

EFFECT OF GUAR GUM AND XANTHAN GUM COMPRESSION COATING ON RELEASE STUDIES OF METRONIDAZOLE IN HUMAN FECAL MEDIA FOR COLON TARGETED DRUG DELIVERY SYSTEMS

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ABSTRACT

Objective: Compression coating with polysaccharides such as guar gum (GG) and xanthan gum (XG) mixture has been pioneer to be useful for microflora activated colon drug delivery systems.

Method: In the present investigation bacteria triggered colon-targeted delivery of metronidazole (MTZ) was developed by compression coating with GG and XG in different coat weights. Rapidly disintegrating core tablets containing 100 mg of MTZ were prepared and compression coated with 150 mg, 250 mg and 350 mg of granules containing a mixture of GG and XG.

Results: Drug release was highly retarded with 350 mg of coat weight, After 24 h of dissolution in human fecal media the cumulative percentage of drug release from the 350 mg compression coated tablets was found to be around $21.0 \pm 1.65\%$. As a result, the coat weight was further reduced to 250 mg. It was perceived that reduction in coat weight to 250 mg did not affect the initial drug release rate in simulated upper gastrointestinal tract (GIT) conditions, but at the end of the 24 h of dissolution the amount of drug release was increased to $84.8 \pm 1.22\%$ in simulated human fecal media. On the other hand, 150 mg compression coated tablets showed $28.56 \pm 1.89\%$ drug release in upper GIT, which indicates uncontrolled drug release within 5 h of the dissolution study.

Conclusion: As a consequence, results of the present investigation shows that compression coated MTZ tablets with 250 mg of guar and xanthan gum coat is most likely to promise in exploiting the MTZ release in colon to treat amoebiasis and other local colonic diseases.

Keywords: colon targeted drug delivery, metronidazole, amoebiasis, human fecal media, guar gum, xanthan gum, simulated colonic media.

INTRODUCTION

During the last few decades there has been interest in developing site specific formulations for targeting drug delivery to the colon. It is a suitable site for safe and slow absorption of drugs which are targeted at the large intestine, designed to act systemically [1]. Nonetheless, colon is a perplexing target for drug delivery and has been driven primarily by the need to improve the treatment of colonic pathologies. Many approaches have been used to surmount these, with mixed rates and have received considerable interest in recent years [2, 3].

The site specific delivery of drugs to the colon can provide major advantages for many pharmaco treatments such as inflammatory bowel disease (IBD) including crohn's disease and ulcerative colitis, majorly colorectal cancer. It has been reported that 1 million Americans are believed to have IBD with 15,000- 30,000 new cases diagnosed annually [4].

To triumph colon specific delivery via oral route, the dosage form should be strict controlling on pre-colon release and spontaneous release at proximal colon. Granting the concept looks quite simple, but difficult to achieve in practice as the colon is the most distal segment of the gastrointestinal tract (GIT). The pharmaceutical strategies which are commonly used to achieve a colon specific drug delivery system include timed releasing system [5, 6] coating with pH sensitive polymer, prodrug and colonic microflora activated delivery systems [2, 7].

The pH dependent systems are designed to release the drug at a particular pH of the gastro intestinal tract (GIT). The site specificity of pH dependent systems is poor because of large variation in the entire pH of the GIT [8, 9]. Among all the systems, the microflora activated delivery systems have been found to be the most promising since the abrupt increase of the bacteria population and associated enzymatic activities in the ascending colon represents a non-continuous event independent of GI transit time and pH. The key principle in microflora activated systems is a series of polysaccharides which evade enzymatic degradation in the small intestine and are predominantly metabolized by colon bacteria, such as xanthan gum, amylose, dextran, pectin and galactomannan [10].

The natural polysaccharides such as guar gum, xanthan gum remain undigested in the stomach and the small intestine and are degraded by the anaerobic microflora of the colon, for example, *bacteroides*, *bifidobacteria species* and *eubacteria*, to smaller monosaccharides, which are then used as energy source by the bacteria. These carriers act as prebiotics (non-digestible food ingredients which can be fermented by gastrointestinal microflora) to the colonic bacteria and encompass in the drug release mechanisms [11]. Not quite all of guar gum based systems were either in matrix or compression coated tablet form [12, 13].

The present investigation is aimed at using these inexpensive, naturally occurring and abundantly available polysaccharides in different levels of coating for colon delivery of model drug MTZ. Guar gum a natural galactomannan polysaccharide obtained from the ground endosperms of *Cyamopsis tetragonolobus*, family Leguminosae [14]. It mainly contains high molecular weight hydrocolloidal polysaccharide, composed of galactan and mannan monomeric units combined through glycosidic linkages and shows degradation in the large intestine due the presence of microbial enzymes [15, 16, 17].

An attempt was made to formulate a compression coated tablet dosage form which comprised of guar and xanthan gum as a compression coating material in different coat weights. In the present investigation we endeavored to use guar gum and xanthan gum mixture as compression coating material in 150mg, 250 mg and 350 mg coat weights. The present paper describes the optimization of coating weight for a colon specific drug delivery with guar gum and xanthan gum mixture and *Invitro* evaluation of drug release testings of the formulation in human fecal media under anaerobic conditions.

MATERIALS AND METHOD

Metronidazole (98-100.3%) was purchased from Jackson Laboratories Pvt. Ltd, Punjab, India. Guar gum and Xanthan gum were purchased from Molychem Manufacturers Pvt. Ltd, Mumbai, India. Cross povidone was purchased from Central Drug house (P) Ltd, New Delhi, India. The acetonitrile used was of high performance liquid chromatography (HPLC) grade (Qualigens), and Triple

distilled water (TD water) used. Talc, starch and magnesium stearate were of pharmacopoeial grade.

Preparation of Core and Compression Coated Tablets

Rapidly disintegrating core tablets consisting of MTZ (100 mg) and a super disintegrant polyvinyl pyrrolidone (cross-PVP) (4 mg) were prepared. A weighed quantity of MTZ required for 20 tablets for each batch (A1, A2 and A3) was mixed thoroughly with the required amount of cross-PVP. The ingredients were mixed thoroughly in a V-cone blender. Quantity weighing 104 mg was taken and compressed individual into tablets using a single punch tableting machine. The compression coat material was prepared using xanthan gum, guar gum in 150mg, 250 mg and 350 mg weight in a ratio of 1:2 (xanthan gum: guar gum). Core tablets were compression coated with different coating mixtures. Initially, 350 mg of coat material was applied on to the core tablets. Further, in an attempt to reduce the coat weight, 250 mg and 150 mg of coat material was applied over the core tablets.

Table 1: It shows composition of metronidazole tablets containing A 1 (150mg) A 2 (250 mg) A 3 (350mg) coat weight

Ingredients	Quantity (mg) present for each compression coated tablet		
	A 1	A 2	A 3
Metronidazole	100	100	100
Cross-PVP	4	4	4
Xanthan gum	50	83	117
Guar gum	100	177	233
Talc	4.25	5.95	7.65
Magnesium stearate	3	4.2	5.4

The ingredients for coating of the core tablet in the quantities mentioned in **Table 1** were wet granulated using starch paste (10%). Granules of the above wet mass were prepared by passing through a sieve with a nominal aperture of 1 mm. The granules were dried for 1 h at a temperature of 50 °C. The dried granules were passed through a sieve with a nominal aperture of 1mm and mixed with talc (1.7%) and magnesium stearate (1.2%). Accurately weighed 350 mg, 250 mg and 150 mg of coat material was applied over the core tablets. For compression coating, 40% of coat weight was placed in the die cavity followed by careful centering of the core tablet and addition of the remainder of coat weight. The coating material was compressed around the core using a single station tableting machine. Compression coated MTZ tablets were tested for thickness, hardness, drug content and drug release characteristics.

Determination of Drug Content

The compression coated tablets were finely powdered and equivalent to 100mg of MTZ was accurately weighed and transferred to 100ml volumetric flask containing approximately 50ml of pH 6.8 phosphate buffer. The flask was shaken to solubilize the drug. The volume made up with pH 6.8 phosphate buffer and mixed thoroughly. The solutions were filtered through 0.22 µ membrane filter. The sample analysis for drug content was done at 319 nm by using RP- HPLC (LC-20AD, Shimadzu, Japan) method described below.

Preparation of Human Fecal Medium for Release Testing

Human fecal slurries have been commonly used to investigate for the fermentation of non-starch polysaccharides (Guar gum and Xanthan gum) used in colon targeted drug delivery systems. The slurries were prepared by homogenising fresh feces 5% w/v (with respect to 200ml volume of dissolution media) obtained from healthy human volunteers (no preceding history of gastrointestinal disorder and had not taken antibiotics for at least three months

prior to the study) 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 fecal dilution of 5% w/v [18]. All the above procedure was carried out by purging carbon dioxide into the dissolution media in order to maintain anaerobic conditions

In vitro Drug Release of Guar Gum and Xanthan Gum Coated Tablet of Metronidazole Using Human Fecal Slurries

Drug release studies in the presence of fresh human fecal slurries were carried out using USP I (basket type) dissolution test apparatus. However, slight modification in the procedure was done. Gradient pH dissolution method was used to evaluate the drug release from formulations meant for colonic drug delivery using human fecal contents. The experiments were carried out in required volume beaker immersed in water maintained in the jars of dissolution test apparatus. Compression coated tablets were subjected to each of the vessels (beakers) containing the dissolution medium. For the first 2 hours, the dissolution study of tablet 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, the study is continued for upto 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 5% w/v of freshly prepared fecal slurries was added to the dissolution media and the study was continued upto 24h under the continuous purging of CO₂ throughout the study. About 1.0ml samples were withdrawn at 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0, 20.0 and 24.0 h respectively from the dissolution medium and it is replaced by the fresh medium which was previously maintained under anaerobic condition. The volume of the sample made upto 10 ml and filtered by using 0.22 µ membrane filter. 20µl of this solution was injected to HPLC (LC-20AD, Shimadzu, Japan). All the studies were repeated for six times for each batch (A1, A2 and A3) and the mean data was recorded.

RP-HPLC Determination of the Metronidazole Content in the Tablet Formulation and the Dissolution Fluids Containing Fecal Content

The quantitative determination of MTZ was analyzed by reverse phase high performance liquid chromatography. The RP-HPLC system consisted of a Shimadzu HPLC system Model LC AD 20 (Japan) with a computerized system controller, and SPD-M20 a UV detector, containing Kinetex 5u C-18 column (250x4.6 mm² ID: particle size 5 µm) was used.

Mobile phase was acetonitrile (ACN) and Triple distilled water (consisting of 0.4% triethylamine and pH adjusted to 3.6 with 5% orthophosphoric acid) used. The filtered mobile phase was pumped at a flow rate of 0.7 ml/min in the ratio of 44:56 (ACN: TD water with pH 3.6). The eluent was detected by UV detector at 319 nm. The retention time was found to be 3.6 min. A standard curve was constructed for MTZ in the range of 10-70 µg/ml. A good linear relationship was observed between the concentration of MTZ and peak area ($r^2=0.999$). The standard curve was used for estimating the content of MTZ in the tablet and the dissolution medium.

Statistical Analysis

The cumulative percent of MTZ released from compression coated tablets (n=6) in the dissolution medium at 24 h with and without human fecal contents was compared and the statistical significance was tested by using Student's *t-test*. A value of P<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Drug Content

Drug content of the guar gum and xanthan gum compression coated tablets of MTZ was found to be 97.5%

Metronidazole Core Tablets

The core tablets of MTZ were prepared by direct compression of the core mix prepared. The core tablets had a diameter of 5.1 ± 0.02 mm and height of 2.6 ± 0.01 mm. The hardness of the core tablets were found to be in the range of 3.5-4.5 kg/cm². These tablets were found to be in limit with the friability test since the weight loss was found

to be less than 0.6%. The disintegration time of the core tablets was found to be 40s as it contains cross-PVP in the core of the tablet.

Compression Coated Tablets

Core tablets were compression coated with guar gum and xanthan gum in a mixture outlined in **Table 1**. The granulation was done using starch paste (10%). The hardness of the tablets was found to be in the range of 4.5- 6.0 kg/cm².

In Vitro Release Studies in Presence and Absence of Human Fecal Media

In this present study, rapidly disintegrating core tablets containing 100 mg of MTZ were prepared and compression coated with 150 mg, 250 mg and 350 mg of granules containing a mixture of GG and XG. Hence an attempt was made to formulate a dosage form, which showed minimal drug release in upper GI tract and ensured maximum drug release in the environments of the colon. The compression coat was designed to undergo bacterial degradation in the colon, exposing the rapidly disintegrating drug containing core in the colon. The core tablets were compression coated with coating mixtures outlined in **Table 1**.

Drug release studies from compression coated tablets with 350mg coat weight, showed $2.2 \pm 1.24\%$ in 5 h transit time. This included 2h dissolution at a pH of 1.2 followed by 3 h dissolution at a pH 6.8. At the end of 24 h dissolution, the amount of drug release was $21.0 \pm 1.65\%$, $35.7 \pm 1.29\%$ without and with human fecal media respectively [Fig.1]. After 24 h of dissolution, upon opening, the tablet swells and gels, the core tablets could be seen. Since the drug release rate was highly retarded at a coat weight of 350mg, it was proposed to reduce the coat weight to 250mg and further 150mg.

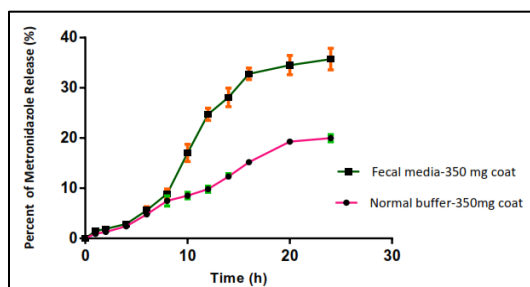


Figure 1: It shows comparative mean % drug release from compression coated tablets with a coat weight of 350 mg

Drug release studies from compression coat weight 250mg (A2) and 150 mg (A3), showed a drug release of $5.6 \pm 1.85\%$, $38.56 \pm 1.89\%$ in 5h transit time respectively. This indicates 150mg coat weight tablets were unable to retard the drug release in upper GI tract. At the end of 24 h dissolution, the amount of drug release was $78.8 \pm 1.22\%$, $86.6 \pm 1.82\%$ respectively in the human fecal medium [Figure 2; Figure 3].

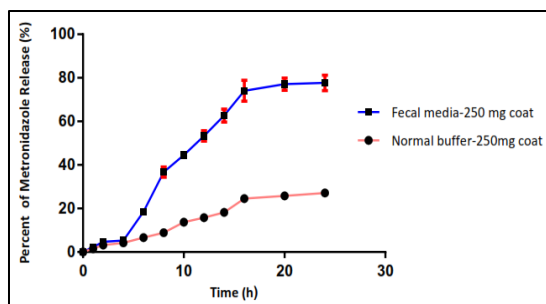


Figure 2: It shows comparative mean % drug release from compression coated tablets with a coat weight of 250 mg

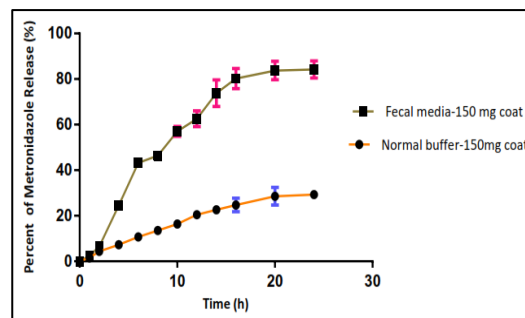


Figure 3: It shows comparative mean % drug release from compression coated tablets with a coat weight of 150 mg

250 mg coat weight with guar and xanthan gum (1:2) promises for colon specific drug delivery because, the compression coat in this case consists of a relatively higher concentration of degradable polysaccharide which will facilitate a faster drug delivery to the colon compared to 350mg coat weight. However the relative potential of the formulations needs to be evaluated in human volunteers.

CONCLUSION

The present investigation was carried out to develop colon targeted drug delivery systems for MTZ for an effective and safe therapy of amoebiasis and local colonic infections. The natural biodegradable polysaccharide composition consisting of xanthan gum as a drug release retarding agent in combination with colon degradable polysaccharides, guar and starch can be successfully used to protect the drug in pre-colon region and abrupt release in proximal colon. Furthermore, under gradient pH conditions (pH=1.2 for 2 h; pH=6.8 for 2 h; pH=6.5 for 20 h). It was observed that after changing the pH of the medium to 6.8 and adding human fecal content, drug release was accelerated. However, in human colonic fluids the microflora will be more when compared to *in vitro* conditions, thus drug release in 250 mg coat tablets will increase a little. The obtained results indicate that, compression coated MTZ tablets with 350 mg of guar gum and xanthan gum did not degrade in simulated colonic fluids whereas formulation coated with 250 mg coating mixture appears to be most promising in exploiting drug release in the colon, which needs to be further proven by *in vivo* experiments.

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