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FORMULATION AND DEVELOPMENT OF DELAYED RELEASE TABLETS OF BETULINIC ACID

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ABSTRACT

Purpose: Colonic drug delivery has gained increased importance for the delivery of drugs, proteins and therapeutic peptides. Drugs to reach the colonic region unchanged they must be protected in upper GIT (stomach & intestinal regions.. The present study is for development of colon targeting Betulinic acid tablets using compression coating method using different combinations of Gums, organic and inorganic solvents. Preformulation, weight variation, content uniformity and invitro dissolution studies were performed using USP apparatus II. Mediums of different pH and microflora medium were used. Results: Hardness 4kg/cm^2 (core) & 5kg/cm^2 (coated), gums in combination showed better results than when used alone. F1, F2, F3 failed in early stages of evaluation tests. Release rates end of 5 hours (F4=3.17%, F5=2.69%, F6=4.04%, F7=1.92%) & at end of 17 hours (F4=82.54%, F5=98.06%, F6=88.16%, F7=99.7%). Conclusion: colon delivery can be achieved using combination of guar gum & xanthan gum combinations or combination of 3 gums including tamarind gum.

KEY WORDS: **Colon targeting, microflora medium, colorectal cancer**

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1. INTRODUCTION

The colon specific drug delivery is valuable in the topical treatment of colonic disorders which include Inflammatory bowel diseases (cronh's disease, ulcerative colitis), Ameobiasis, Colon carcinomas along with some infections. The delivery of drug to colon is useful for systematic absorption of drugs especially proteins and peptides which are degraded in upper GIT. In addition the colon has a longer retention time and appears to be highly responsive to agents that enhance the absorption of poorly absorbed drugs.[1] various approaches for colon targeting includes

1. Time dependent
2. pH dependent systems
3. Microflora activities[2]

Enzymatic activities associated with the microflora of the colon are best method to be used as a tool for colon specific drug delivery. Colonic diseases are majorly associated with pain, swelling, inflammation and tumors effects to which different drugs are prescribed for each individual symptoms. The drug of choice is Betulinic acid because of its cumulative properties of anti-ulcer, anti-inflammatory and anti-tumor. Anti-ulcer: by stimulating mucus and mucus cell production. Anti-inflammatory: by deducing the levels of COX-2, nitric oxide, TNF, IL-I, & malondiaaldehyde in edema paw tissue and activities of super oxide dismutase, glutathione peroxidase, glutathione reductase in liver. Anti-tumor: by its anti proliferative and apoptosis- inducing mechanisms. Betulinic acid is class II drug according to bcs classification. Several polysaccharides are being investigated as carriers for colon-specific drug delivery. The polysaccharides that are under active investigation include pectin and its salts, chondroitin sulfate, amylase, chitosan, guar gum etc. colon drug delivery systems were developed for mebendazole, metronidazole, and celecoxib etc. In light of this information it was planned to develop colon targeting delivery systems for Betulinic acid to provide effective and safe therapy. The present study aims at utilizing hpmc, guar gum, xanthan gum, tamarind gum and their combinations. Solvents used are water and iso propyl alcohol.

2. MATERIALS AND METHODS

2.1 MATERIALS

Betulinic acid, cyclo dextrin's, microcrystalline cellulose, crosscaramellose sodium, spray dried lactose, starch paste, magnesium stearate, talc, Guar gum, Xanthan gum, Tamarind gum, HPMC, distilled water.(provided by Pharmtech solutions pvt ltd)

Dissolution apparatus USP II (basket type), disintegration (PLT-279), U.V apparatus (T 60 U), HPLC (Azulent 1200 series).

2.2 METHODS

(1) Solubility enhancement using cyclodextrins

Betulinic acid is a highly lipophilic drug and insoluble in water or aqueous medium. Due to its insoluble nature the dissolution of drug in the solvent medium is not achieved. To enhance the dissolution property of the drug, drug molecules are complexes with cyclo dextrin, which have hydrophilic outer surface and inner hydrophobic region in which the drug is entrapped. Thus the drug will not be in direct contact with the medium. Drug and cyclo dextrin were taken in 1:1 ratio using iso propyl alcohol as medium at a concentration of 50ml/25mg of drug. Cyclodextrin are kept under continuous stirring to which drug is added after a period of 2 hours. Stirring is continued for 5 hours, stopped and kept aside for 3 days. Which is again stirred at slow rates filtered and dried at temperature of 50^{o}c till all the iso propyl alcohol traces gets evaporated. [4,5.]

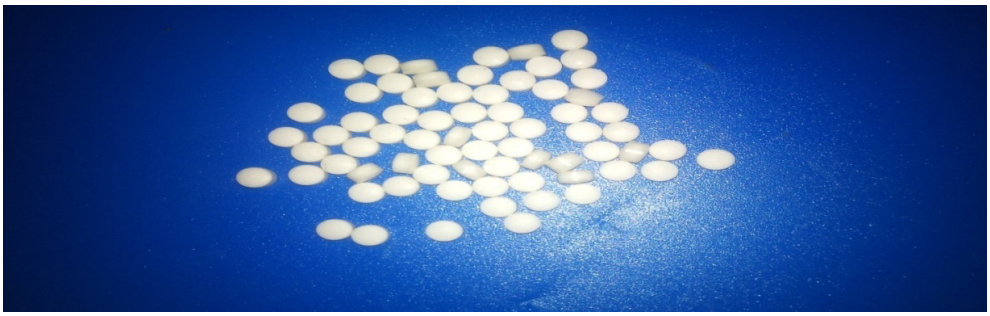
(2) Preparation of core tablets

The core tablets of Betulinic acid for compression coating with various gums were prepared using direct compression technique. Each core tablet consists of 50mg of drug, starch as binder, micro crystalline cellulose as direct compression vehicle, croscarammelose sodium as disintegrant, talc and magnesium

stearate (2:1) as lubricant and glidants. The drug, croscaramelose sodium, mcc, starch paste were mixed thoroughly to which magnesium stearate and talc are added which are then passed through #30. The uniformity of mixing was assessed by conducting content uniformity test on power sample. The mixture was compressed into tablets with an applied force of 4000 kg using 5-mm flat punches using single station tablet machine (Larsen & toubro, MK-1). The core tablets were tested for hardness, thickness, content uniformity, friability, disintegration. [2]

Table 1
Content of the core tablet

s.no	Content	Quantity
1	drug	50mg
2	ccs	6mg
3	mcc	6mg
4	starch	10mg
5	mg. stearate	1mg
6	talc	2mg

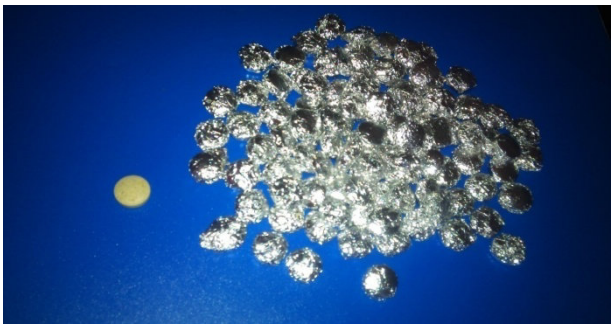


(3) Coating using compression coating method

After suitable tests core tablets were compression coated with different coat formulations F1 to F7 of different gums and combinations as in table 2. Compression coating is done by wet granulation technique. In wet granulation, all the excipients were mixed thoroughly which includes gums, hpmc, starch paste, lactose as mentioned in table 2, passed through #30. Talc and mg.stearate were added post to granulation. About 47% of the coat formulation was placed in die cavity (diameter 12mm). The Betulinic acid core tablet (diameter 5mm) was then carefully placed in the center of the die cavity, which was filled with the remainder of the coat formulation. It was then compressed around the core tablets at an applied force of 5000kg using a 12-mm round, flat punch. These are then tested for hardness, thickness, drug content and drug release. [1, 2& 6]

Table 2
Composition of different gum coat formulation for compression over Betulinic acid core tablets

s.no	contents	f1	f2	f3	f4	f5	f6	f7
1	guar gum	375			187.5	250		125
2	xanthan gum		375		187.5	125	250	125
3	tamarind gum			375			125	125
4	Hpmc[9]	75	75	75	40	40	40	40
5	starch paste	65	65	65	60	60	60	60
6	lactose				40	40	40	40
7	mg.stearate	5	5	5	5	5	5	5
8	talc	5	5	5	5	5	5	5




(4) Preparation of different pH and microflora medium

Tablets should be tested in different pH solutions because of different environments of gastric, intestine and colonic regions, tablet must pass through GI tract crossing stomach and intestine unaffected into the colonic region and release the drug based on mechanism of degradation by microflora in the colonic region. Solutions are prepared resembling the gastric, intestinal and colonic region which include

i. 0.1N HCL solution
ii. 7.4 pH solution
iii. 5 pH solution
iv. Microflora medium

Microflora medium consists of harmless or attenuated bacterial cultures of staphylococcus, lacto bacillus, and streptococcus. These microbial cultures are incubated in pH 5 solution which resembles the colonic environment. This solution is bubbled with CO2.

3. EVALUATION OF TABLETS

Both core and compression coated tablets were evaluated for weight variation, thickness, friability, drug content and invitro drug release studies. Hardness of tablets are tested using Monsanto hardness tester. Friability was determined in a Roche friabilator. The thickness of tablets was measured by Vernier calipers. Weight variation was performed according to official method.

***3.1 INVITRO* STUDIES**

The *Invitro* drug release studies were carried out under conditions mimicking mouth to colon transit using USP II dissolution test apparatus (basket type) at 50 rpm. Test was carried out for a total period of 17 hours using HCL (pH1.2) solution (900ml) as dissolution medium at 37^{o} c for first 2 hours, pH sorensen's phosphate buffer solution (900ml) for next 3 hours and pH 5 micro flora medium for rest of the period. 1ml of sample was withdrawn at end of each 30 mins for first 5 hours and for every 2 hours for the remaining period. The withdrawn sample was replaced by same volume of fresh pre-warmed medium. To the samples (1ml), respective mediums were added to makeup volume up to 10ml. the drug content was analysed measuring the absorbance at 254nm using UV/VIS spectrometer. [7]

3.2HPLC

3.2(a) ASSAY

Assay is an indicative of the amount of drug present in the dosage form. Here it gave the insight information about the substances of the process and about effect of changes. Mobile phase was prepared by mixing specified amount of acetonitrile and water filtered through 0.45micrometer nylon membrane filtered and degassed. The prepared sample was injected into the column and chromatogram and responses. Column used was kromasil C18 at a wave length of 210nm.

3.2(b) COMPATIBILIYT STUDEIS

Principle: Every compound shows its own specific peak point when run through the column, unless they are changed or deteriorates or complexes with other excipients or compounds in vicinity. The present hplc compatibility study works on same principle, which is used to study the effect of excipients on drug and their interactions with other excipients and changes(if any) occurs in the compounds.

Method: 6 tablets from the batch were selected randomly, triturated in a neat and clean motor. The powdered sample which includes drug and

other excipients were run through hplc using appropriate method and mobile phase. Results were noted. Same test was also performed with another 6 tablets but after powdered tablets were kept under accelerated conditions for a specific period of time.

3.2(c) STABILITY STUDIES

The optimum formulation was subjected to stability testing at $27^0C \pm 2^0C$ 65% RH, $40^0C \pm 2^0C$ 75% RH and in refrigerator at $05\text{-}08^0C$ for 4 weeks. The tablets were analysed for drug content.

4. RESULTS AND DISCUSSION

4.1 Core tablets of betulinic acid

The core tablets of betulinic acid were prepared by direct compression method using ccs as disintegrant, starch and lactose as binder and diluent. The weight of core tablet was fixed lowest level, i.e. 60mg. the mean drug content of betulinic acid core tablets was found to be 99.2-98.6% of total drug amount indicating uniformity of drug content in the formulation. The hardness of core tablets of betulinic acid was found to be in range of 4-5kg/cm^2. The core tablets of betulinic acid were also found to comply with the friability test since the weight loss was found to be less than 0.5%. The thickness of the tablets was found to be 4mm & diameter 5mm. The core tablets were found to disintegrate with in 5 mins. Thus excipients i.e. super disintegrant, binder and diluent contributed for required characteristics of betulinic acid tablets for compression coating with different gums and their combinations.

4.2 Gum compression coated betulinic acid tablets

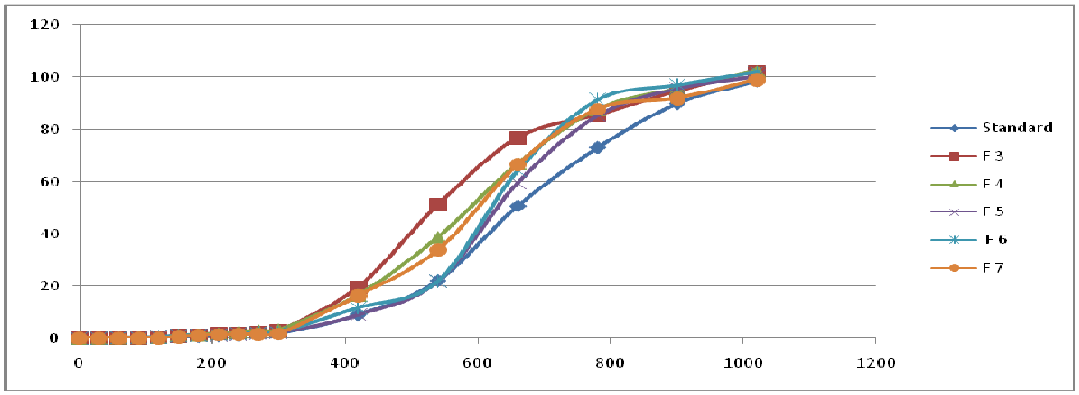
The coat formulation containing various gums and proportions of guar gum, xanthan gum and tamarind gum were prepared by wet granulation technique since these gums has poor compressibility and flow properties. The gum granules were prepared using starch paste as binder (table 2). The compression coated tablets were prepared by applying maximum compression force and the hardness of tablets was found to be in the range of 5-6 kg/cm^2. Thickness of the coat of compression coated betulinic acid tablets were 5mm & diameter 12mm measured by screw gauge. The final weight of compression coated tablet was found to 525mg which is constant in each formulation, indicating uniformity. Formulations F1, F2 and F3 were not up to the mark as per ICH guide lines, as were failed to acquire desired DT and hardness.

4.3 Invitro drug release studies

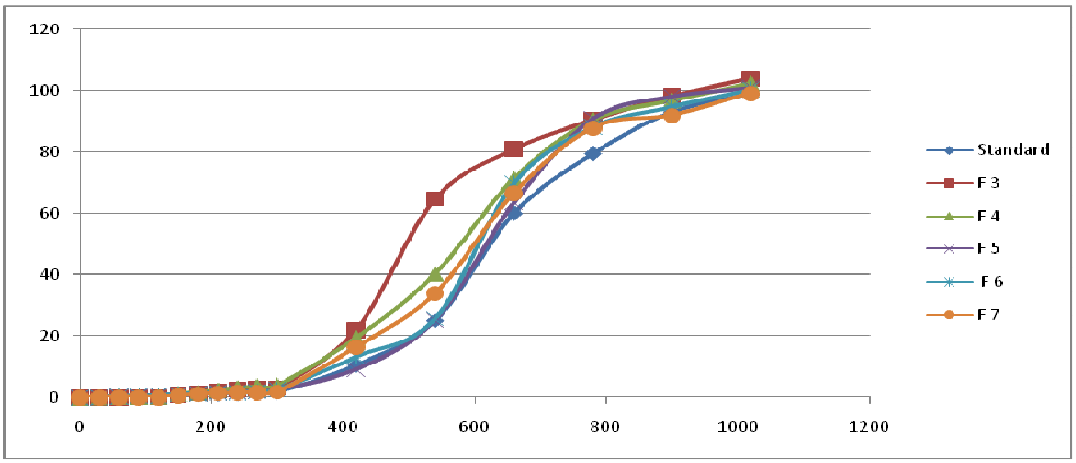
The F4, F5, F6, F7 formulations released 3.17, 2.69, 4.04, and 1.92% of betulinic acid, respectively at the end of 5-hours dissolution study. This indicates that a minimal amount of the drug is released from the compression-coated formulations in the physiological environment of stomach and small intestine. The release of drug mainly depends on the swelling property and enzymatic action of colonic bacteria. The formulation F7 containing the combination of guar gum, xanthan gum, and tamarind gum in compression coat over betulinic acid tablet released least amount of drug in physiological environment of stomach and intestine, indicating it as better formulation. The formulations of betulinic acid containing either combinations of guar gum, xanthan gum and tamarind gum retained their physical integrity.

The release of drug at the end of 17 hours study from F4 = 82.54%, F5 = 98.06, F6 = 88.16% and F7 = 99.7%indicating better release by formulation of F5 and F7. The compression coated F4, F5, F6 & F7 formulations released more than 50 %of drug by the end of 17 hours dissolution study. In the first 5 h of dissolution F4&F6 released nearly 3.17 and 4.04 % of drug respectively which may cause deleterious effects on stomach and small intestine. Hence it is beneficial to select formulation which release minimum amount i.e., F5&F7 which released 2.69 and 1.92% respectively. Thus F5 and F7 were proved to be better formulations. However these formulations need to be evaluated over humans for better understanding. Other drug release is considered as standard due to absence of oral betulinic acid formulation.

Graph 1



Graph 2

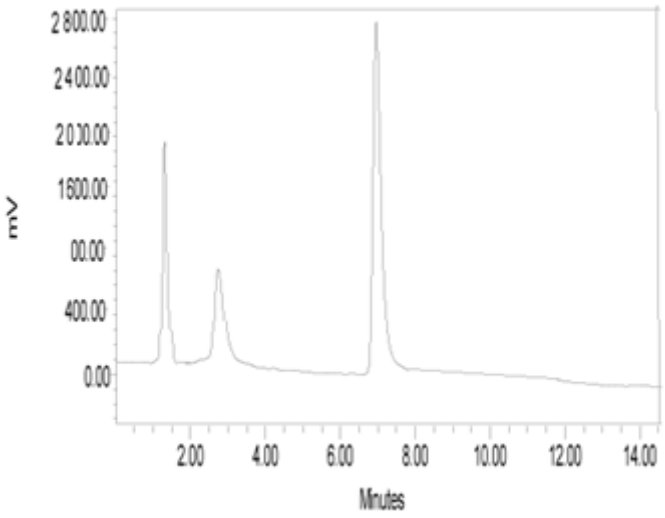


Invitro dissolution profile of the formulation of betulinic acid, mean percent betulinic aacid released from compression coated tablets of formulation F3 to F7 of guar gum, xanthan gum and tamarind gum.

4.4 Stability studies

Betulinic acid delayed release tablets were evaluated at accelerated and normal conditions for their stability for about a period of 4 weeks. There was no change in the physical properties of the tablets; dissolution studies also indicated a release of above 90% of the drug content. Assay using HPLC indicated the presence of 95% of unchanged drug. Due to this results conclusion was made that no interaction between the excipients and drug was observed and maintaining the integrity of the formulation.

Graph 2



4.5 HPLC

Assay was performed to test the purity of drug, its compatibility with excipients and to confirm the stability studies. Test was performed using Kromasil C18 100 * 4.6mm, 5µm column at a flow rate of 1.7mL/min at a wave length of 210nm. Injected volume was about 25µL. Every compound showed its respective peak as per standard usp data. A straight peak was also observed between 6.5 -7 mins along with some smaller peaks. Indicating the undeteriorated & unchanged product of betulinic acid and other excipients.

5. CONCLUSION

The present study was mainly done to formulate a novel colon targeted oral drug delivery for betulinic acid which provides a targeted action at the required site. Different trials were taken by making use of various specialized solvents, disintegrants, diluents and non micronized drug. The physical parameters such as weight, hardness, thickness, friability, disintegration and dissolution and drug content were evaluated. Two of the formulations i.e. F5&F7 showed better results for retention and release of drug as required. F5 formulation included the combination of guar gum and xanthan gum at a concentration of 2:1 respectively and F7 combination of guar gum, xanthan gum and tamarind gum at a concentration of ratio 1:1:1 respectively along with other excipients at different concentrations (table 2). F5 released 2.69 and F7 1.92 % of drug in stomach and intestinal region and a maximum of 98.06 & 99.7% by end of 17 hours release study. Tablets of successful trail batch have been charged in the stability chambers for stability studies for a period of 4 weeks. This showed no change in physical appearance, drug content or dissolution pattern. Based on HPLC studies there was no possible interaction between betulinic acid, gums and other excipients. Drug release followed zero order and erosion kinetics.

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