape and surface features of bhasma scanning electron microscopy was used. The sample of bhasma was prepared by lightly sprinkling the bhasma on a double adhesive tape, which was fixed on an aluminium stub for analysis using SEM. The stubs were coated with gold using a gold sputter coater in a high vacuum evaporator, and sample was analysed by SEM at 15 kV.

#### **4.3.2.4 Particle size analysis**

Particle size of bhasma was analysed using a laser diffraction particle size analyser (Zetasizer Nano ZS90). Bhasma were suspended in the cavity of the particle size analyser containing distilled water, and subjected to dynamic light scattering (DLS) to obtain the particle size of bhasma.

#### **4.3.2.5 XRD (X-ray diffraction analysis)**

The X-ray diffraction pattern of raupya bhasma was recorded on X-ray diffractometer (X'pert pro Netherland) using  $\text{CuK}\alpha$  radiation,  $\lambda = 1.5405980 \text{ \AA}$ , filtered by nickel foil over the range  $20.0 - 80.0^\circ$ . Tension and current applied were 40 KV and 30 mA respectively.

#### **4.3.2.6 TGA**

TGA tool was utilized to determine degradation behaviour of drug at exposure of temperature, and the drug amount with no effect of temperature using Perkin Elmer TGA7

## **4.4 Anti-inflammatory activity of silver bhasma**

### **4.4.1 Preparation of test solution**

Test solution of raupya bhasma of varying concentration (20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml) was prepared in phosphate buffer of pH 7.4.

### **4.4.2 Preparation of standard solution**

Standard solution of diclofenac sodium of varying concentration (20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml) was prepared in phosphate buffer of pH 7.4.

### **4.4.3 Evaluation of in vitro anti-inflammatory activity**

In vitro anti-inflammatory activity of raupya bhasma against denaturation of protein was carried out as per method described by Mizushima and Kobayashi<sup>3</sup>. The 5ml of reaction mixture comprised of 0.2 ml of egg albumin (obtained from hen's egg), 2.8 ml of phosphate buffer saline (pH 7.4) and 2 ml of different concentrations of raupya bhasma so as to obtain the final concentration of 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml. Triple-distilled water in equal volume served as control. Subsequently, the mixtures were incubated using BOD incubator (Navyug, India Ltd) at (37±2) °C for 30 min and heated for 15 min at 70°C. On cooling, the absorbance was measured at 660 nm (Shimadzu 1800, Japan) by using vehicle as blank. Ostwald viscometer was used to determine the viscosity of each sample. Various concentration (20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml) of Diclofenac sodium was used as reference drug and treated likewise for determination of absorbance and viscosity. The percentage inhibition of protein denaturation was determined by using formula given as follow:

$$\% \text{ inhibition} = (V_t / V_c - 1) \times 100$$

Where,  $V_t$  = absorbance of test sample,  $V_c$  = absorbance of control. The drug concentration for 50% inhibition (IC<sub>50</sub>) was determined by plotting percentage inhibition with respect to control against treatment concentration.

## **4.5 In vitro anticancer activity of raupya bhasma**

In vitro anti-cancer activity was performed against colon cancer cell line HCT 116 and compared with standard drug (5FU).

### **4.5.1 Cell culture, growth conditions and treatments-**

Human colon cancer HCT-116 cell line was obtained from European Collection of Cell Cultures (ECACC, Sigma Aldrich). The cell line was grown in minimum essential medium (MEM) supplemented with 10% heat inactivated fetal bovine serum (FBS), penicillin (100 units/ml), streptomycin (100 µg/ml), L-glutamine (0.3 mg/ml) and NaHCO<sub>3</sub> (3.8mg/ml). Cells were grown in CO<sub>2</sub> incubator (Thermocon Electron Corporation, USA) at 37°C in an atmosphere of 95% air and 5% CO<sub>2</sub> with 98% humidity. Cells grown in monolayer cultures were detached with trypsin (0.1% w/v) / EDTA (1mM) solution. Soon after, cells were ready to detach, the trypsin / EDTA solution was removed. Cells were dispersed gently by pipetting in complete growth medium, centrifuged at 200 xg, 4°C for 5min. The required cell suspension (0.6x10<sup>4</sup>/100µl) was distributed into 96 well plate and incubated in CO<sub>2</sub> incubator. After 16h cells were refreshed with fresh complete DMEM medium. Cells grown in semi-confluent stage (approx. 70% confluent) were treated with tested material while the untreated control cultures received only the respective vehicle (< 1%).

### **4.5.2 Cell proliferation assay (MTT)**

MTT assay is a quantitative colorimetric method for the determination of cell survival and proliferation. The assessed parameter is the metabolic activity of viable cells. Metabolically active cells reduce pale yellow tetrazolium salt (MTT) to a dark blue water-insoluble formazan, which can be, after solubilization with DMSO, directly quantified. The absorbance of the formazan directly correlates to the number of viable cells. The HCT 116 cells were plated in 96-well plates at a density of 6000 cell/well in 100µL of medium per well over night. Next day cultures were incubated with different concentrations of test material and incubated for 48 h. The dye at a concentration of 2.5mg/ml [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] (MTT) was added for 4 h. The supernatant was aspirated and MTT-formazon crystals dissolved in 150µL of DMSO; OD was measured at λ540 (reference wavelength, λ620) on an

ELISA reader (BioTek.). Cell growth was calculated by comparing the absorbance of treated versus untreated cells.

Cellular morphology of prostate cancer PC-3 cells was also visualized under inverted microscope (Olympus-1X70, magnification 20X).

## **4.6 Drug excipient extraction**

### **4.6.1 On the basis of visual observation**

Visual observations are the elementary tool to decide the compatibility of the formulation, drug polymer interactions forming colour when incompatibility occur in drug-polymers and polymer-polymers during the compatibility studies. Difference between formulation features like colour, powder form, agglomerates, dryness, and hygroscopicity of drug-polymers and polymer-polymer after and before compatibility studies were noted by the visual inspection to determine the compatibility of various excipients with the drug in the formulation.

Under this specified amount of drug and the excipients (guar gum, xanthan gum) were weighed separately, mixed in ratio 1:1 and then filled in separate glass vials. The vials were then stored under two different conditions at 2-8°C and at 40°C with 75% RH in a humidity chamber at a temperature of 40°C/75% RH for one month. Vials containing pure drug and pure polymers were also kept in similar conditions. Observation were recorded on 0th day, 7th day, 15th day and 30th day.

### **4.6.2 On the basis of FTIR spectroscopy**

Drug polymer compatibility a study was carried out to determine the possibility and extent of interaction of RB with various polymers used in the formulation like guar gum and xanthan gum. The drug was mixed with each of the above polymer in 1:1 ratio in glass vials and analysed by FTIR spectroscopy. The spectrum was recorded for RB, guar gum, xanthan gum, RB + guar gum, RB + xanthan gum, raupya bhasma + guar gum + xanthan gum. Samples were prepared in KBr disk (2mg sample in 200mg KBr) with a hydraulic press by applying a pressure of 8-9 tons for 2 min. The disks were scanned over a wave number range of 4000–400 cm<sup>-1</sup> and the resolution was kept as 4 cm<sup>-1</sup>.

## 4.7 Formulation and evaluation of microsphere for colon targeted drug delivery

### 4.7.1 Formulation of microsphere

Microspheres of raupya bhasma were formulated using emulsification polymerisation method<sup>4</sup>. Aqueous dispersion of guar gum and xanthan gum was dispersed in a 100 ml of cold water containing the drug (0.5g) and kept for swelling for 2 h). After swelling, the mixture of drug and polymer were dispersed in castor oil (100 ml) containing 2 g of tween 80 and 0.1% silicon oil with continuous stirring using a mechanical stirrer at a speed of 4000 rpm. After thorough uniform mixing, 0.2 mL of concentrated H<sub>2</sub>SO<sub>4</sub> added was followed by 3 ml of glutaraldehyde. The mixture was stirred at a constant speed for 4 h. at 50°C. The microspheres were collected by sedimentation followed by decantation of oil. The microspheres were washed with several fractions of isopropyl alcohol. The formulation was optimised using 22 factorial design as shown in Table 4.3

**Table 4.3: Composition of formulation of microsphere**

Formulation	Composition		
	Guar gum (g)	Xanthan gum (g)	Raupya bhasma (g)
F1	1	0.5	0.5
F2	0.5	1	0.5
F3	0.5	0.5	0.5
F4	1	1	0.5

### 4.7.2 Characterisation of microsphere

#### 4.7.2.1 Percentage yield

To calculate the % yield, the prepared formulation was divided by the total amount of polymer and raupya bhasma taken in the preparation of the formulation by using given formula:

$$\text{Percentage yield} = \frac{\text{Actual yield of formulation}}{\text{Total weight of polymers and drug}} \times 100$$

#### **4.7.2.2 Determination of raupya bhasma included in formulation**

The amount of raupya bhasma in microsphere was determined by placing microsphere in phosphate buffer saline (PBS, pH 7.4) for 48 h at 37°C with vigorous stirring. The concentration of raupya bhasma was determined using ICP-MS. The percentage of loading efficiency and content was expressed in the following equation.

$$\text{Loading efficiency (\%)} = \frac{\text{Weight of loaded drug in microsphere}}{\text{Initial feeding weight of drug}} \times 100$$

$$\text{Loading content (\%)} = \frac{\text{Weight of loaded drug in microsphere}}{\text{Weight of microsphere}} \times 100$$

#### **4.7.2.3 Surface associated drug content**

Microspheres of raupya bhasma were assessed for drug content which was associated with surface of formulation. For the purpose, each batch of 100 mg of formulation was shaken in 20 ml of 0.1N hydrochloric acid for 5 min and filtered using Whatman filter paper. Surface associated drug content in filtrate was determined using ICP-MS. All the investigations were conducted thrice (n=3).

$$\text{Surface associated drug content} = \frac{\text{Amount of drug present in filtrate}}{\text{Amount of drug used in formulation}} \times 100$$

#### **4.7.2.4 Particle Size Analysis**

Particle size of microsphere was analysed by a laser diffraction particle size analyser, microspheres of raupya bhasma were suspended in the cavity of the particle size analyser containing double distilled water, and the particle size was calculated by means of the software.

#### **4.7.2.5 Morphology**

The morphology of microsphere of raupya bhasma were evaluated using scanning electron microscopy (SEM) (JEOL 100-CX USA Inc, Peabody, MA).

Scanning electron microscopy (SEM) was used to determine shape and surface characteristics of microspheres. The samples of microsphere of raupya bhasma for



evaluation using SEM were prepared by lightly sprinkling the microsphere on a double adhesive tape, which was fixed on an aluminium stub. The aluminium stubs were coated with gold using a gold sputter coater in a high vacuum evaporator, and samples were observed by SEM at 10kV.

#### **4.7.2.6 Equilibrium Swelling Studies of formulation**

Accurately weighed formulation (100 mg) was transferred in PBS (pH 7.4) and kept for swelling until its weight became constant. The formulation was taken out and blotted with filter paper and the weight difference was measured. The degree of swelling ( $\alpha$ ) of formulation was calculated using the given formula:

$$\alpha = \frac{w_g - w_o}{w_o}$$

Where,  $w_o$  and  $w_g$  are the initial weight of the microspheres and the weight of the microspheres at equilibrium swelling in the medium respectively.

#### **4.7.2.7 Flow properties**

##### **4.7.2.7.1 Angle of repose**

Angle of repose was determined by funnel method. Formulation was poured through a funnel that can be elevated vertically until extreme cone height ( $h$ ), was obtained. Diameter of heap, ( $D$ ), was determined. Following formula was used to calculate angle of repose.

$$\tan \theta = \frac{h}{r},$$

$$\theta = \tan^{-1} \left( \frac{h}{r} \right)$$

Where,  $\theta$  = Angle of repose,  $h$  = height of the pile (cm) and  $r$  = radius of the pile (cm).

### **4.7.3 In vitro release of study of microsphere**

#### **4.7.3.1 Preparation of Dissolution Media**

##### **4.7.3.1.1 Preparation of fresh human fecal content medium**

Human fecal slurry (freshly prepared) is usually used to evaluate fermentation of polysaccharides (non-starch). Human faecal slurries were prepared by homogenisation of fresh faeces in 0.1 M sodium phosphate buffer (pH 6.8) by supplying CO<sub>2</sub> to maintain

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anaerobic conditions. Freshly prepared human fecal slurry was finally added to the dissolution media to give a final dilution of 4% w/v. All the above mentioned processes were carried out in anaerobic condition by supply of carbon dioxide<sup>5</sup>.

#### 4.7.3.1.2 Preparation of goat caecal medium

Fresh caecal content of goat was procured from local market of Phagwara and kept in desiccator under anaerobic conditions. Accurately weighed caecal content were suspended in the pH 6.8 buffer under anaerobic condition. Finally 4 % w/v caecal medium was prepared by adding dissolution media. All the above procedures were carried out anaerobically in presence of carbon dioxide<sup>6</sup>.

#### 4.7.3.1.3 Preparation of Probiotic culture medium

Dissolution using probiotic culture is required to active probiotic in suitable media.

Prebiotic and probiotic capsule brand name: Velgut (Eris life sciences Pvt.Ltd.)

#### **Composition of culture**

*Bifidobacterium breve*

*Bifidobacterium longum*

*Bifidobacterium infantis*

*Lactobacillus acidophilus*

*Lactobacillus plantarum*

*Lactobacillus casei*

Total 5 billion

*Lactobacillus rhamnosus*

*Streptococcus thermophilus*

*Saccharomyces boulardi*

Fructooligosaccharide-100mg

#### 4.7.3.1.4 Preparation of media

A 5% w/v tempered grade honey was added to 12 % w/v non-fat dry milk (NDM) in a volumetric flask of 100 ml capacity and final volume was adjusted up to 100 ml with sterile water. Then mixture was subjected to the process of pasteurization (heating up to 70 °C for 30 minutes and cooled to 37 °C). A capsule of probiotic powder was transferred in few ml of sterile water and was inoculated in the pasteurized milk media.

Pasteurised milk media containing probiotics was incubated at 37 °C for 48 h under anaerobic conditions. The bacteria were counted in culture media. It was found to be  $9.8 \times 10^{10}$  CFU/ml ( $10^{11}$ - $10^{12}$  CFU/ml normal micro biota count in colon). The probiotic media was added to dissolution media for evaluation of drug release of microbial triggered drug delivery to colon<sup>7</sup>.

#### **4.7.3.2 In vitro drug release using human fecal slurries**

Dissolution studies for colon specific drug delivery were carried out using human fecal slurries (freshly prepared) for formulation F4. Basket type dissolution test apparatus (USP I) with minor alteration in process was used for evaluation of release of raupya bhasma in the presence of human fecal slurries (freshly prepared). Gradient pH dissolution method using human fecal contents was used to assess the release of raupya bhasma from formulations meant for drug delivery to colon.

The experiments were conducted using beaker (250 ml) submerged in water containing pots of dissolution apparatus. A capsule was transferred to each pot having the dissolution medium. The in vitro release of formulation was started in 150 ml media of pH 1.2 using 100 rpm at  $37 \pm 0.5^\circ\text{C}$  for the first 2 h. After 2 h the pH of the dissolution media was maintained to 6.8 by addition of 50ml phosphate buffer and sodium hydroxide solution (q.s.). The study was continued for 4 h. At the end of 4 h, the media was made anaerobic by degassing with carbon dioxide for 15 min. Undissolved oxygen in the media was removed by supply of carbon dioxide to media. After that 4% w/v fecal slurries (freshly prepared) was transferred to the dissolution media and the experiment was continued upto 24h under the nonstop purging of carbon dioxide. Approximate 1.0ml samples were withdrawn at interval of 1.0, 2.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0, 20.0 and 24.0 h from the dissolution medium and the same volume was substituted by the fresh medium which was previously kept in anaerobic condition. The sample volume was adjusted to 10 ml and followed by filtration using 0.22 micron membrane filters and was subjected to ICP-MS analysis. All the studies were conducted six times and the mean data was recorded.

#### **4.7.3.3 In vitro drug release using goat caecal content**

Dissolution studies for colon specific drug delivery were carried out using human goat caecal content (freshly prepared) for formulation F4. Basket type dissolution test

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apparatus (USP I) with minor alteration in process was used for evaluation of release of raupya bhasma in the presence of goat caecal content (freshly prepared). Gradient pH dissolution method using goat caecal content was used to assess the release of raupya bhasma from formulations meant for drug delivery to colon.

The experiments were conducted using beaker (250 ml) submerged in water containing pots of dissolution apparatus. One capsule was transferred to each pot having the dissolution medium. The in vitro release of formulation was started in 150 ml media of pH 1.2 using 100 rpm at  $37 \pm 0.5^\circ\text{C}$  for the first 2 h. After 2 h the pH of the dissolution media was maintained to 6.8 by addition of 50 ml phosphate buffer and sodium hydroxide solution (q.s.). The study was continued for 4 h. At the end of 4 h, the media was made anaerobic by degassing with carbon dioxide for 15 min. Undissolved oxygen in the media was removed by supply of carbon dioxide to media. After that 4% w/v goat caecal content (freshly prepared) was transferred to the dissolution media and the experiment was continued up to 24 h under the nonstop purging of carbon dioxide. Approximate 1.0 ml samples were withdrawn at interval of 1.0, 2.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0, 20.0 and 24.0 h from the dissolution medium and the same volume was substituted by the fresh medium which was previously kept up in anaerobic condition. The sample volume was adjusted to 10 ml and followed by filtration using 0.22 micron membrane filters and was subjected to ICP-MS analysis. All the studies were conducted six times and the mean data was recorded.

#### **4.7.3.4 In vitro drug release using probiotic culture**

Dissolution studies for colon specific drug delivery were carried out using probiotic culture for formulation F4. Basket type dissolution test apparatus (USP I) with minor alteration in process was used for evaluation of release of raupya bhasma in the presence of probiotic culture. Gradient pH dissolution method using probiotic culture contents was used to assess the release of raupya bhasma from formulations meant for drug delivery to colon.

The experiments were conducted using beaker (250 ml) submerged in water containing pots of dissolution apparatus. One capsule was transferred to each pot having the dissolution medium. The in vitro release of formulation was started in 150 ml media of pH 1.2 using 100 rpm at  $37 \pm 0.5^\circ\text{C}$  for the first 2 h. After 2 h the pH of the dissolution

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media was maintained to 6.8 by addition of 50 ml phosphate buffer and sodium hydroxide solution (q.s.). The study was continued for 4 h. At the end of 4 h, the media was made anaerobic by degassing with carbon dioxide for 15 min. Undissolved oxygen in the media was removed by supply of carbon dioxide to media. After that entire volume of prepared probiotic culture ( $9.8 \times 10^{10}$  CFU/ml) was transferred to the dissolution media and the experiment was continued up to 24 h under the nonstop purging of carbon dioxide. Approximate 1.0 ml samples were withdrawn at interval of 1.0, 2.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0, 20.0 and 24.0 h from the dissolution medium and the same volume was substituted by the fresh medium which was previously kept up in anaerobic condition. The sample volume was adjusted to 10 ml and followed by filtration using 0.22 micron membrane filters and was subjected to ICP-MS analysis. All the studies were conducted six times and the mean data was recorded.

*The studies on the above-mentioned formulation were also carried out in the same way without adding probiotic culture, goat caecal content and human fecal contents i.e normal buffer media.*

## **4.8 Formulation of coated granules of RB and their evaluation for colon targeted delivery**

### **4.8.1 Development of formulation (Guar gum and eudragit FS 30D coated raupya bhasma loaded granule core)**

#### **4.8.1.1 Preparation of RB loaded granule core**

The granules were prepared by mixing raupya bhasma, guar gum and xanthan gum in a ratio of 1: 1.5: 1.5. V-cone blender was used for mixing of ingredients. Wet granulation of the prepared mass was carried out using purified water. The damp mass was passed over sieve number 12. The prepared granules were dried at 45°C for an h in a hot air oven.

#### **4.8.1.2 Coating of the Prepared Granules**

The prepared granules were coated up to 20% using 1% guar gum solution in an accelacota coating pan. Guar gum acted as a prompting mechanism for the release of medicament in the colon by colonic micro flora. Further, the granules were coated with

40% Eudragit FS30D to eliminate the release of medicament in stomach and small intestine, by doing so the medicament will release only in the colon<sup>8</sup>.

## **4.8.2 Characterisation of coated granules**

### **4.8.2.1 Percentage yield**

To calculate % yield, prepared formulation was divided by the total amount of polymer and raupya bhasma taken in the preparation of the formulation. Percentage yield was calculated using given formula:

$$\text{Percentage yield} = \frac{\text{Actual yield of formulation}}{\text{Total weight of polymers and drug}} \times 100$$

### **4.8.2.2 Determination of raupya bhasma included in formulation**

The amount of raupya bhasma in formulation was determined by placing microsphere in phosphate buffer saline (PBS, pH 7.4) for 48 h at 37°C with vigorous stirring. The concentration of raupya bhasma was determined using ICP-MS. The percentage of loading efficiency and content was expressed as given in the following equation.

$$\text{Loading efficiency (\%)} = \frac{\text{Weight of loaded drug in microsphere}}{\text{Initial feeding weight of drug}} \times 100$$

$$\text{Loading content (\%)} = \frac{\text{Weight of loaded drug in microsphere}}{\text{Weight of microsphere}} \times 100$$

### **4.8.2.3 Surface associated drug content**

Formulation of raupya bhasma was assessed for drug content which was associated with surface of formulation. For the purpose, each batch of 100 mg of formulation were shaken in 20 ml of 0.1N hydrochloric acid for 5 min and filtered using Whatman filter paper. Surface associated drug content in filtrate was determined using ICP-MS. All the investigation were conducted thrice (n=3).

$$\text{Surface associated drug content} = \frac{\text{Amount of drug present in filtrate}}{\text{Amount of drug used in formulation}} \times 100$$

#### **4.8.2.4 Equilibrium Swelling Studies of formulation**

Accurately weighed formulation (100 mg) was transferred in PBS (pH 7.4) and kept for swelling until its weight became constant. The formulation was taken out, blotted with filter paper and the weight difference was measured. The degree of swelling ( $\alpha$ ) of formulation was calculated using the given formula:

$$\alpha = \frac{w_g - w_o}{w_o}$$

Where,  $w_o$  and  $w_g$  are the initial weight of the microspheres and the weight of the microspheres at equilibrium swelling in the medium respectively.

#### **4.8.2.5 Flow properties**

##### **4.8.2.5.1 Angle of repose**

Angle of repose was determined by funnel method. Formulation was poured through a funnel that can be elevated vertically until extreme cone height ( $h$ ), was obtained. Diameter of heap, ( $D$ ), was determined. Following formula was used to calculate angle of repose.

$$\tan \theta = h / r,$$

$$\theta = \tan^{-1} (h / r)$$

Where,  $\theta$  = Angle of repose,  $h$  = height of the pile (cm) and  $r$  = radius of the pile (cm).

### **4.8.3 In vitro release of coated granules**

#### **4.8.3.1 Preparation of Dissolution Media**

##### **4.8.3.1.1 Preparation of fresh human fecal content medium**

Human fecal slurries (freshly prepared) is usually used to evaluate fermentation of polysaccharides (non-starch). Human faecal slurries were prepared by homogenisation of fresh faeces in 0.1 M sodium phosphate buffer (pH 6.8) by supplying  $\text{CO}_2$  to maintain anaerobic conditions. Freshly prepared human fecal slurries was finally added to the dissolution media to give a final dilution of 4% w/v. All the above mentioned processes were carried out in anaerobic condition by supply of carbon dioxide<sup>5</sup>.

4.8.3.1.2 Preparation of goat caecal medium

Fresh caecal content of goat were procured from local market of Phagwara and kept in desiccator under anaerobic conditions. Accurately weighed caecal content were suspended in the pH 6.8 buffer under anaerobic condition. Finally 4 % w/v caecal medium were prepared by adding dissolution media. All the above procedure were carried out anaerobically in presence of carbon dioxide <sup>6</sup>.

4.8.3.1.3 Preparation of Probiotic culture medium

Dissolution using probiotic culture is required to active probiotic in suitable media.

Prebiotic and probiotic capsule brand name: Velgut (Eris life sciences Pvt.Ltd.)

**Composition of culture**

*Bifidobacterium breve*

*Bifidobacterium longum*

*Bifidobacterium infantis*

*Lactobacillus acidophilus*

*Lactobacillus plantarum*      Total 5 billion

*Lactobacillus casei*

*Lactobacillus rhamnosus*

*Streptococcus thermophilus*

*Saccharomyces boulardi*

Fructooligosaccharide-

100mg

4.8.3.1.4 Preparation of media

A 5% w/v tempered grade A honey was added to 12 % w/v non-fat dry milk (NDM) in a 100 ml volumetric flask and volume was made up to 100 ml with sterile water. Then mixture was subjected to the process of pasteurization (heating up to 70 °C for 30 minutes and cooled to 37 °C). A capsule of probiotic powder was transferred in few ml of sterile water and was inoculated in to the pasteurized milk media. Pasteurised milk media containing probiotics was incubated at 37 °C for 48 h under anaerobic conditions. The bacteria were counted in culture media. It was found to be  $9.8 \times 10^{10}$  CFU/ml ( $10^{11}$ - $10^{12}$  CFU/ml normal microbiota count in colon). The probiotic media was added to



dissolution media for evaluation of drug release of microbially triggered drug delivery to colon<sup>7</sup>.

#### **4.8.3.1.5 In vitro drug release of formulation using human fecal slurries**

Dissolution studies for colon specific drug delivery were carried out using human fecal slurries (freshly prepared). Basket type dissolution test apparatus (USP I) with minor alteration in process was used for evaluation of release of raupya bhasma in the presence of human fecal slurries (freshly prepared). Gradient pH dissolution method using human fecal contents was used to assess the release of raupya bhasma from formulations meant for drug delivery to colon.

The experiments were conducted using beaker (250 ml) submerged in water containing pots of dissolution apparatus. One capsule was transferred to each pot having the dissolution medium. The in vitro release of formulation was started in 150 ml media of pH 1.2 using 100 rpm at  $37 \pm 0.5^\circ\text{C}$  for the first 2 h. After 2 h the pH of the dissolution media was maintained to 6.8 by addition of 50 ml phosphate buffer and sodium hydroxide solution (q.s.). The study was continued for 4 h. At the end of 4 h, the media was made anaerobic by degassing with carbon dioxide for 15 min. Undissolved oxygen in the media was removed by supply of carbon dioxide to media. After that 4% w/v fecal slurries (freshly prepared) was transferred to the dissolution media and the experiment was continued upto 24h under the nonstop purging of carbon dioxide. Approximate 1.0ml samples were withdrawn at interval of 1.0, 2.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0, 20.0 and 24.0 h from the dissolution medium and the same volume was substituted by the fresh medium which was previously kept up in anaerobic condition. The sample volume was adjusted to 10 ml and followed by filtration using 0.22 micron membrane filters and was subjected to ICP-MS analysis. All the studies were conducted six times and the mean data was recorded.

#### **4.8.3.2 In vitro drug release using goat caecal content**

Dissolution studies for colon specific drug delivery were carried out using human goat caecal content (freshly prepared). Basket type dissolution test apparatus (USP I) with minor alteration in process was used for evaluation of release of raupya bhasma in the presence of goat caecal content (freshly prepared). Gradient pH dissolution method

using goat caecal content was used to assess the release of raupya bhasma from formulations meant for drug delivery to colon.

The experiments were conducted using beaker (250 ml) submerged in water containing pots of dissolution apparatus. One capsule was transferred to each pots having the dissolution medium. The in vitro release of formulation was started in 150 ml media of pH 1.2 using 100 rpm at  $37 \pm 0.5^\circ\text{C}$  for the first 2 h. After 2 h the pH of the dissolution media was maintained to 6.8 by addition of 50 ml phosphate buffer and sodium hydroxide solution (q.s.). The study was continued for 4 h. At the end of 4 h, the media was made anaerobic by degassing with carbon dioxide for 15 min. Undissolved oxygen in the media was removed by supply of carbon dioxide to media. After that 4% w/v goat caecal content (freshly prepared) was transferred to the dissolution media and the experiment was continued up to 24 h under the nonstop purging of carbon dioxide. Approximate 1.0 ml samples were withdrawn at interval of 1.0, 2.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0, 20.0 and 24.0 h from the dissolution medium and the same volume was substituted by the fresh medium which was previously kept up in anaerobic condition. The sample volume was adjusted to 10 ml and followed by filtration using 0.22 micron membrane filters and was subjected to ICP-MS analysis. All the studies were conducted six times and the mean data was recorded.

#### **4.8.3.3 In vitro drug release of formulation using probiotic culture**

Dissolution studies for colon specific drug delivery were carried out using probiotic culture. Basket type dissolution test apparatus (USP I) with minor alteration in process was used for evaluation of release of raupya bhasma in the presence of probiotic culture. Gradient pH dissolution method using probiotic culture contents was used to assess the release of raupya bhasma from formulations meant for drug delivery to colon.

The experiments were conducted using beaker (250 ml) submerged in water containing pots of dissolution apparatus. One capsule was transferred to each pots having the dissolution medium. The in vitro release of formulation was started in 150 ml media of pH 1.2 using 100 rpm at  $37 \pm 0.5^\circ\text{C}$  for the first 2 h. After 2 h the pH of the dissolution media was maintained to 6.8 by addition of 50 ml phosphate buffer and sodium hydroxide solution (q.s.). The study was continued for 4 h. At the end of 4 h, the media was made anaerobic by degassing with carbon dioxide for 15 min. Undissolved oxygen

in the media was removed by supply of carbon dioxide to media. After that entire volume of prepared probiotic culture ( $9.8 \times 10^{10}$  CFU/ml) was transferred to the dissolution media and the experiment was continued up to 24 h under the nonstop purging of carbon dioxide. Approximate 1.0 ml samples were withdrawn at interval of 1.0, 2.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0, 20.0 and 24.0 h from the dissolution medium and the same volume was substituted by the fresh medium which was previously kept up in anaerobic condition. The sample volume was adjusted to 10 ml and followed by filtration using 0.22 micron membrane filters and was subjected to ICP-MS analysis. All the studies were conducted six times and the mean data was recorded.

*The studies on the above-mentioned formulation were also carried out in the same way without adding probiotic culture, goat caecal content and human fecal contents i.e normal buffer media.*

#### **4.9 References**

1. Krishna G. "Rasendrasara Samagrah (Sanskrit)," 1<sup>st</sup> ed. Varanasi (India): Chaukhamba Prakashan, 1967, pp 87-93.
2. Chaturvedi R and Jha CV (2011) Standard manufacturing procedure of rajat bhasma. Ayu 32: 566-571.
3. Mizushima Y and Kobayashi M (1968) Interaction of anti-inflammatory drugs with serum preteins, especially with some biologically active proteins. J of Pharma Pharmacol 20:169-173.
4. Chaurasia M, Chaurasia MK, Jain NK, Jain A, Soni V, Gupta Y, Jain SK (2006) Cross-Linked Guar Gum Microspheres: A Viable Approach for Improved Delivery of Anticancer Drugs for the Treatment of Colorectal Cancer. AAPS PharmSciTech 7 (3) E1-E9.
5. Kotla NG, Shivapooja A, Muthyala J et.al. (2013) Effect Of Guar Gum And Xanthan Gum Compression Coating On Release Studies Of Metronidazole In Human Fecal Media For Colon Targeted Drug Delivery Systems. Asian J Pharm Clin Res 6: 315-318.
6. Shabir S, Shaheeda and Ramanamurthy KV (2012) Formulation and evaluation of chitosan sodium alginate microcapsules of 5-fluorouracil for colorectal cancer. Int j res pharma chem 2: 7-19.

7. Wilhelm H, Holzapfel and Petra Haberer (1998) Overview of gutflora and probiotics. *Int J Food Microbiol* 41:85-101.
8. JI CM, Xu HN and WU W (2009) Guar Gum as Potential Film Coating Material for Colon-specific Delivery of Fluorouracil. *Journal of biomaterials applications* 23: 311-329.
9. Wadekar MP, Rode CV, Bendale YN (2005) Preparation and characterization of a copper based Indian traditional drug: Tamra bhasma. *J Pharm Biomed Anal* 39: 951-55.
10. X Gao, H Feng and J Ma (2010) Analysis of the dielectric constants of the Ag<sub>2</sub>O film by spectroscopic ellipsometry and single oscillator model,” *Physica B: Condens Matter* 405: 1922-1926.
11. Opie EL (1999) On the relation of necrosis and inflammation to denaturation of proteins. *J Exp Med* 115: 597-608.
12. Umaphathy E, Ndebia EJ and Meeme A (2010) An experimental evaluation of *Albuca setosa* aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. *J Med Plants Res* 4: 789-795.
13. Jagtap VA, Agasimundim YS and Jayachandran E (2011) In vitro anti-inflammatory activity of 2-amino-3-(substitutedbenzylidene-carbohydrazide)-4,5,6,7-tetrahydrobenzothiophenes. *J Pharm Res* 4: 378-379.
14. Anson ML and Mirsky AE (1932) The effect of denaturation on the viscosity of protein systems. *Gen Physiol* 15: 341-350.
15. Williams LAD, Connar AO and Latore L (2008) The in vitro anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals, in the early stages of the drug discovery process. *West Indian Med J* 57: 327-331.
16. Sinko PJ (2011) *Martin's Physical Pharmacy and Pharmaceutical Sciences*, 6<sup>th</sup> ed. Lippincotts Williams and Wilkins, Baltimore.

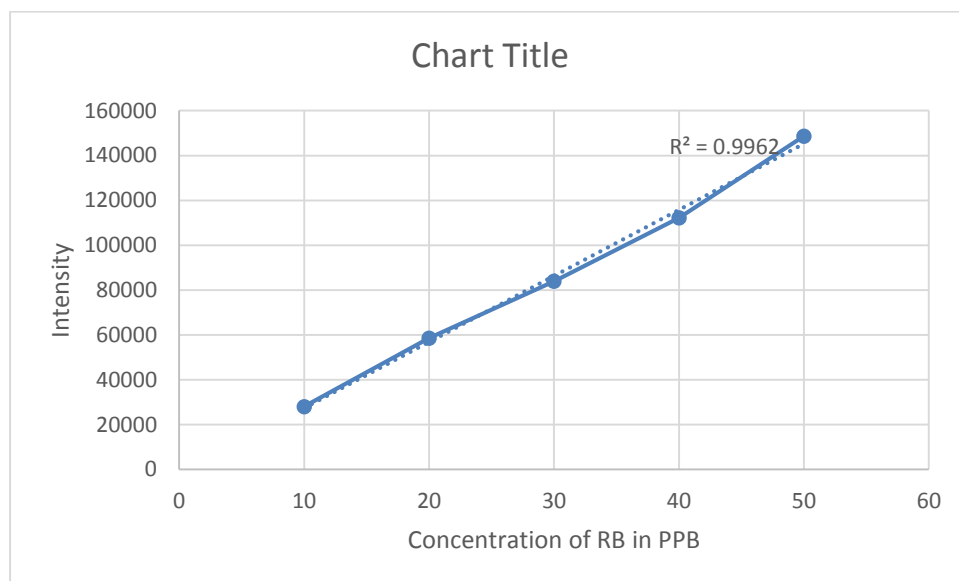
## 5 RESULTS AND DICUSSION

### 5.1 Standard Plot

Inductive coupled plasma mass spectroscopy was used for quantitative analysis of silver. Standard plot of raupya bhasma was plotted between intensity versus concentration (Table 5.1 and Figure.5.1).

**Table 5.1: Standard plot of Bhasma (Conc vs Intensity)**

Conc (PPB)	Intensity
10	28033
20	58615
30	83916
40	112232
50	148666



**Figure 5.1: Standard plot of raupya bhasma (Conc Vs Intensity)**

### 5.2 Preparation and characterisation of raupya bhasma

Prepared bhasma was found to be satisfactory and demonstrated all positive results as per ancient method of evaluation. The result of evaluation of bhasma by traditional method is shown in Table-5.2. The silver content determined using ICP-MS was found

to be 63.054%. Flame test showed no fumes indicating that bhasma was free from organic substance. The particle size of bhasma was found to be 323.8 nm (Figure 5.2) by zeta sizer (laser diffraction analyser). Shape of bhasma (Figure 5.3 and 5.4) was found to be microcrystalline, irregular, using scanning electron microscopy (SEM). The thermo gram (Figure 5.5) of the raupya bhasma was obtained in the temperature range of 30–960°C in air atmosphere which showed 20.515% weight loss at temperature between 60 to 624.63°C, 6.784% weight loss in between 624.63 to 811.71°C and 2.740% weight loss in between 811.72 to 995.83 °C. The XRD pattern of bhasma (Figure 5.6) indicated nature of bhasma as crystalline and mainly composed of argentous and argentic oxide.

**Table 5.2: Traditional method of evaluation of bhasma**

<b>Test</b>	<b>Observation</b>	<b>Inference</b>
<b><i>Nishchandravam</i></b> Bhasma was observed in bright sunlight to detect the presence and absence of lustre.	No metallic lustre was observed.	Silver was completely converted into its compound.
<b><i>Rekhapurnata</i></b> A little amount of bhasma was rubbed in between the index finger and thumb to observe if particles can fill furrows of fingertips or not.	Particles of bhasma filled in furrows of finger.	Size of prepared bhasma was fine.
<b><i>Varitara test</i></b> A small amount of bhasma was sprinkled over the stagnant water surface	Bhasma floated over surface of water	Size of prepared bhasma was very fine.
<b><i>Unama test</i></b> It is further assessment of varitara test. A grain of rice was kept carefully on floating bhasma.	Rice grain floated over film of bhasma on surface of water.	Further confirmed that size of prepared bhasma was very fine.

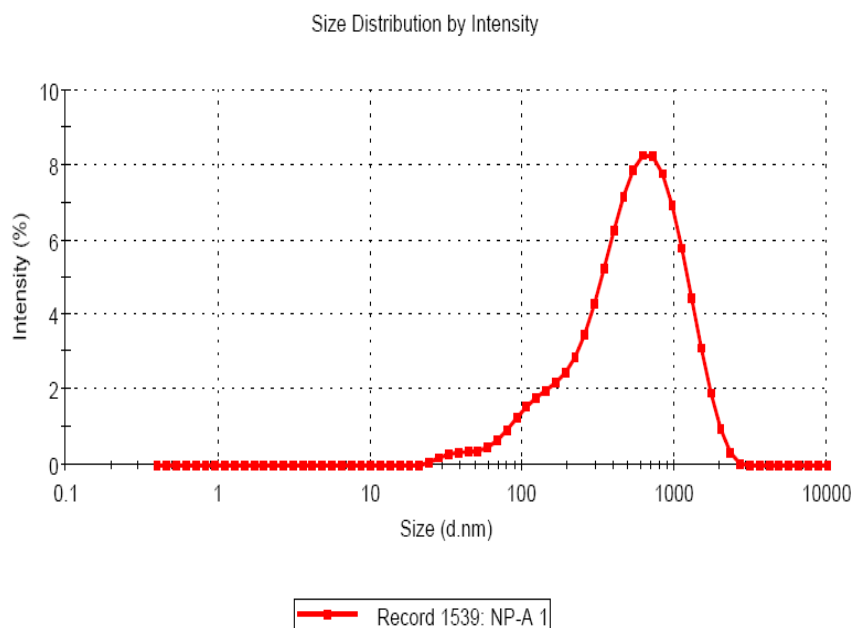


Figure 5.2: Particle size using Zetasizer

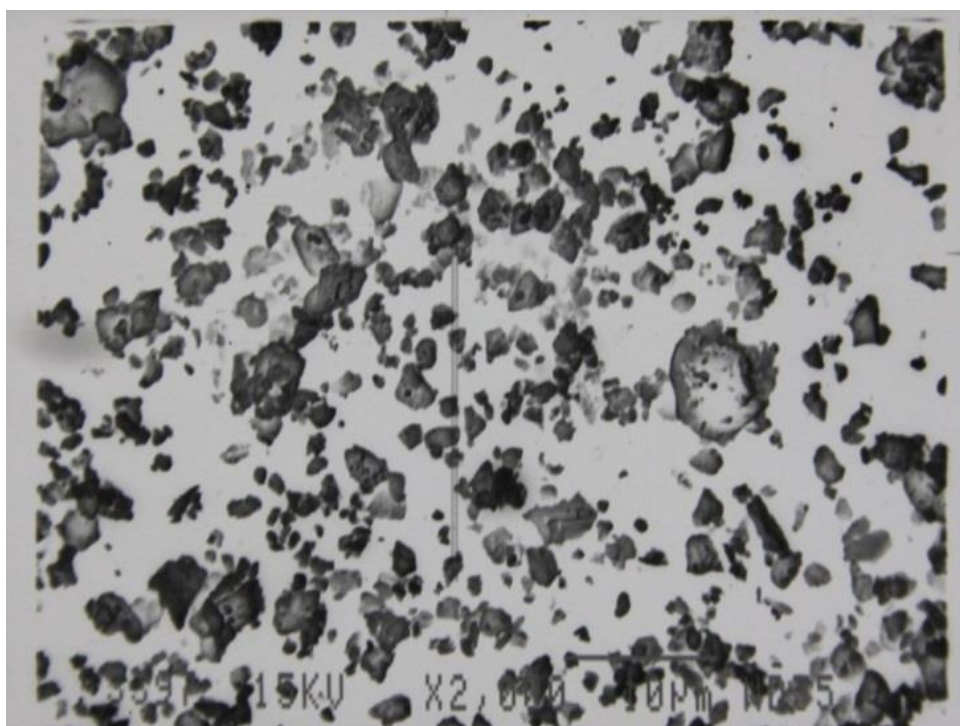


Figure 5.3: SEM of raupya bhasma (X 2500)

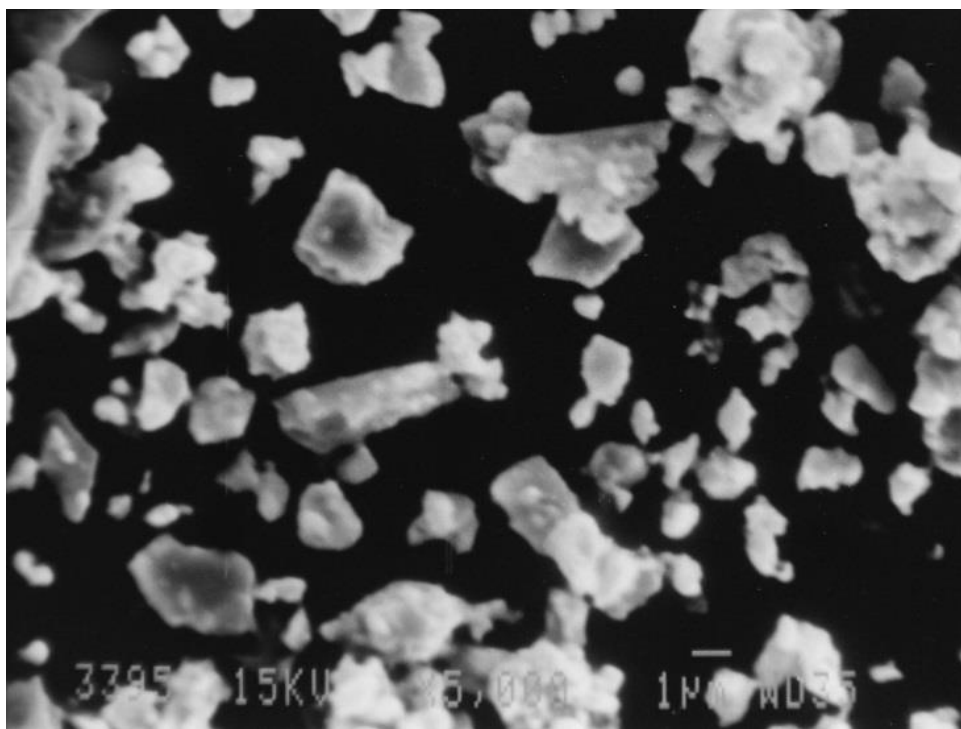


Figure 5.4: SEM of raupya bhasma (X 5000)

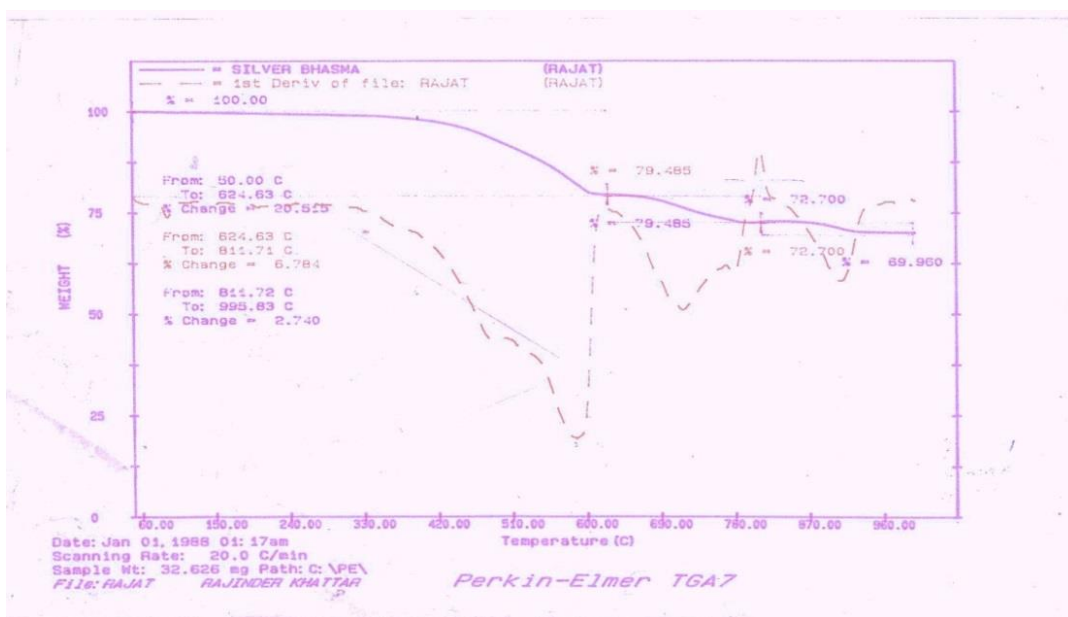
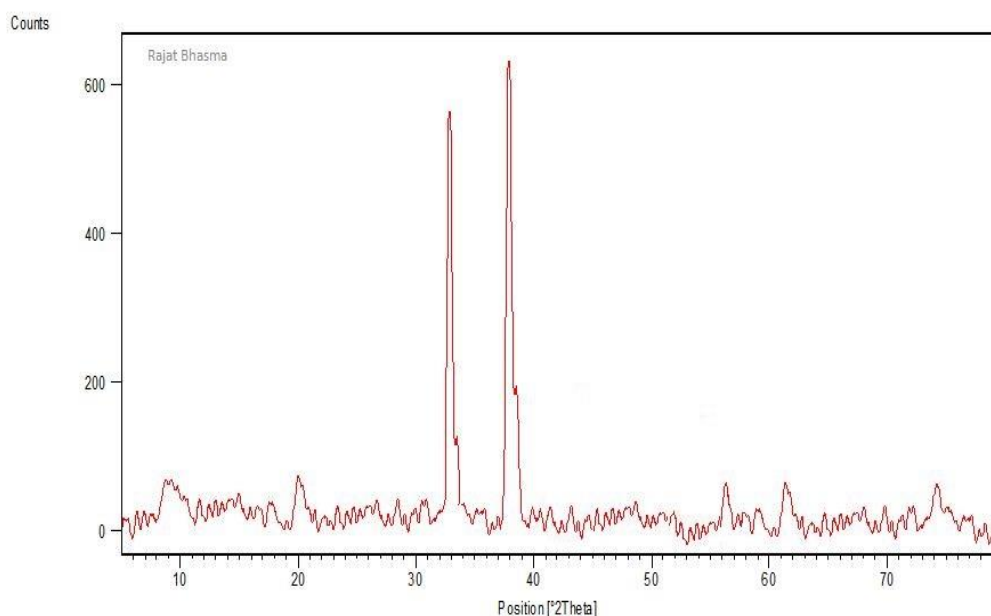


Figure 5.5: TGA pattern of raupya bhasma





**Figure 5.6: XRD pattern of raupya bhasma**

Heating of silver foil during sodhana may lead to increase in tension causing expansion of silver foil followed by cooling in liquid media lead to decrease in tension and increase in compression force. Repeated heating and cooling process may lead to brittleness, reduction in hardness and finally reduction in particle size. Repeated calcination during marana may lead to oxidation of metal. Oxidation of metal may lead to conversion of the zero valent metal into a form with higher oxidation state. The best significant characteristic of this synthesis (traditionally known as ‘bhasmikiran’) is that the toxic nature (i.e. systemic toxicity causing stomach pain, nausea, vomiting etc.) of the resulting compound of meal was entirely vanished while persuading the therapeutic potential into it<sup>8</sup>. Reduced in particle size may lead to increase in bioavailability, moreover reduction in dose was directly related to reduction in toxicity of bhasma.

The colour of prepared bhasma was found to be black that was similar to reported colour of standard raupya bhasma. As silver sulphide and oxide are black in colour so raupya bhasma may be the mixture of both<sup>9</sup>. The prepared bhasma was found to be odourless, tasteless, fine in touch without any metallic sound. Metal was characterised by its metallic lustre. When this prepared bhasma was seen under day light as well as sun light it did not show any metallic lustre indicating that all silver was completely

converted into bhasma (ash). Rekhapuran test was found to be positive as bhasma filled in between the furrows of finger which indicated that bhasma was very fine in particle size. Varitara test was found to be positive as the bhasma floated on surface of water, which further indicated that the metal was completely converted into ash. Even the better quality of bhasma was insured by unama test as rice grain also floated over bhasma. As no smoke was produced when bhasma was subjected to nirdhum test, indicated that prepared bhasma was free from organic impurities. The result of traditional method of evaluation is summarised in Table 5.2. There is some limitation of traditional method of evaluation as it does not give information about silver content in prepared bhasma, presence of organic substance, particle size, shape and crystalline nature. To consider this, an attempt has been made to increase the acceptability of bhasma. Silver content was determined using ICP-MS method and was found to contain 63.054% silver by ICP-MS. FT-IR spectra of raupya bhasma indicated that it was free from organic matter. The lack of organic matter in bhasma is further evidence of appropriate calcination during the preparation of raupya bhasma. The particle size of bhasma was found to be 323.8 nm by zeta sizer (laser diffraction analyser), Figure 5.2. Shape of bhasma was found to be microcrystalline irregular, due to aggregation of particle as the particle size was found to be of 1  $\mu\text{m}$  (1000 nm) using SEM (Figure 5.3 and 5.4). However the average particle size determined by laser diffraction analyser was found to be 323.8 nm.

As repeated calcination cycle was required in preparation of bhasma, it was necessary to perform thermo gravimetric analysis. The thermo gram of the raupya bhasma was obtained in the temperature range of 30–960°C in presence of air atmosphere which showed 20.515% weight loss at temperature between 60 to 624.63°C, 6.784% weight loss in between 624.63 to 811.71°C and 2.740% weight loss in between 811.72 to 995.83 °C. The TGA thermo gram (Figure 5.5) can be used as a standard for quality of raupya bhasma. The X-ray diffraction of raupya bhasma is shown in Figure 5.6. Diffraction peak at  $2\theta = 33^\circ$  and  $38.5^\circ$ , were obtained. The high intensity of X-ray diffraction in the XRD pattern indicated that the raupya bhasma was crystalline in nature. The  $2\theta$  value indicated that raupya bhasma was composed of oxide of silver<sup>10</sup>.

### 5.3 Anti-inflammatory activity

The in vitro anti-inflammatory effect of raupya bhasma was evaluated against denaturation of protein (egg albumin). The results obtained for inhibition of protein and change in viscosity is summarized in Table 5.3. The current findings showed a concentration dependent inhibition of albumin denaturation by raupya bhasma throughout the concentration range of 20 to 100µg/ml. Diclofenac sodium was used as a reference which also exhibited concentration dependent affect (20 to 100µg/ml) inhibition of protein denaturation (Table 5.4).The effect of diclofenac sodium for anti-inflammatory activity was found to be less when compared with raupya bhasma. This was further established by comparing their IC50 values. Raupya bhasma possessed IC50 value 43.2 µg/mL whereas that of diclofenac sodium was found to be 46.1 µg/ml.

**Table 5.3: Effect of roupya bhasma on denaturation of protein**

Concentration (µg/ml)	% inhibition	Viscosity(cp)
Control	-	-
20	28.9196±0.0852	0.628±0.027
40	47.5154±0.152	0.785±0.039
60	60.5934±0.106	0.815±0.016
80	71.0142±0.0569	0.880±0.023
100	73.6988±0.126	0.970±0.015

Data is presented as Mean±SEM (n=5)

**Table 5.4: Effect of diclofenac sodium on denaturation of protein**

Concentration(µg/ml)	% inhibition	Viscosity
Control	-	-
20	25.4704±0.121	0.657 ±0.012
40	44.4752±0.0730	0.740±0.015

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## Chapter 5: Results and Discussions

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Concentration( $\mu\text{g/ml}$ )	% inhibition	Viscosity
60	56.2944 $\pm$ 0.0632	0.823 $\pm$ 0.012
80	66.38361 $\pm$ 0.0494	0.86 $\pm$ 0.021
100	71.4242 $\pm$ 0.0628	0.97 $\pm$ 0.012

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Data is presented as Mean $\pm$ SEM (n=5)

The prepared bhasma was evaluated for in vitro anti-inflammatory activity as there are definite problems in use of animals for biological activity of new chemical entity (NCE), like ethical problems and the lack of justification for their use when invitro methods are existing for investigation. To consider this, in the current research, the denaturation of protein bioassay was chosen for in vitro evaluation of anti-inflammatory activity of raupya bhasma. During protein denaturation, the secondary and tertiary structures are lost by application of external stress or compound, such as strong acid, strong base, concentrated inorganic salt, organic solvent and heat. Most biological proteins lose their biological function when denatured. It is a proven fact that denatured protein is the causative factor of inflammation<sup>10,11</sup>. Therefore, new chemical entity which can inhibit denaturation of proteins could be used for development of formulation for treatment of inflammation<sup>12</sup>. The IC<sub>50</sub> values of RB indicated that it was more active as compared to diclofenac sodium. The inhibition of denaturation of protein was also supported by the viscosity change. Denaturation of protein may lead to increase in viscosity of solution<sup>12</sup>. In the contemporary research, the comparatively high viscosity of control validated this statement. Presence of raupya bhasma prevented this, involving inhibition of denaturation of protein. The decrease in viscosity was noted as compared to control where no raupya bhasma was added. The viscosity was reduced with decrease in concentration of raupya bhasma as well as diclofenac sodium<sup>13</sup>. From literature it is clear that non-steroidal anti-inflammatory drugs stabilized albumin (prevent denaturation) on elevated temperature at physiological pH<sup>15</sup>. Therefore, from the results of the present preliminary study it may be concluded that raupya bhasma possessed marked in vitro anti-inflammatory activity.

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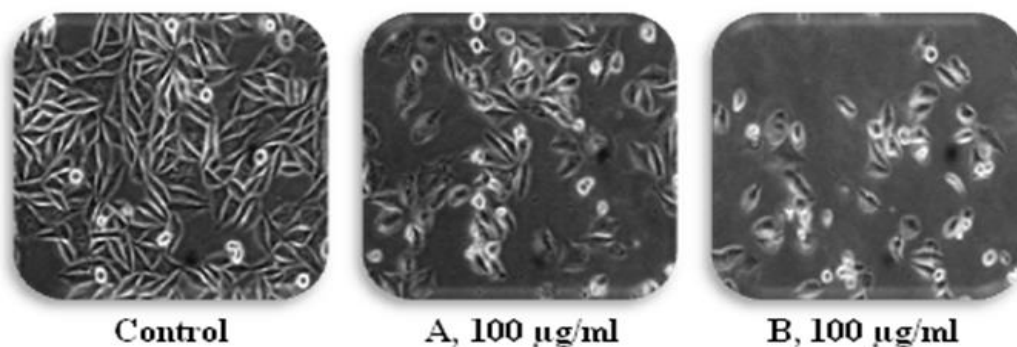
However, supplementary conclusive research should be carried out to establish the mechanism behind the anti-inflammatory action of raupya bhasma.

### **In vitro anticancer activity**

Raupya bhasma possessed marked activity against colon cancer. It was confirmed by cell growth inhibition (Table.5.5). Raupya bhasma inhibited 24% cell growth, however standard drug (5-FU) had inhibited only 11 % cell growth.

**Table 5.5: Percentage cell-growth inhibition**

Sample	Conc.	% Cell growth inhibition
		HCT-116 (Colon)
Standard (5-FU)	100µg/ml	11
Test (Raupya Bhasma)	100µg/ml	24



**Figure 5.7: Percentage cell growth inhibition**

A= Standard Drug; B= Test drug

## **5.4 Drug Polymer Interaction**

### **5.4.1 Visual observation**

The compatibility study of raupya bhasma (RB) with different excipients [polymers: guar gum (GG)-xanthan gum (XG)] is shown in Table 5.6.

**Table 5.6: Compatibility studies with different excipients**

Sample	Ratio	Initial Observation		Condition day and temperature							
		Color	Lump formation	0 <sup>th</sup> day 2-8°C 40°C/ RH=75%		7 <sup>th</sup> day 2-8°C 40°C/ RH=75%		15 <sup>th</sup> day 2-8°C 40°C/ RH=75%		30 <sup>th</sup> day 2-8°C 40°C/ RH=75%	
RB	-	Black	No	√	√	√	√	√	√	√	√
GG	-	Creamy	No	√	√	√	√	√	√	√	√
		White									
XG	-	Creamy	No	√	√	√	√	√	√	√	√
		White									
RB+	1:1	Blackish	No	√	√	√	√	√	√	√	√
GG		grey									
RB+	1:1	Blackish	No	√	√	√	√	√	√	√	√
XG		grey									
RB+	1:1:1	Blackish	No	√	√	√	√	√	√	√	√
GG		grey									
+XG											

√ Represent no change in physical appearance.

From the results of drug-excipient compatibility study, it was observed that there was neither any change in colour, nor any lump formation in any of the mixtures at different temperature and humidity conditions. Therefore, it confirmed that the raupya bhasma was compatible with excipients used in the formulation.

#### 5.4.2 FTIR spectroscopy

The IR spectra of raupya bhasma drug and excipients were taken and compared with those of the mixtures. If any interaction or incompatibility occurs between drug-polymer and polymer-polymer, there would be change in the spectral peak pattern and peak location in the IR spectra of the component mixture than the peak pattern and peak location obtained of individual components. If no change in spectral peak pattern and peak location in the IR spectra of the component mixture and the peak pattern and peak location obtained of individual components is observed, it indicates that drug-polymer and polymer-polymer were compatible without any interaction (Figure 5.8 -5.13).

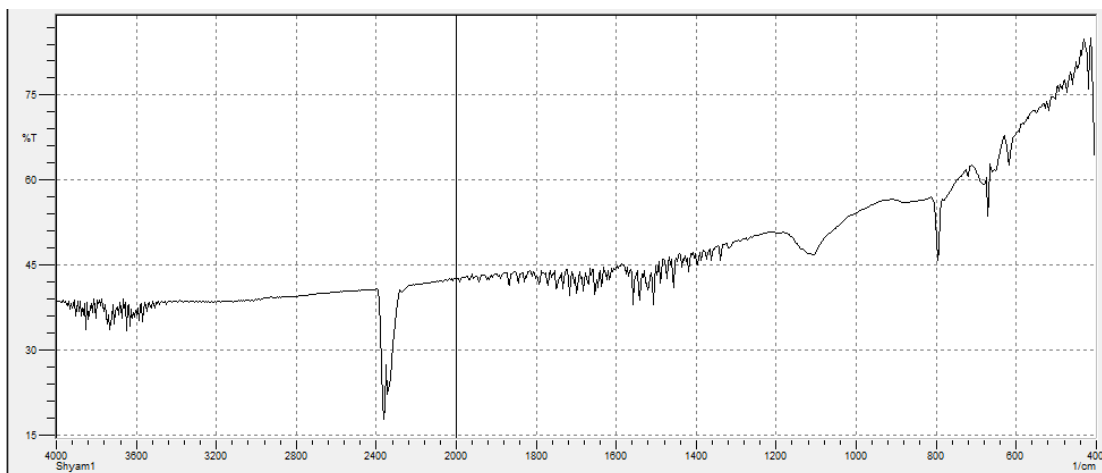


Figure 5.8: FTIR spectra of Raupya bhasma

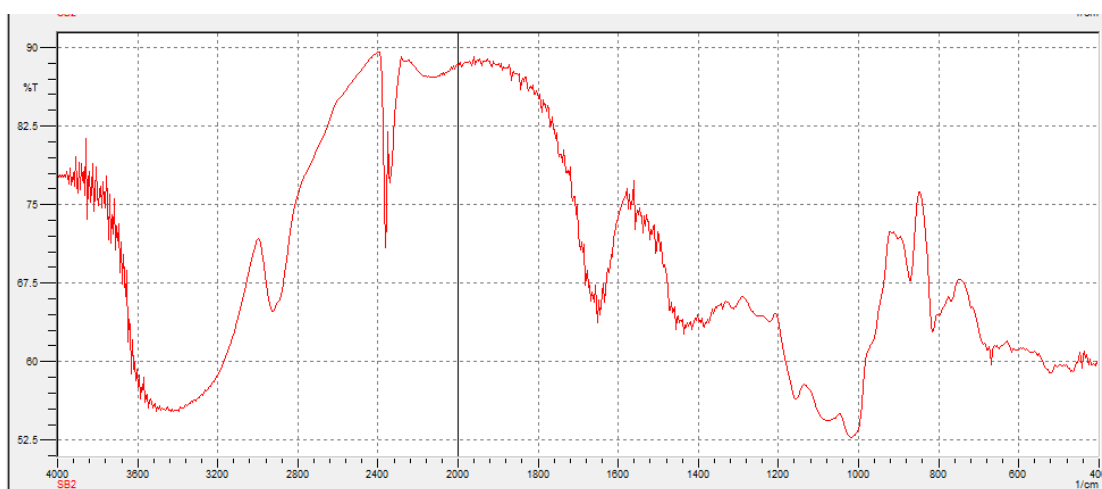


Figure 5.9: IR spectra of Guargum

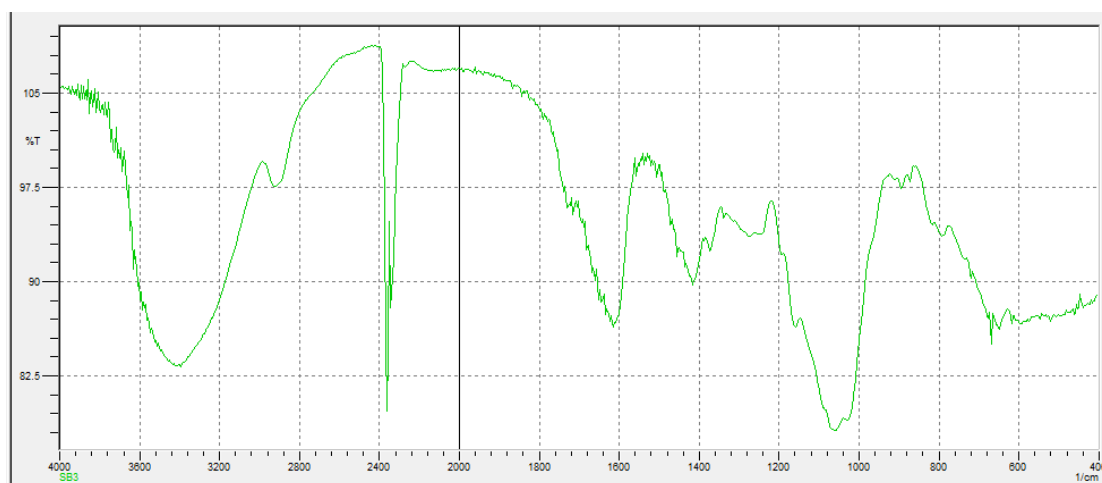


Figure 5.10: FTIR spectra of Xanthan Gum

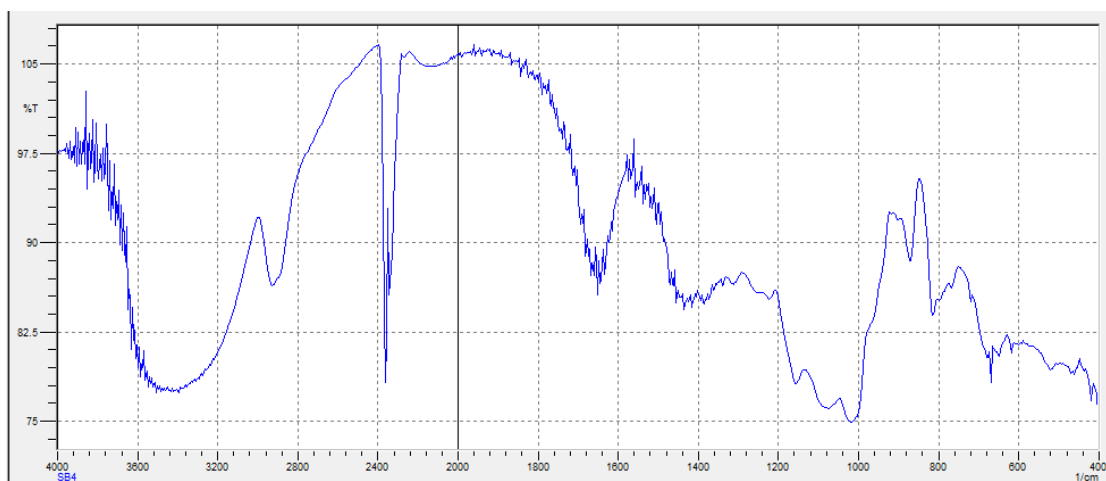


Figure 5.11: FTIR spectra of raupya bhasma + guar gum

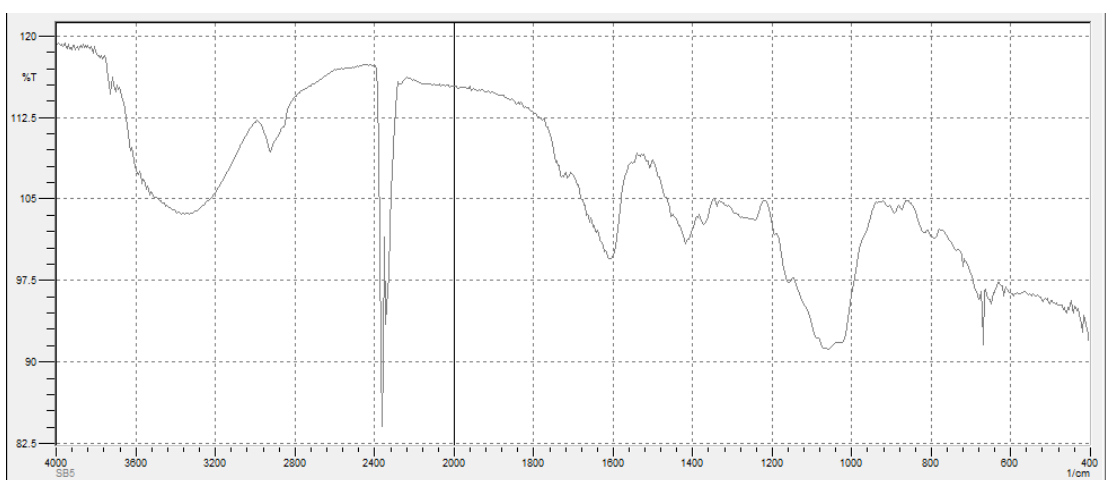


Figure 5.12: FTIR spectra of raupya bhasma+ xanthan gum

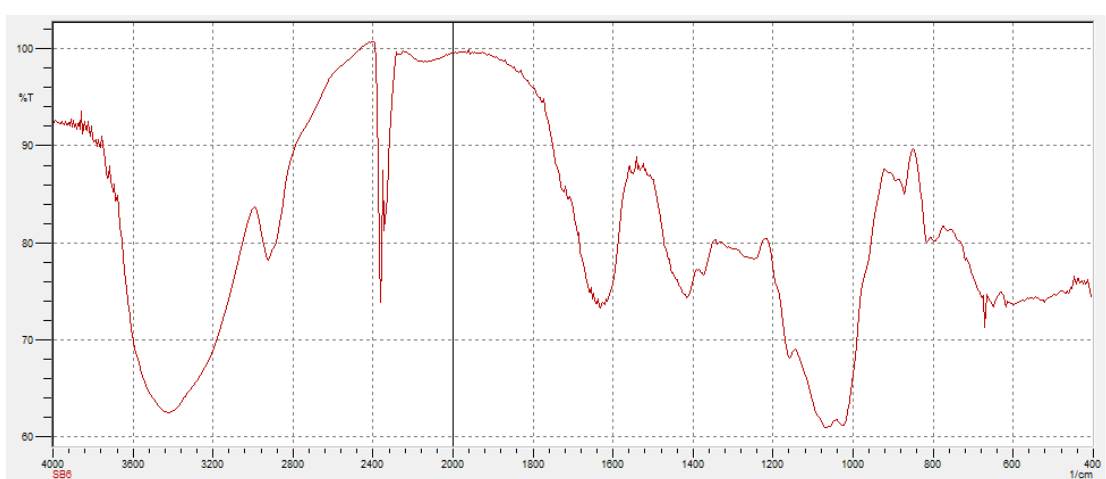


Figure 5.13: FTIR spectra of raupya bhasma + guar gum + xanthan gum



From the comparative FTIR spectral study, for compatibility study of: raupya bhasma, xanthan gum, guar gum + xanthan gum, raupya bhasma+guar gum and raupya bhasma+ guar gum + xanthan gum, it may be concluded that there was no significant difference in the FTIR spectra of physical mixtures of raupya bhasma+ xanthan gum, raupya bhasma + guar gum, and IR spectra of + guar gum + xanthan gum when compared to FTIR spectra of individual components.

## **5.5 Formulation and evaluation of microsphere**

Microsphere was prepared by emulsification polymerisation technique using guar gum and xanthan gum as polymer. Xanthan gum and guar gum were chosen for encapsulation purpose to retard the release of drug before reaching to colon. Xanthan gum or guar gum has been used independently in colon-specific drug delivery but when used in combination enhanced gel properties and drug release-retarding tendency drastically increased<sup>16</sup>.

Cross-linking of microsphere using glutaraldehyde may lead to hardening which is temperature-induced cross-linking in acidic medium.

The determination of size, size distribution and morphology are very important characteristics to be verified in the development of formulation. The homogeneity of size in a formulation is the indicative of stability and some behaviour of the system. The morphology of drug loaded raupya bhasma microspheres were analysed using SEM. The Figure 5.18 shows that microsphere had a spherical shape with relatively narrow size distribution and furthermore, no large aggregates were observed. Measurement of particle size by zeta sizer is based on the principle of dynamic light scattering (DLS) and found to be 599.6nm (Figure 5.19). DLS (also known as PCS- Photo Correlation spectroscopy) determine brownian motion and co-relates this to the particles size. It can be achieved by enlightening the particles with a laser and investigating the intensity of fluctuations in the scattered light. DLS makes use of particles ability to scatter light and their natural brownian motion when suspended in fluid, water in this case. Particle size is calculated based on an estimate of the particles diffusion coefficient while suspended in a medium. The relationship between the size of a particle and its speed is due to brownian motion can be understand by the Stokes-Einstein equation. Particle diffusion rates are inversely proportional to particle size<sup>17</sup>.

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Percentage yield, loading efficacy, loading content, surface associated drug content, degree of swelling and angle of repose of microsphere (F1 to F4) are reported in Tables 5.7 - 5.10 respectively. The swelling index of guar gum and xanthan gum was very high which drastically decreased due to cross linking. Cross-linking restricts the free access of water to the polymer hydroxyl group, which in turn decreases the swelling properties of the cross-linked polymer. The ideal fate of microsphere in vivo is to eventually release the contents to the surrounding biological fluid. In vitro dissolution testing provides a valuable tool for investigating drug release mechanism. Also, drug release testing is a fundamental part of drug product development and manufacturing is also employed as a quality control tool to monitor batch to batch consistency of the drug release. In vitro drug release study of microspheres in the presence of fecal content was carried out as follows: The fecal content was collected from healthy human volunteer. As the condition of GIT is anaerobic so anaerobic condition is maintained by supply of nitrogen gas which prevent the death of microorganism in aerobic condition. The percentage cumulative release of microspheres in different release media during different time period is shown in Table 5.11-5.14 and Figure 5.14-5.17. In polysaccharide based drug delivery system, polysaccharide polymer protects the drug from the surroundings of stomach and small intestine, and is capable to deliver the drug to the colon. As guar gum and xanthan gum are prebiotics, microflora of colon feed on them and drug gets released in colon. Use of activated probiotics instead of faecal content gives better result and act in a similar way. More over use of probiotics as dissolution media is a new concept and can replace other biological media because of compliance and easy availability.

**Table 5.7: Characterization of microsphere (F1)**

<b>Parameter</b>	<b>Value</b>
Percentage yield	83.25±0.43
Loading efficacy%	85.32±0.75
Loading content%	20.28±0.63
Surface associated drug content %	0.77±0.41
Degree of swelling%	34.84±0.41
Angle of repose	25.32±19

**Table 5.8: Characterization of microsphere (F2)**

Parameter	Value
Percentage yield	81.52±0.37
Loading efficacy%	83.48±0.29
Loading content%	19.92±0.33
Surface associated drug content %	0.98±0.32
Degree of swelling%	35.55±0.32
Angle of repose	25.13±17

**Table 5.9: Characterization of microsphere (F3)**

Parameter	Value
Percentage yield	89.47±0.12
Loading efficacy%	86.94±0.27
Loading content%	21.56±0.14
Surface associated drug content %	0.68±0.31
Degree of swelling%	33.25±0.31
Angle of repose	25.62±22

**Table 5.10: Characterization of microsphere (F4)**

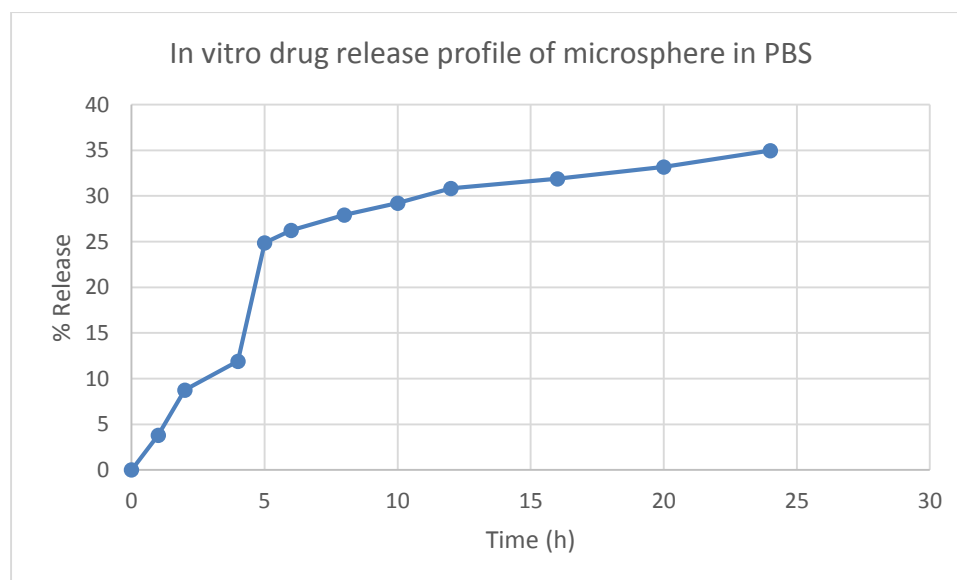
Parameter	Value
Percentage yield	94±0.16
Loading efficacy%	97±0.38
Loading content%	23.12±0.11
Surface associated drug content %	0.23±0
Degree of swelling%	31±0.01
Angle of repose	25.56±37

On the basis of characterisation of formulation F1, F2, F3 and F4, it was concluded that F4 is best among all microspheres. Further studies were conducted only for formulation F4.

**Table 5.11: In vitro drug release profile of microsphere in PBS**

Time in h	% Release
0	0
1	3.81±0.16
2	8.75±0.19
4	11.90±0.18
5	24.87±0.13
6	26.23±0.22
8	27.91±0.27
10	29.22±0.28
12	30.83±0.32
16	31.87±0.33
20	33.18±0.42
24	34.96±0.37

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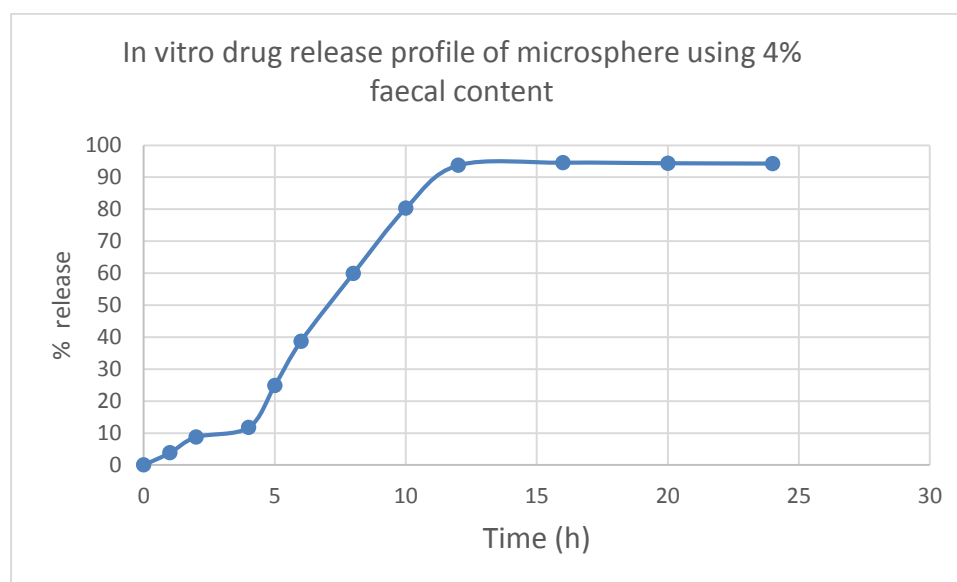


**Figure 5.14: In vitro drug release profile of microsphere using 4% faecal content**

**Table 5.12: In vitro drug release profile of microsphere using 4% faecal content**

Time in h	4% faecal content
0	0
1	3.81±0.12
2	8.74±0.17
4	11.74±0.21
5	24.85±0.26
6	38.71±0.34
8	59.93±0.56
10	80.29±0.38
12	93.73±0.76
16	94.56±0.51
20	94.39±0.40
24	94.28±0.79

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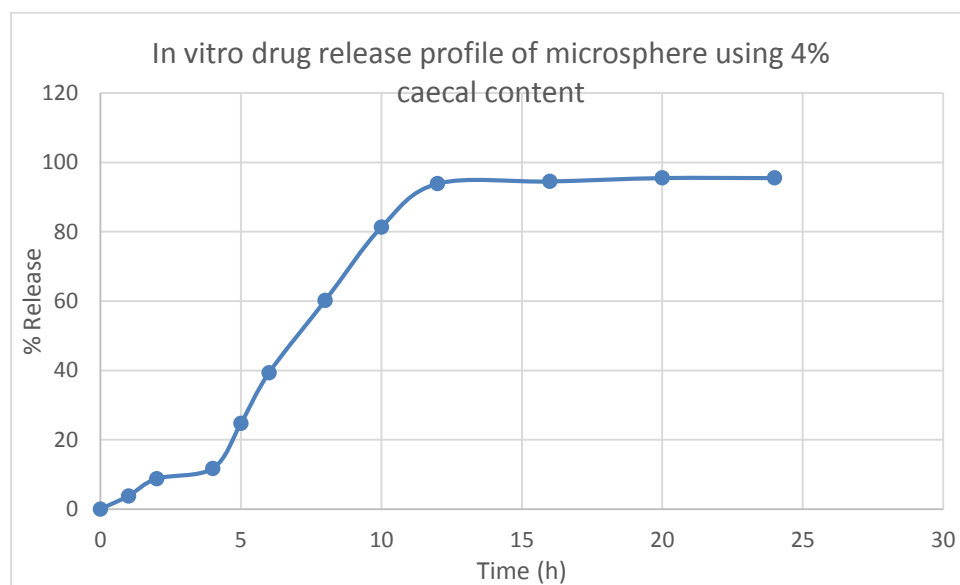


**Figure 5.15: In vitro drug release profile of microsphere using 4% faecal content**

**Table 5.13: In vitro drug release profile of microsphere using 4% caecal content**

Time in h	4% caecal content
0	0
1	3.809±0.11
2	8.75±0.14
4	11.74±0.76
5	24.76±0.28
6	39.33±0.33
8	60.18±0.59
10	81.36±0.34
12	93.89±0.77
16	94.51±0.54
20	95.50±0.42
24	95.50±0.78

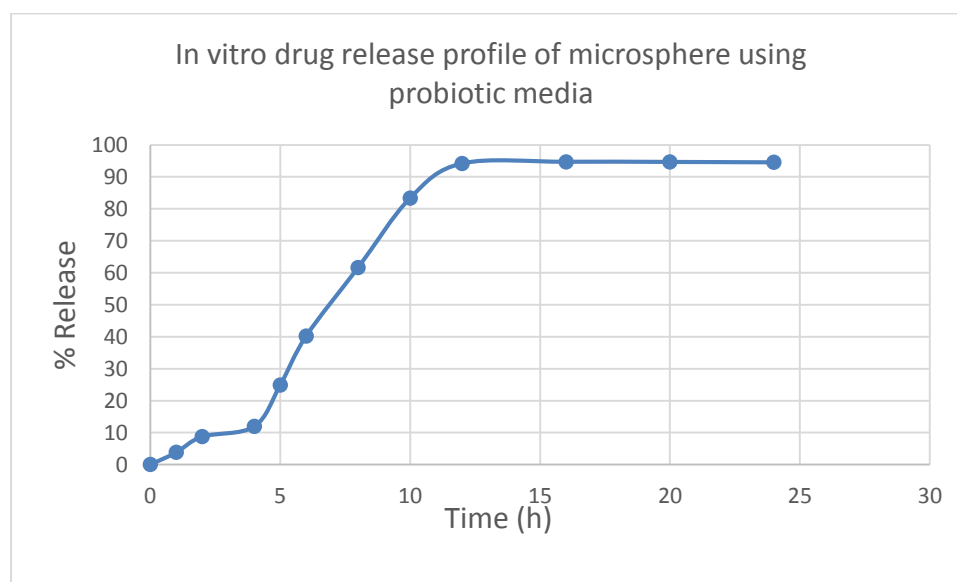
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**Figure 5.16: In vitro drug release profile of microsphere using 4% caecal content**

**Table 5.14: In vitro drug release profile of microsphere using activated probiotic media**

Time in h	Probiotics $9.8 \times 10^{10}$ CFU/ml
0	0
1	$3.81 \pm 0.10$
2	$8.76 \pm 0.14$
4	$11.91 \pm 0.29$
5	$24.83 \pm 0.22$
6	$40.20 \pm 0.48$
8	$61.63 \pm 0.39$
10	$83.36 \pm 0.71$
12	$94.18 \pm 0.47$
16	$94.70 \pm 0.76$
20	$94.64 \pm 0.48$
24	$94.52 \pm 0.82$



**Figure 5.17: In vitro drug release profile of microsphere using probiotic media**

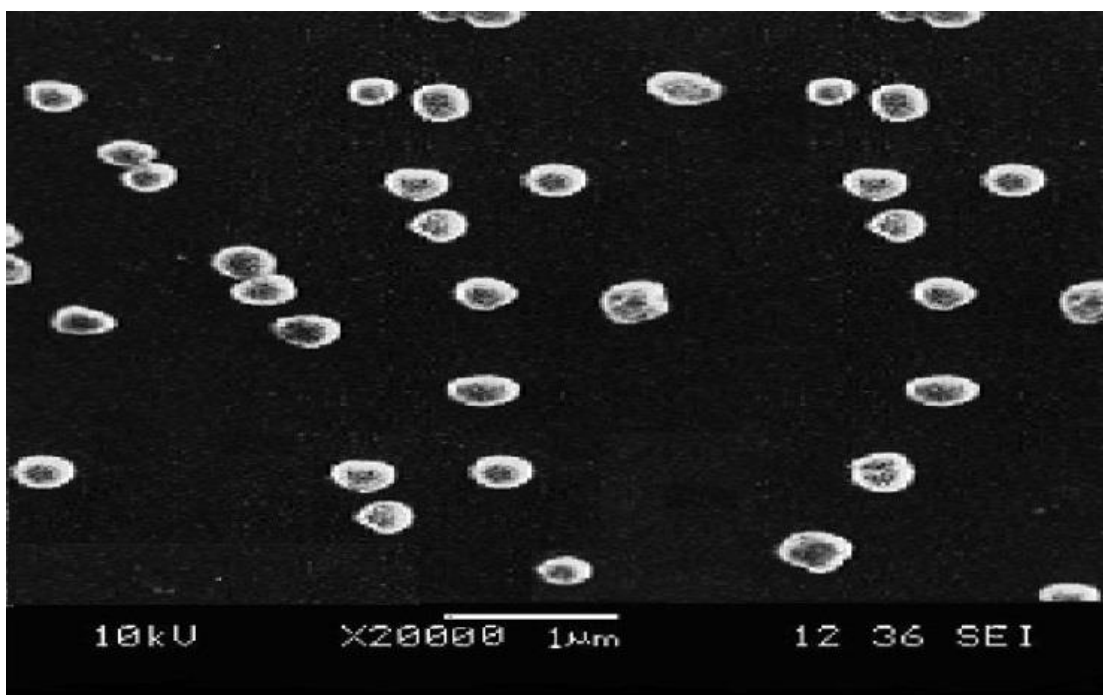


Figure 5.18: SEM image of microsphere showed a good distribution and small size of microsphere

	Diam. (nm)	% Intensity	Width (nm)
<b>Z-Average (d.nm):</b> 599.5	<b>Peak 1:</b> 716.2	88.6	31.24
<b>Pdl:</b> 0.595	<b>Peak 2:</b> 17.16	8.8	3.329
<b>Intercept:</b> 1.02			
<b>Result quality</b> Refer to quality report			

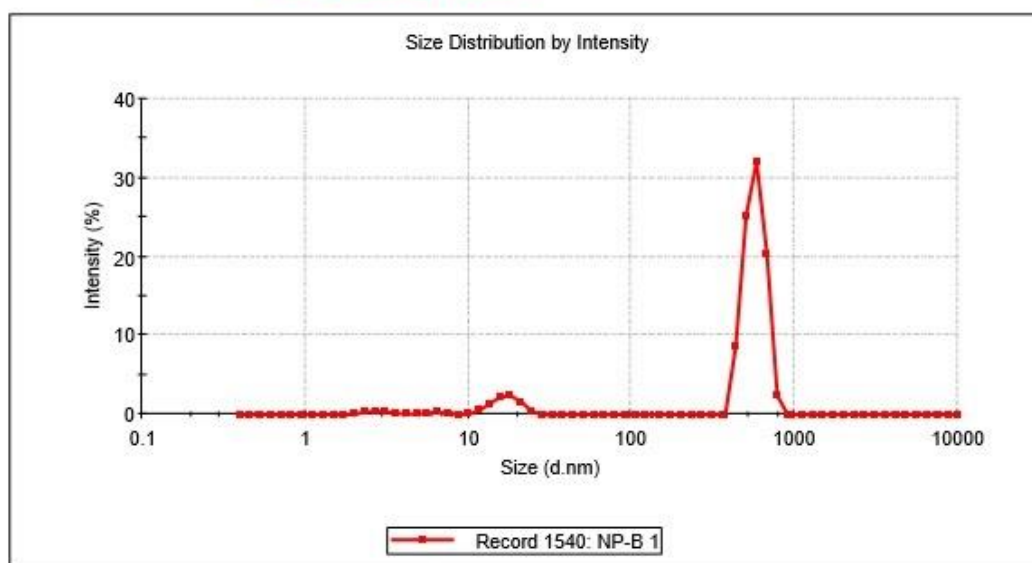


Figure 5.19: Particle size determination by zeta sizer



## **5.6 Formulation of coated granules of raupya bhasma and their evaluation for CTDD**

### **5.6.1 Preparation of formulation**

Formulation was prepared by coating of granules with guar gum followed by eudragit FS30D and was found to be near to spherical. Eudragit FS30D protects the core drug in GIT due to fluctuation of pH and helps in delivering the drug to colon. Guar gum is a well-known example of prebiotic, GIT flora feed over guar gum coating and the core is exposed. The granules contain guar gum and xanthan gum on which GIT flora can feed.

Percentage yield, loading efficacy, loading content, surface associated drug content, degree of swelling and angle of repose of formulation is reported in Table 5.15. In vitro release of granules in PBS is shown in Table 5.16 and in vitro release of coated granules in different media is shown in Tables 5.17- 5.20.

Coated granules deliver the drug to colon by dual mechanism. Eudragit FS 30D layer may lead to delivery of drug to colon, moreover guar gum layer also play important role and deliver the drug due to feeding of microbes on guar gum.

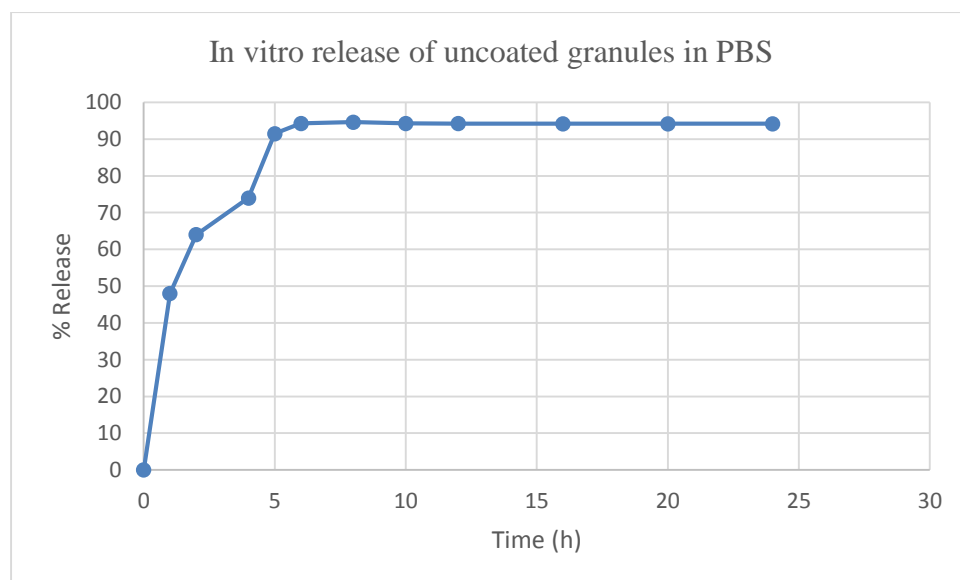
**Table 5.15: Characterization of coated granules**

<b>Parameter</b>	<b>Value</b>
Percentage yield	93±0.22
loading efficacy%	98.32±0.31
loading content%	14.5±0.32
surface associated drug content %	0.1±0
degree of swelling%	19
angle of repose	25±.23

**Table 5.16: In vitro release of uncoated granules in PBS**

Time in h	PBS
0	0
1	48.32±0.23
2	64±0.14
4	74±0.33
5	91.5±0.66
6	94.27±0.83
8	94.61±0.37
10	94.29±0.36
12	94.24±0.41
16	94.20±0.39
20	94.18±0.36
24	94.17±0.33

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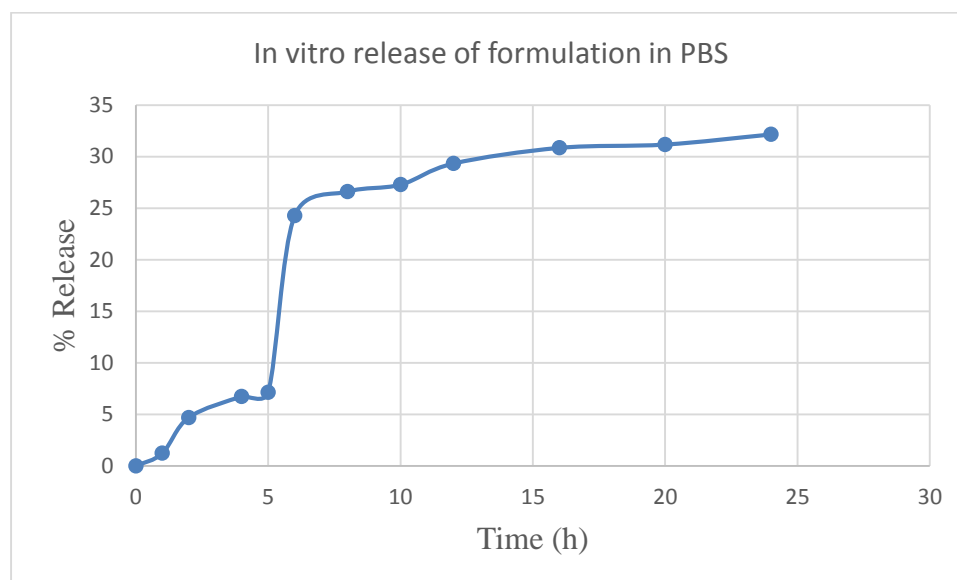


**Figure 5.20: In vitro release of uncoated granules in PBS**

**Table 5.17: In vitro release of formulation in PBS**

Time in h	% drug release
0	0
1	1.24±0.09
2	4.71±0.14
4	6.74±0.13
5	7.15±0.18
6	24.27±0.23
8	26.61±0.32
10	27.29±0.33
12	29.34±0.36
16	30.86±0.41
20	31.18±0.39
24	32.16±0.46

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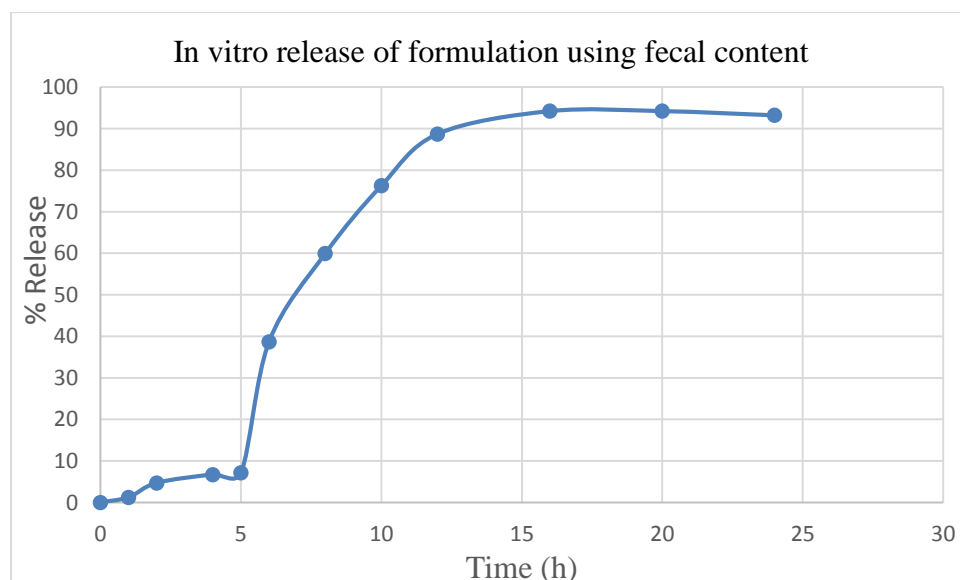


**Figure 5.21: In vitro release of formulation in PBS**

**Table 5.18: In vitro release of formulation using fecal content**

Time in h	4% faecal content
0	0
1	1.24±0.14
2	4.71±0.17
4	6.73±0.21
5	7.15±0.26
6	38.71±0.64
8	59.93±0.33
10	76.29±0.82
12	88.73±0.71
16	94.22±0.64
20	94.22±0.42
24	93.21±0.46

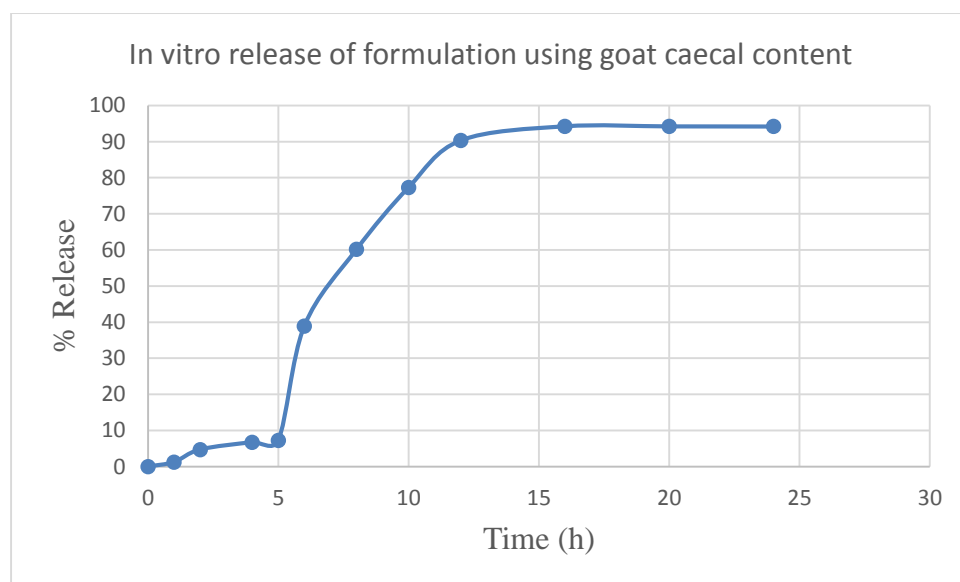
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**Figure 5.22: In vitro release of formulation using fecal content**

**Table 5.19: In vitro release of formulation Using goat caecal content**

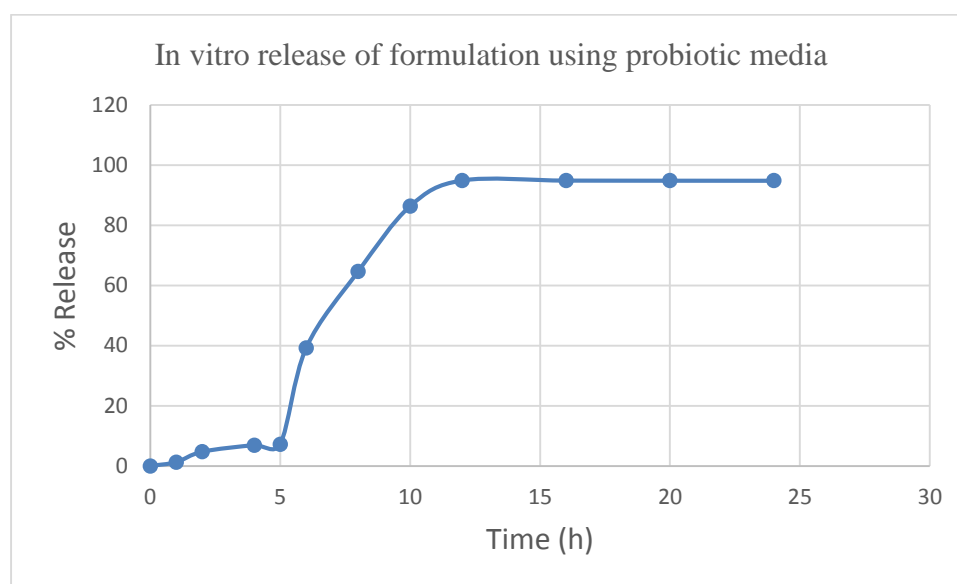
Time in h	4% caecal content
0	0
1	1.25±0.03
2	4.72±0.07
4	6.74±0.16
5	7.24±0.18
6	38.92±0.24
8	60.14±0.32
10	77.33±0.48
12	90.34±0.28
16	94.26±0.63
20	94.24±0.72
24	94.20±0.68



**Figure 5.23: In vitro release of formulation using goat caecal content**

**Table 5.20: In vitro release of formulation using probiotic media**

Time in h	Probiotics $9.8 \times 10^{10}$ CFU/ml
0	0
1	1.25±0.06
2	4.72±0.14
4	6.91±0.27
5	7.23±0.21
6	39.20±0.45
8	64.63±0.38
10	86.36±0.34
12	94.90±0.86
16	94.85±0.88
20	94.82±0.79
24	94.80±0.80



**Figure 5.24: In vitro release of formulation using probiotic media**

The normal transit time in the stomach is 2h. which may vary, while in the small intestine it is relatively constant around 3h. For the colon targeted drug release, the lag time should be similar to the time taken for the system to reach the colon. The lag time of 5h. is considered sufficient on the basis of relatively constant transit time in the small

intestine (3h). In case of uncoated granules, more than 90% drug was released in PBS in 5h (Table 5.16 Figure 5.20), therefore further dissolution studies were not carried out in presence of caecal and faecal content. As granules are matrix system and swelling of guar gum and xanthan gum was very high, the drug released without degradation of polymer by diffusion. To prevent the release of drug before reaching the colon, the granule was coated with guar gum followed by eudragit FS30D. The outer eudragit FS30D coating defends the system against in vitro release, profile reflects that coated granule of raupya bhasma is a better formulation as compared to microsphere. Gastrointestinal environment dissolves rapidly in distal small intestine, where a lumen pH of over 7 triggers the dissolution of the enteric polymer. The inner guar gum coating works as a time-controlled retardant and offers additional protection to the granules until it is degraded by microbes at the proximal colon. In vitro results (Tables 5.17-5.20 and Figures 5.21-5.24) indicate that guar gum followed by eudragit FS30D coating is a feasible coating material to achieve colon specific drug delivery.

## **5.7 References**

1. Krishna G. "Rasendrasara Samagrah (Sanskrit)," 1<sup>st</sup> ed. Varanasi (India): Chaukhambha Prakashan, 1967, pp 87-93.
2. Chaturvedi R and Jha CV (2011) Standard manufacturing procedure of rajat bhasma. *Ayu* 32: 566-571.
3. Mizushima Y and Kobayashi M (1968) Interaction of anti-inflammatory drugs with serum preteins, especially with some biologically active proteins. *J of Pharma Pharmacol* 20:169-173.
4. Chaurasia M, Chaurasia MK, Jain NK, Jain A, Soni V, Gupta Y, Jain SK (2006) Cross-Linked Guar Gum Microspheres: A Viable Approach for Improved Delivery of Anticancer Drugs for the Treatment of Colorectal Cancer. *AAPS PharmSciTech* 7 (3) E1-E9.
5. Milojevic S, Newton JM and Cummings JH (1996) Amylose as a coating for drug delivery to the colon: Preparation and In vitro evaluation using 5-aminosalicylic acid pellets. *J. Control Release* 38:75-84.
6. Shabir S, Shaheeda and Ramanamurthy KV (2012) Formulation and evaluation of chitosan sodium alginate microcapsules of 5-fluorouracil for colorectal cancer. *Int j res pharma chem* 2: 7-19.

7. Wilhelm H, Holzapfel and Petra Haberer (1998) Overview of gutflora and probiotics. *Int J Food Microbiol* 41:85-101.
8. Ji CM, Xu HN and WU W (2009) Guar Gum as Potential Film Coating Material for Colon-specific Delivery of Fluorouracil. *Journal of biomaterials applications* 23: 311-329.
9. Wadekar MP, Rode CV, Bendale YN (2005) Preparation and characterization of a copper based Indian traditional drug: Tamra bhasma. *J Pharm Biomed Anal* 39: 951-55.
10. X Gao, H Feng and J Ma (2010) Analysis of the dielectric constants of the Ag<sub>2</sub>O film by spectroscopic ellipsometry and single oscillator model,” *Physica B: Condens Matter* 405: 1922-1926.
11. Opie EL (1999) On the relation of necrosis and inflammation to denaturation of proteins. *J Exp Med* 115: 597-608.
12. Umaphathy E, Ndebia EJ and Meeme A (2010) An experimental evaluation of *Albuca setosa* aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. *J Med Plants Res* 4: 789-795.
13. Jagtap VA, Agasimundim YS and Jayachandran E (2011) In vitro anti-inflammatory activity of 2-amino-3-(substitutedbenzylidinecarbohydrazide)-4,5,6,7-tetrahydrobenzothiophenes. *J Pharm Res* 4: 378-379.
14. Anson ML and Mirsky AE (1932) The effect of denaturation on the viscosity of protein systems. *Gen Physiol* 15: 341-350.
15. Williams LAD, Connar AO and Latore L (2008) The in vitro anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals, in the early stages of the drug discovery process. *West Indian Med J* 57: 327-331.
16. Panigrahi AK, Annapurna MM, Himasanka K. (2012) Pmatrix tablet for colon specific drug delivery. *Int J Pharm Sci Res* 3(10): 3842-3846.
17. Sinko PJ (2011) *Martin's Physical Pharmacy and Pharmaceutical Sciences*, 6<sup>th</sup> ed. Lippincotts Williams and Wilkins, Baltimore.



## 6 CONCLUSION

In the present investigation an attempt has been made to prepare and characterise the raupya bhasma. Characterisation of raupya bhasma using modern analytical techniques enhances its acceptability in modern medicine. On the basis of analysis using Zeta sizer it was concluded that the size of raupya bhasma lies in nano range. It means raupya bhasma is the ancient concept of silver nanomedicine/nanoparticle. SEM data confirms that raupya bhasma is crystalline in nature. It was further supported by XRD. On the basis of XRD pattern, it is also concluded that raupya bhasma is composed of oxide of silver. FT-IR data confirms that bhasma is free from any organic impurity. Silver content was determined using ICP-MS method. Thermogram of TGA can be used as a standard for evaluating raupya bhasma. The study is important because of the fact that raupya bhasma was standardised for the first time by modern analytical techniques which can be used as a standard to ensure the quality.

Anti-inflammatory activity of raupya bhasma was determined using protein denaturation method, using egg albumin obtained from hen's egg. It was found that raupya bhasma possesses better anti-inflammatory activity than diclofenac sodium. From the  $IC_{50}$  values it became evident that raupya bhasma is more active than diclofenac sodium, being effective in lower concentrations. The anti-denaturation effect was further supported by the change in viscosity. It has been reported that the viscosity of protein solution increases on denaturation.

The present investigation is significant because of the fact that anti-inflammatory activity of raupya bhasma has been reported for the first time.

Ulcerative colitis is a condition of inflammation of colon, raupya bhasma could be a suitable candidate for treatment of ulcerative colitis. Moreover, it could also be used for other inflammatory diseases like oxidative stress, tumour necrosis factor alpha, interleukins which are related to denaturation of proteins and are the mediator of inflammation, in addition also the causative factors of cancer.

Anti-cancer (colon cancer) activity of raupya bhasma was determined by MTT assay using HCT 118 cell lines and it was found that raupya bhasma possess better anti-cancer activity as compared to 5-FU. The anti-cancer activity of raupya bhasma was determined for the first time in this study.

The study is also important because formulation of raupya bhasma (microsphere and coated granules) was prepared for the first time for targeting to colon. It enhances the efficacy at lower dose.

In vitro drug release has been carried out using human faecal content and goat caecal content. But both methods are not convenient either at laboratory scale or at industry scale. So the present study realises the usage of probiotic culture in dissolution media to mimic the colonic microflora conditions in order to evaluate in vitro drug release behavior of formulations meant for colonic delivery. The novel approach exploits development of dissolution media used for evaluating drug release of pharmaceuticals meant for colon specific drug delivery. More specifically, it pertains to the development of animal sparing dissolution media for testing the drug release of polysaccharide based formulations used for colon specific drug delivery.

## APPENDIX

- i. *Shyam Baboo Prasad, Vidhu Aeri, Yashwant. Current Understanding of Synthesis and Pharmacological Aspects of Silver Nanoparticles, American Journal of Phytomedicine and Clinical Therapeutics 2013; 1 (7) 536-547.*
- ii. *Shyam Baboo Prasad, Yashwant, Madhurima Bhargava, Vidhu Aeri. Development of quality standards of ancient silver based nanomedicine: raupya (silver) bhasma. Indo American Journal of Pharm Research 2013;3(10) 8205-8210.*
- iii. *Shyam Baboo Prasad, Yashwant, Vidhu Aeri. Formulation and Evaluation of Dosage form of Raupya (Silver) bhasma for colon targeted drug delivery, American Journal of Pharmatech Research 2013; 3 (6) 318-326.*
- iv. *Shyam Baboo Prasad, Yashwant, Vidhu Aeri. In vitro anti-inflammatory activity of Raupya (Silver) Bhasma, Journal of chemical and pharmaceutical research 2013; 6 (9) 194-197.*
- v. *Shyam Baboo Prasad, Aeri V, Yashwant. Bhasma: Traditional Concept of Nanomedicine and Their Modern Era Prospective. International Journal of Pharmaceutical and clinical research 2013; 5 (4) 150-154.*
- vi. *Shyam Baboo Prasad, Aeri V, Yashwant. Approaches of Colon targeted drug delivery. International journal of drug delivery and technology 2013; 3: 1: 8-11.*
- vii. *Shyam Baboo Prasad, Vidhu Aeri, Yashwant, "Role of Traditional and Alternative Medicine in Treatment of Ulcerative Colitis" International Journal of Pharmacognosy and Phytochemical Research 2013; 5(3); 248-253.*
- viii. *Shyam Baboo Prasad, Hitesh Verma, Vidhu Aeri, Yashwant, "Probiotics: A Medieval To Modern Era Prospective" International Journal of Toxicological and Pharmacological Research 2013; 5(3): 63-68.*

## Current Understanding of Synthesis and Pharmacological Aspects of Silver Nanoparticles

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### ABSTRACT

Silver nanoparticles (SNPs) are one of the most frequently used nanomaterials because of their antimicrobial properties. It can be prepared by physicochemical and biological method. Silver ions has toxic effects on many pathogens, including bacteria, viruses, and fungi. Because of relatively low toxicity of SNPs in humans, it has been used in various medical applications. Decrease in particle size of silver nanoparticles may leads to increase in antibacterial activity which has been associated with the increasing surface area to mass ratio. As various diseases is triggered by these microorganism so pharmacological evaluation of silver nanoparticles may be fruitful in those disease which occurs due to bacteria, fungi and virus. The present review deals with various method of preparation and reported preclinical activity of silver and its derivatives.

**Keywords:** Silver nanoparticles, Nano crystalline silver, Nanomedicine, Metallic nanoparticles, Silver.

### INTRODUCTION

Nanotechnology is emerged as a fastest growing field with numerous applications in science and technology for manufacturing new materials. Nanotechnology is defined as the design, characterization and application of structures, devices and systems by controlling shape and size at 1 to 100 nm<sup>1</sup>. Modern era is of nanomedicine owing to their various therapeutic applications with more efficacies and lesser side effects. The popularity is due to their potential for achieving specific process and selectivity in

pharmacological action<sup>2</sup>. Metallic nanoparticles, including gold, silver, iron, zinc and metal oxide nanoparticles, have shown great promise in biomedical application, due to their large surface area to volume ratio<sup>2</sup>.SNPs or nanosilver (NS) are emerging as one of the fastest growing product in nanotechnology industry. In daily life NS is used in room spray, wall paints, water purifier and laundry detergent.SNPs are also incorporated in textiles for manufacturing of cloth, vests, underwear and socks. It is estimated that of all nano



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## DEVELOPMENT OF QUALITY STANDARDS OF ANCIENT SILVER BASED NANOMEDICINE: RAUPYA (SILVER) BHASMA

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### ABSTRACT

Health practitioner afraid to use metal as medicine due to reported severe toxicity. However Ayurvedic system of medicine particularly Rashshastra described about metal based medicine to cure various ailments. From ancient time bhasma is used in various disease and found to be free from toxicity. As per Ayurvedic physician bhasma may be toxic if it is not prepared as per standard method mention in Rashshastra. Raupya bhasma is silver based nanomedicine of ancient Ayurveda which is used to strengthen brain, liver, heart and memory. It is also used as immunomodulator and aphrodisiac. Due to lack of scientific data over Raupya bhasma it is not as popular as other silver nanomedicine. To consider above mention fact an attempt has been taken to prepare Raupya bhasma according to ancient literature and their characterisation by modern analytical techniques. In this work, we present a systematic characterization of this traditional drug using various techniques like inductive coupled plasma mass spectroscopy (ICP-MS), X-ray diffraction (XRD), thermo gravimetric analysis (TGA), scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR) and zeta sizer. The silver content in bhasma was found to be 63.054%. The nature of bhasma was found to be microcrystalline irregular having particle size 323.8 nm. TGA analysis indicates about loss of weight with temperature. The results obtained were found to satisfactory and confirm the traditional evaluation process by modern method. In addition, some specific findings were also made which could be used as standard data for quality control of Raupya bhasma.

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### Formulation and Evaluation of Dosage form of Raupya (Silver) bhasma for colon targeted drug delivery

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#### ABSTRACT

The potential of guar gum as a film coating material for colon targeted delivery of raupyabhasma is assessed in this study. The granules was prepared by mixing raupyabhasma, guar gum and xanthan gum which was coated by guar gum and pH-sensitive polymer eudragit FS30D sequentially around drug-loaded granules. The outer eudragit FS30D coating defends the system against gastrointestinal environment and dissolves rapidly in distal small intestine, where a lumen pH of over 7 triggers the dissolution of the enteric polymer. The inner guar gum coating works as a time-controlled retardant and offers additional protection of the granules until it is degraded by microbes at the proximal colon. In vitro results indicate that guar gum followed by eudragit FS30D coating is a feasible coating material to achieve colon specific drug delivery.

**Keywords:** Colon targeting, Microbially triggered drug delivery to colon, Polysaccharide based drug delivery, Colon targeted drug delivery, Silver nanoparticles, Bhasma etc.

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### ***In vitro* anti-inflammatory activity of Raupya (Silver) Bhasma**

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#### **ABSTRACT**

*Formulation of silver nitrate and silver sulphadiazine is choice of drug in topical treatment of burns and related inflammation. Ancient silver based nanomedicine of Ayurveda is raupya bhasma (RB) used in treatment of different ailments but RB is still not explored to their anti-inflammatory activity. To consider this an attempt has been made to evaluate in-vitro anti-inflammatory activity of RB against denaturation of protein. Denaturation of tissue proteins is one of the well-documented causes of inflammation. Different concentration of RB was incubated with egg albumin in specified experimental conditions and subjected to determination of absorbance and viscosity to assess the in-vitro anti-inflammatory property using diclofenac sodium as standard against denaturation of protein. RB inhibited protein denaturation in dose dependent manner. The effect of RB as anti-inflammatory agent was found to be better than standard (diclofenac sodium) as the IC50 value of RB and diclofenac sodium are 43.2 µg/mL and 46.1 µg/mL respectively. Inhibition of denaturation of protein was further confirmed by change in viscosity. On the basis of present research it is concluded that RB possessed marked anti-inflammatory activity against the denaturation of protein.*

**Keywords:** Raupya bhasma, Silver nanoparticle, Anti-inflammatory activity, In vitro anti-inflammatory activity, Inflammation.

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#### **INTRODUCTION**

Inflammation is a process of body defence mechanism, which is associated with pain and involves the increase of vascular permeability, increase of protein denaturation and membrane alteration. Inflammation may be due to chemical agent, physical agents and microbes. It is characterized by swelling, redness, pain, heat, and loss of function of injured area[1]. Injury of cells may leads to release of kinins, prostroglandins and histamine. The release of these mediators causes vasodilation, increase in permeability of the capillaries which may lead to increased blood flow to injured site. Inflammation can be classified as both acute and chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues [1,2]. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation is known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process. Current drugs available for treatment of inflammation such as opioids and non-steroidal anti-inflammatory drugs (NSAIDs) are not useful in all cases of inflammatory disorders, because of their severe side effects. As a result, a search for other alternatives seems necessary and beneficial [1,2,3]. From the history of civilization traditional medicines were used to cure human ailments in every possible condition. In modern era we have the option to use them over the synthetic molecules because traditional drugs have lesser side effects [4]. Modern era is of nanomedicine owing to their various therapeutic applications with more efficacies and lesser side effects. The popularity is due to their potential for achieving specific process and selectivity in pharmacological action[5]. Bhasma the ancient concept of nanomedicine is used for treatment of various chronic



## Bhasma: Traditional Concept of Nanomedicine and Their Modern Era Prospective

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### ABSTRACT

Bhasma is used as medicine from ancient time for treatment of various disease. But health professional afraid to use metal product as medicine. As per Ayurvedic alchemist bhasma is safe to use only when it is prepared as per protocol mention in Rasashastra. The quality of bhasma can be assured by ancient Ayurvedic literature but it is not accepted in modern society. To trigger the research in field of bhasma present review deals with general method of preparation, characterisation and reported preclinical activity of bhasma.

**Key words:** Bhasma, Nanomedicine, Nanoparticle, Metallic nanoparticles, Herbomineral, Ayurveda

### INTRODUCTION

Nano medicine getting popularity day by day owing to their various therapeutic applications with more efficacies and lesser side effects<sup>1</sup>. The popularity is due to their specific and selective pharmacological action. Bhasma the ancient concept of nano medicine is used treatment of various chronic ailments since 7<sup>th</sup> century BC. From the history of civilization traditional medicines were used to cure human ailments in every possible condition. In modern era we have the option to use them over the synthetic molecules because they have lesser side effects<sup>2,3,4</sup>. Bhasma is the calcination product of inorganic and organic substances. Bhasma as a medicine is a mystery due to severe side effect associated with metal when administered internally<sup>5</sup>. As per ayurvedic physician bhasma is nontoxic if metal is processed according to ancient ayurvedic literature. The rational pharmaceutical and therapeutically approach of Ayurveda in general and Rasa shastra in particular has transformed metal into medicinal form. The processes of shodhana (purification/potentiation) and marana (calcinations/detoxification – treatment with that quantum of energy which is needed for physico-chemical conversion of raw materials to Bhasma: a therapeutic form) which are very individualized in terms of material, media, method and absolute medicinal form<sup>6</sup>. The bhasmas are taken along with milk, butter, honey, or ghee which makes these elements easily assimilable, eliminating their harmful effects and enhancing their biocompatibility<sup>7</sup>. Our ancient literature describes various method to ensure the quality of bhasma. In current few year tremendous work has been carried out to ensure the quality of bhasma. The present review deals with ancient as well as modern method of preparation of bhasma, therapeutic application of almost all bhasma and their method of characterisation

by traditional method ( as per ancient literature) and using modern analytical techniques.

Preparation of Bhasma: Bhasma can be prepared by putapaka method and kupipakwa method<sup>3,7</sup>. Summarised method of preparation is shown in figure-1

In recent time burning (Calcination) process is done in crucible at specific temperature as per nature of metal and the remaining procedure is kept same for preparation of bhasma.

Bhasma as nano medicine: Bhasma is consider as biologically produced nanomedicine as the size of individual particle is found in nano range. Heating of metal during sodhana may leads to increase in tension causing expansion of metal foil followed by cooling in liquid media leads to decrease in tension and increase in compression force. Repeated heating and cooling process may leads to brittleness, reduction in hardness and finally reduction in particle size. It is confirmed by various research carried out for characterisation of bhasma. The size of swarna bhasma and silver bhasma were found to be of 56 and 16 nm respectively<sup>7</sup>.

Chemical nature of bhasma: Bhasma is produced by the process of calcination of metal and minerals. Calcination of metal may leads to conversion of metal into its metallic oxide<sup>8,9</sup>. Major chemical composition of bhasma is reported in table-1.

Evaluation of bhasma<sup>10,11</sup>: The quality of bhasma can be evaluated by traditional method of evaluation (Bhasma pariksha). Evaluation can be done by physical and chemical test

Physical test

*Nishachandratva*: Bhasma can be observed under bright sunlight to detect the presence and absence of lustre. A good quality of bhasma should be free from metallic lustre indicating metal is completely converted in to ash.

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## Approaches for Targeted Drug Delivery to Colon

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### ABSTRACT

The conventional drug delivery system for colonic disease may leads to absorption of drug across biological membrane of gastrointestinal tract (GIT). The absorption of drug throughout GIT may leads to increase in dose and associated side effects. Colon targeted drug delivery (CTDD) is a method of delivering medication to a patient in a manner that increases concentration of the medication in colon relative to other part of GIT. The aim of CTDD is to prolong, localize, target and have protected drug interaction to diseased tissue. The present review deals with primary as well as recent approaches of delivery of drug to colon.

**Key words:** Colon targeting, Colon targeted drug delivery, Targeted drug delivery, Drug delivery, colon.

### INTRODUCTION

The aim of targeted drug delivery (TDD) is selective and effective localization of drug into the target at therapeutic concentrations with limited or no access to non-target sites. A targeted drug delivery system is chosen in drugs having instability, low solubility, short half-life, large volume of distribution, poor absorption, low specificity and low therapeutic index<sup>1,2</sup>. TDD may provide maximum therapeutic activity by preventing degradation or inactivation of drug during transit to the target site. It can also minimize adverse effects because of inappropriate disposition and minimize toxicity of potent drugs by reducing dose. The colon is a site where both local and systemic delivery of drugs can take place. Local delivery allows topical treatment variety of bowel diseases such as ulcerative colitis, Crohn's disease, amebiasis, colon cancer and local treatment of colonic pathologies<sup>3,4</sup>. The colon specific drug delivery system (CDDS) can be used for systemic delivery of protein and peptide drugs. However, treatment can be made effective if the drugs can be targeted directly into the colon, thereby reducing the systemic side effects<sup>5</sup>.

Primary Approaches for Colon targeted drug delivery: Primary approaches that are used for colon targeted drug delivery (CTDD) are as follow

**pH Sensitive Polymer Coated Drug Delivery:** The pH Sensitive polymer coated drug delivery to colon can be achieved as the pH along the gastrointestinal tract (GIT) varies as shown in table 1. This can be accomplished by means of coating that are intact at lower pH of the stomach but that will dissolved at neutral pH of the colon. These polymer used for coating should be resistant to the acidic condition of the stomach but ionize and get dissolved above a certain threshold alkaline pH found in small intestine. Thus it is possible to apply same concept to deliver drugs to the terminal of ileum or colon by use of

enteric polymers with a relatively high threshold pH for dissolution and subsequent drug release. The most frequently used polymer for this purpose is methacrylic acid and methylmethacrylate that dissolve at pH 6 (Eudragit L) and pH 7 (Eudragit S) have been investigated. But the pH of the distal is 6. This delivery system thus has a inclination to release the drug load prior to reaching the colon. To overcome the problem of premature drug release, a copolymer of methacrylic acid, methyl methacrylate and ethyl acrylate (Eudragit FS) which dissolve at slower rate and at higher threshold pH 7 to 7.5 was reported. One must question the impact of gastrointestinal disease on targeting performance since patient with ulcerative colitis are known to have markedly low colon pH<sup>6,7,8</sup>. Polymer used in pH Sensitive Polymer Coated Drug Delivery is shown in Table 1.

**Time dependent drug delivery:** In this approach, drug release to colon from the system after a predetermined lag time. The normal transit time in the stomach is 2 hr. which may vary, while in the small intestine it is relatively constant around 3hr. For the colon targeted drug release the lag time should similar to the time taken for the system to reach the colon. The lag time of 5 hr. is considered sufficient on the basis of relatively constant transit time in the small intestine (3hr). The lag time rely upon the gastric motility and size of the dosage form. One of the most primitive methods is the Pulsincap device. This device consists of a non-disintegrating half capsule body sealed at the open end with a hydrogel plug, which is enclosed by a water-soluble cap. The whole unit is coated with an enteric polymer to avoid the difficult of variable gastric emptying. When the capsule pass in the small intestine, the enteric coating dissolves and the hydrogel plug starts to swell. The quantity of hydrogel is adjusted so that it pops out only after the specified period of time to release the contents. In another approach, organic acids are filled into the body of

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Review Article

Role of Traditional and Alternative Medicine in Treatment of  
Ulcerative Colitis

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**Abstract**

Currently available treatment options for ulcerative colitis (UC) in modern medicine have several adverse effects. Therefore, there is a need to develop safe and effective treatment modalities for ulcerative colitis (UC). Traditional and alternative medicine play an important role in the management of UC as they were developed from the experience of people which passed from one generation to next since history of civilisation. This article presents a review on some reported traditional and alternative medicine for UC.

**Key words:** Ulcerative colitis, Inflammatory bowel disease, Colitis, Colon, Inflammation, Traditional medicine, Herbal medicine etc.

**Introduction**

Inflammatory bowel disease (IBD) is a general term for a group of chronic inflammatory disorders of unknown etiology involving the gastrointestinal tract. Chronic IBD may be divided into two major groups, ulcerative colitis (UC) and Crohn's disease (CD), clinically characterized by recurrent inflammatory involvement of intestinal segments with several manifestations often resulting in an unpredictable course<sup>1</sup>. Ulcerative colitis is an inflammatory chronic disease primarily affecting the colonic mucosa; the extent and severity of colon involvement are variable. In its most limited form it may be restricted to the distal rectum, while in its most extended form the entire colon is involved. However, 80% of the patients present with disease extending from the rectum to the splenic flexure, and only 20% have pancolitis<sup>2</sup>. Although the causes of IBD remain unclear, considerable progress has been made recently in the identification of important pathophysiologic mechanisms, and further and newer knowledge has been obtained from recent studies concerning their epidemiology, natural history, diagnosis and treatment.

**Symptoms**

Initial symptoms of ulcerative colitis include diarrhoea, blood in stool, pain, weight loss, arthralgia, fever, loss of appetite, ophthalmopathies, nausea, vomiting, abscesses, fistulae and lymph node swelli<sup>3</sup>. Symptoms of mild, moderate and chronic UC is reported in table 1

**Epidemiology**

Ulcerative colitis is usually associated with recurrent attacks with complete remission of symptoms in the interim. The disease is more common in Caucasians than in Blacks or Orientals with an increased incidence (three to six fold) in Jewish. Both sexes are equally affected. The peak occurrence of both diseases (UC and CD) is between ages 15 and 35, it has been reported in every

decade of life. A familial incidence of IBD is currently recorded. In Asia, Africa and South America, cases of UC is reported less as compared to European country. Breast feeding, smoking and appendectomy are associated with reduced risk of UC. Depression, western diet, left-handedness may increase risk of UC<sup>4</sup>.

**Pathophysiology**

The cause of UC still remains unclear, but on the basis of research in recent years point to an over stimulation or inadequate regulation of the mucosal immune system as a major pathophysiologic pathway, and particular emphasis has been given to either the study of mucosal inflammation or immunologic reactions. When the disease is active, the lamina propria of the mucosa becomes highly infiltrated with a mixture of acute and chronic inflammatory cells. There is a predominant increase in mucosal Immunoglobulin G (IgG) production, evidence of complement activation, and activation of macrophages and T cells. This immunological activity is associated with the release of a vast array of cytokines, kinins, leukotriene, platelet activating factor (PAF) and reactive oxygen metabolites. These mediators not only serve to amplify the immune and inflammatory response, but they also have direct effects on epithelial function, on endothelial function (which may increase permeability and lead to ischemia), and on repair mechanisms, thus increasing collagen synthesis. In addition, many of the cytokines (interleukins 1 and 6, tumour necrosis factor) will activate an acute phase response, resulting in fever and a rise in serum acute phase proteins<sup>5,6,7</sup>.

**Diagnosis**

The diagnosis of UC is made on clinical suspicion and confirmed by biopsy, stool examinations, sigmoidoscopy or colonoscopy, or barium radiographic examination<sup>8</sup>.

**Complication**

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## Probiotics: A Medieval To Modern Era Prospective

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### ABSTRACT

Probiotics have been perceived as a solution to many of the life style related problems. In modern era the quality of water and food supply may affect the intestinal micro flora. Chlorinated water and preservatives added to foods may lead to an alteration in the normal micro biota of intestine. Probiotics, however, lead to reversal of this altered microbial picture back to normal. They tend to maintain the delicate balance existing between the gastro intestinal tract and the immunological system of the body. Whenever this balance is disturbed, a disease develops. Probiotics competitively inhibit the over stimulation of immune system by pathogenic bacteria by adhering themselves to the gastro intestinal mucosa in place of pathogenic bacteria and thus inhibiting their colonization. They have proved to be beneficial in the case of allergies/eczema, diarrhoea, hyperlipidaemia, Acquired Immune Deficiency Syndrome, liver cirrhosis, gastric ulcer, hypertension, inflammation, arthritis, inflammatory bowel disease, peptic ulcer and cancer etc. It is also beneficial in case of antibiotic resistance or antibiotic associated side effects. The present review deals with the updated information about the role of probiotics in health and disease.

**Key words:** Probiotics, *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Saccharomyces*, Healthcare

### INTRODUCTION

Pharmaceuticals have not been able to completely control the global morbidity and mortality in case of both acute and chronic diseases. Hence, search for the other alternatives has always been there<sup>1</sup>. The old age quote of Hippocrates becomes most pertinent in the current health scenario i.e. "let food be thy medicine and medicine be thy food"<sup>2</sup>. In the late 90's, microbiologists identified the difference between the micro flora of the diseased human beings and those of normal human beings. The beneficial micro flora were termed as "probiotics"<sup>3</sup>. There are billions of bacteria present in human Gastro Intestinal Track (GIT) forming about 1 kg of the human weight, which includes both harmful as well as beneficial bacteria. Together they are called as gut flora. Delicate balance between the harmful and the beneficial bacteria is responsible for maintenance of health. When this balance is disturbed, the person becomes diseased. One of the ways to regain this balance is the external administration of probiotics (beneficial bacteria) into the body of the diseased person. Probiotics include a large number of different types of bacteria that are normal inhabitants of human GIT. The most common among them are various species of *Lactobacilli* and *Bifidobacteria*. They reside in small intestine and colon. Probiotics have been able to attract the maximum attention among several food supplements as they have additional benefits beyond their nutritional value<sup>4</sup>. In 1965, Stillwell and Lilly introduced the term "probiotics"<sup>5</sup>. The term is made up of two words Latin preposition *pro* means "for" and the Greek adjective *βιοτικός* means "biotic". Hence, it means "for favour of life". According to World health organisation (WHO) and Food and Agriculture Organization (FAO) it

is defined as "living microorganism intended for administration into the host body in adequate amount so as to confer health benefits"<sup>6</sup>.

History: It has been known since long that there are benefits of using fermented milk products and poultices of bread moulds. But Ellie Metchnikoff started the probiotic therapy via fermented milk products in 1907. In 1915, the therapy was used for the treatment of urogenital infections. However, in the intertwining period of 7-8 decades less study is reported on probiotics due to an increased interest in antibiotics. These were labelled as "alternative medicines". Recently there has been a resurgence of probiotics due to demand of consumers for better treatment. This resurgence can also be attributed to development of resistance against antibiotics<sup>6</sup>.

Probiotic criteria: An organism must fulfil the following criteria in order to be considered as probiotics: There should be high cell viability, and should be able to survive in low pH. Even if strain cannot colonize in gut, it should have the ability to persist. They should have the ability to adhere to the epithelium of GIT so as to overcome the flushing effect due to peristalsis. They should have the ability to interact or to send signals to the immune cells associated with GIT. They should be capable of being isolated from humans. They should have processing resistance. They should be non-pathogenic. They should have positive influence on local metabolic activities.

A dose of five billion colony forming units are generally recommended for adequate health benefits. Probiotics should be Generally Recognized as Safe (GRAS). Probiotics preparations involve the use of both single as well as mixture of microorganisms<sup>6</sup>.

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