AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

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

Shyam Baboo Prasad[1],Yashwant[1], Vidhu Aeri[2*]

1. School of Pharmaceutical Sciences, Lovely Professional University, Phagwara (Punjab).
2.Faculty of Pharmacy, JamiaHamdard, Hamdard Nagar, (New Delhi).

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.

*Corresponding Author Email: vidhuaeri@yahoo.com
Received 23 October 2013, Accepted 28 October 2013

Please cite this article in press as: Aeri V. *et al.*, Formulation and Evaluation of Dosage form of Raupya (Silver) bhasmafor colon targeted drug delivery. American Journal of PharmTech Research 2013.

INTRODUCTION

The antibacterial property of silver has been well-known for thousands of years with the ancient Greeks cooking from silver pot. Silver nitrate was used to treat ulcers in 17th and 18th century.[1,2] More recently silver is used as a biocide to prevent infection in burns, traumatic wounds and diabetic ulcers.[3,4] Recently it is proven that silver nanoparticles (SNPs) have anti-inflammatory activity.[5,6] It also possess activity against ulcerative colitis and colon cancer.[7,8]Raupyabhasma (RB) is the ancient concept of silver nanomedicine used in treatment of various ailments and found to be free from toxicity at therapeutic doses.[5,9] To consider this an attempt has been taken to develop a formulation meant for delivery of drug to colon. Conventional oral drug delivery generally does not provide rate-controlled release to target site. In most of the cases, conventional drug delivery offers sharp increase in drug concentration often achieving toxic level and following a relatively short period at the therapeutic level of the drug concentration finally drops off until re-administration. In order to achieve maximum therapeutic efficacy, it is necessary to deliver the drug to the target site in the optimal amount for the required period of time. It can be achieve by targeted drug delivery system.[10]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, amoebiasis, colon cancer and local treatment of colonic pathologies. [11,12] However, treatment can be made effective if the drugs can be targeted directly into the colon, thereby reducing the systemic side effects.[13]Targeting to colon can be achieved by coating of drug with PH sensitive polymer. In disease condition the pH of GIT can alter and drug can be released prior to reaching colon.[10] Another approach to target the drug to colon is time dependent drug delivery system in which 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 major draw backs of this system is that the drug can reach to colon earlier if GIT motility is more and can reach later if GIT motility is less.[10] CTDD can also be achieved by microbially triggered Drug Delivery. Because of the presence of the biodegradable enzymes only in the colon, the use of biodegradable polymers for colon-specific drug delivery seems to be a more site- specific approach as compared to other approaches.[14]The varieties of enzymes, mainly of bacterial origin present in the colon, are essential for the biotransformation of the prodrugs. Microbially triggered drug delivery involve prodrug approaches of drug delivery and

polysaccharide based drug delivery. Drawbacks of the prodrug approach is that it is not applicable to all types of drug. In polysaccharide based drug delivery system polysaccharide polymer protect the drug from the surroundings of stomach and small intestine, and are capable to deliver the drug to the colon. On reaching the colon, they undergo assimilation by micro-organism or degradation by enzyme or break down of the polymer back bone leading to a subsequent reduction in their molecular weight and thereby loss of mechanical strength. They are then unable to hold the drug entity any longer.[15]

MATERIAL AND METHODS

Raupyabhsama of baidyanath Ayurved ltd. was procured from Prakash Pharmacy Jalandhar, India), Guar gum (Central Drug House, India), Xanthan gum (Central Drug House, India), Eudragit FS30D (Evonic industry, Germany)

Development of formulation (Guar gum and eudragit FS 30D coated raupyabhasma loaded granule core)

Preparation of RB loaded granule core

The granules were prepared by mixing raupyabhasma, guar gum and xanthun gum in a ratio of 1: 1.5: 1.5. The ingredients were mixed thoroughly in a V-cone blender. Wet granulation of the prepared mass was carried out using purified water. The damp mass was passed through sieve number 12. The prepared granules were dried for 1 hr. in a hot air oven at 45°celsius.

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 triggering mechanism for the drug release in the colon by colonic micro flora. Further, the granules were coated with 40% with Eudragit FS30D to retard drug release in stomach and small intestine so that the drug can only release in the colon[8].

Characterisation of coated granules

Percentage yield

To calculate % yield prepared formulation was divided by the total amount of all the polymer and drug used in the preparation of the formulation, which give the total percentage yield. It was calculated by using following equation:

Percentage yield = Actual yield of formulation / Total weight of polymers and drug X 100

Determination of raupyabhasma included in formulation

The amount of raupyabhasma in microsphere was determined by placing microsphere in phosphate buffer saline (PBS, pH 7.4) for 48 hours at 37°C with vigorous stirring. The

concentration of raupyabhasma were determined using ICP-MS. The percentage of loading efficiency and content was expressed as with following equation.

Loading efficiency (%) = Weight of loaded drug in microsphere / Initial feeding weight of drug x100

Loading content (%) = Weight of loaded drug in microsphere/ Weight of microsphere x 100

Surface associated drug content

Formulation was evaluated for surface associated drug content on the surface of formulation. From each batch, 100 mg of formulation were shaken in 20 ml of 0.1N Hcl for 5 min and then filtered through Whatman filter paper. The amount of drug present in filtrate was determined ICPMS and calculated as a percentage of total drug content. All the experiments were conducted in triplicate (n=3).

Surface associated drug content = Amount of drug present in filtrate/ Amount of drug used in formulation x100

Equilibrium Swelling Studies of formulation

A pre weighed amount (100 mg) of formulation were placed in PBS (pH 7.4) and allowed to swell up to a constant weight. The formulation were removed and blotted with filter paper, and their changes in weight were measured. The degree of swelling (α) was then calculated from the following formula:

$$\alpha = wg - wo / wo$$

Where, wo is the initial weight of the microspheres and wg is the weight of the microspheres at equilibrium swelling in the medium.

Flow properties

Angle of repose

This was determined by using funnel method. Powder was poured from a funnel that can be raised vertically until a maximum cone height (h), was obtained. Diameter of heap, (D), was measured. The angle of repose was calculated by the following equations.

$\tan \theta = h / r,$

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

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

In vitro release of coated granules

Preparation of Dissolution Media

Preparation of fresh human faecal content medium

Freshly prepared human faecal slurries have been commonly used to investigate the fermentation

of non-starch polysaccharides. The slurries were prepared by homogenising fresh feces (4% w/v with respect to 200 ml volume of dissolution) obtained from healthy human volunteers in anaerobic 0.1 M sodium phosphate buffer (pH 6.8) under anaerobic conditions. These were finally added to the dissolution media to give a final faecal dilution of 4% w/v. All the above procedure was carried out under carbon dioxide in order to maintain anaerobic conditions[6].

Preparation of goat caecal medium

Fresh caecal content of goat were procured from local market of phagawara, punjab and kept in desiccator under anaerobic condition. Accurately weighed caecal content were suspended in the pH 6.8 buffer continuously bubbled with carbon dioxide. These were finally added to the dissolution media to give a final caecal dilution of 4 % w/v. All the above procedures were carried out under carbon dioxide in order to maintain anaerobic conditions.

In vitro drug release of formulation using human fecal slurries

Drug release studies in the presence of fresh human faecal slurries were carried out using USP I (basket type) dissolution test apparatus. However, slight modification in the procedure was done. Gradient pH dissolution method is used to evaluate the drug release from formulations meant for colonic drug delivery using human faecal contents.

The experiments were carried out in 250 ml beaker immersed in water maintained in the jars of dissolution test apparatus. Six capsules of formulation were subjected to each of the vessels (beakers) containing the dissolution medium. For the first 2 hours, the dissolution study of formulation is carried out in 150ml pH 1.2 Hcl buffer using 100 rpm at 37 ± 0.5°c. Afterwards the pH of the dissolution media is adjusted to 6.8 using 50 ml pH 6.8 phosphate buffer and sodium hydroxide and the study is continued for up to 4 h. At the end of the fourth hour, the media is degassed using carbon dioxide gas to remove undissolved oxygen and to maintain anaerobic conditions inside the medium for 15 min. Then the 4% w/v of freshly prepared faecal slurries is added to the dissolution media and the study is continued up to 24h under the continuous purging of CO_2 throughout the study. About 1.0 ml samples were withdrawn at 1.0, 2.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0, 20.0 and 24.0 h respectively from the dissolution medium and it is replaced by the fresh medium which was maintained under anaerobic condition. The volume of the sample made up to 10 ml and were filtered by using 0.22 micron membrane filters and was subjected to ICP-MS analysis. All the studies are repeated six times and the mean data is recorded.

In vitro drug release using goat caecal content

Drug release studies in the presence of rat caecal content were carried out using USP I (basket

type) dissolution test apparatus. However, slight modification in the procedure was done. Gradient pH dissolution method is used to evaluate the drug release from formulations meant for colonic drug delivery using human faecal contents. The experiments were carried out in 250 ml beaker immersed in water maintained in the jars of dissolution test apparatus. Six capsules of microsphere were subjected to each of the vessels (beakers) containing the dissolution medium. For the first 2 hours, the dissolution study of microsphere capsules is carried out in 150ml pH 1.2 Hcl buffer using 100rpm at 37 ± 0.5°c. Afterwards the pH of the dissolution media is adjusted to 6.8 using 50ml pH 6.8 phosphate buffer and sodium hydroxide and the study is continued for up to 4 h.

At the end of the fourth hour, the media is degassed using carbon dioxide gas to remove undissolved oxygen and to maintain anaerobic conditions inside the medium for 15 min. Then the 4 % w/v of rat caecal content is added to the dissolution media and the study is continued up to 24h under the continuous purging of CO_2 throughout the study. About 1.0ml samples were withdrawn at 1.0, 2.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0, 20.0 and 24.0 h respectively from the dissolution medium and it is replaced by the fresh medium which was maintained under anaerobic condition. The volume of the sample made up to 10 ml and were filtered by using 0.22 micron membrane filters and subjected to ICPMS analysis. All the studies are repeated six times and the mean data is recorded.

The studies on the aforementioned formulation were also carried out in the same manner without adding rat caecal content and/or human faecal contents i.e normal buffer media.

RESULTS AND DISCUSSION

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 of drug to colon. Guar gum is a well-known example of prebiotic, GIT flora feed over guar gum coating then core is exposed. Again granule contains guar gum and xanthan gum on which GIT flora can feed and finally drug released to colon.

Table 1: Characterization of coated granules

Parameter	Value
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

Percentage yield, loading efficacy, loading content, surface associated drug content, degree of swelling and angle of repose of formulation is reported in table 1. In vitro release of granules in PBS is shown in table 2 and in vitro release of coated granules is shown in table 3.

Table 2: 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

Table 3: In vitro release of formulation in PBS, faecal content and probiotic media

Time in h	PBS	4% faecal content	4% caecal content
0	0	0	
1	1.24±0.09	1.24±0.14	1.25±0.03
2	4.71±0.14	4.71±0.17	4.72±0.07
4	6.74±0.13	6.73±0.21	6.74±0.16
5	7.15±0.18	7.15±0.26	7.24±0.18
6	24.27±0.23	38.71±0.64	38.92±0.24
8	26.61±0.32	59.93±0.33	60.14±0.32
10	27.29±0.33	76.29±0.82	77.33±0.48
12	29.34±0.36	88.73±0.71	90.34±0.28
16	30.86±0.41	94.22±0.64	94.26±0.63
20	31.18±0.39	94.22±0.42	94.24±0.72
24	32.16±0.46	93.21±0.46	94.20±0.68

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).[10] In case of uncoated granules more than 90% drug released in PBS up to 5 hour, so further dissolution studies did not carried out in presence of caecal and faecal content. As granules was matrix system and swelling of guar gum and xanthan gum is very high so drug released without degradation of polymer by diffusion. To prevent the release of drug before reaching to colon the granule is coated with guar gum followed by eudragit FS30D. 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.[15]

CONCLUSION

On the basis of current finding it is concluded that coating of granule with guar gum followed by eudragit FS30D has the potential to be used as coating material for colon-specific drug delivery.

ACKNOWLEDGEMENT

The authors are thankful to management of Lovely Professional University for providing necessary facilities for the present study.

REFERENCES

1. Bhattacharya R, Mukherjee P. Biological properties of "naked" metal nanoparticles. Adv drug deliv rev 2008; 60: 1289-1306.
2. Klasen HJ Historical review of the use of silver in the treatment of burns. I. Early uses. Burns 2000; 26: 117-130.
3. Wadhera A, Fung M. Systemic argyria associated with ingestion of colloidal Silver. Dermatol Online J 2005; 11: 12.
4. Atiyeh BS, Costagliola SN, Hayek SA. Effect of silver on burn wound infection control and healing: review of the literature. Burns 2007; 33: 139–148.
5. Prasad SB, Yashwant, Aeri V. In vitro anti-inflammatory activity of Raupya (Silver) Bhasma. J Chem Pharm Res 2013; 5:194-197.
6. Patricia LN,JianFei W, Edward E, Tredget MD , Robert EB. Anti-inflammatory activity of nanocrystalline silver in a porcine contact dermatitis model. Nanomed2008; 4: 241-251.
7. Kailash CB, Paul JS. Effects of Nanocrystalline Silver (NPI 32101) in a Rat Model of Ulcerative Colitis. Dig Dis Sci 2007; 52: 2732–2742.
8. Satapathy SR, Mohapatra P, Preet R, Das D, Sarkar B, Choudhuri T, Wyatt MD, Kundu CN. Silver-based nanoparticles induce apoptosis in human colon cancer cells mediated through p53.Nanomed 2013; 8: 1307-1322.

9. Prasad SB, Yashwant, Aeri V. Bhasma: Traditional Concept of Nanomedicine and Their Modern Era Prospective. Inter Journal of Pharmaceutical and Clinical Research 2013; 5: 150-154.
10. Prasad SB, Aeri V, Yashwant.Approaches for Targeted Drug Delivery to Colon. International Journal of Drug Delivery Technology 2013; 31: 8-11.
11. Philip AK, Philip B. Colon Targeted Drug Delivery Systems: A Review on Primary and Novel Approaches. Oman Med J 2003; 25: 70-78.
12. Prasad SB, Aeri V, Yashwant. Role of Traditional and Alternative Medicine in Treatment of Ulcerative Colitis. International Journal of Pharmacognosy and Phytochemical Research 2013; 5: 248-253.
13. Tortora GJ, Derrickson BH. Principles of anatomy and physiology.12th ed., Wiley International 2008; 922-923.
14. Prasad SB, Verma H, Aeri V, Yashwant. Probiotics: A Medieval To Modern Era Prospective. International Journal of Toxicological and Pharmacological Research 2013; 5: 63-68.
15. Hu Z, Kimura G, Mawatari S, Shimokawa T, Yoshikawa Y and Takada K. New preparation method of intestinal pressure-controlled colon delivery capsules by coating machine and evaluation in beagle dogs. J Control Release 1998; 56: 293-302.