Colon-targeted quercetin delivery using natural polymer to enhance its bioavailability

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ABSTRACT

The aim of the present study is to develop a polymer (Guar Gum)-based matrix tablet (using quercetin as a model drug) with sufficient mechanical strength, and promising *in vitro* mouth-to-colon release profile. By definition, an oral colonic delivery system should retard drug release in the stomach and small intestine, and allow complete release in the colon. By drug delivery to the colon would therefore ensure direct treatment at the disease site, lower dosing, and fewer systemic side effects. Quercetin is antioxidant in nature and used to treat colon cancer, but they have poor absorption in the upper part of the gastrointestinal tract (GIT). As a site for drug delivery, the colon offers a near neutral pH, reduced digestive enzymatic activity, a long transit time, and an increased responsiveness to absorption enhancers. By achieving a colon-targeted drug delivery system, the absorption of quercetin may be increased, which leads to better bioactivity in fewer doses.

Key words: Colon cancer, quercetin, guar gum, antioxidant

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INTRODUCTION

In recent times, colon-targeted drug delivery systems have gained importance, for the systemic delivery of protein and peptide drugs.[1] Drug delivery to the colon is desired not only for oral delivery of peptide and proteins, but also to treat different diseases associated with the colon such as, irritable bowel syndrome, colon cancer, colitis, and ulcerative colon.[2] Drug targeting to the colon is also useful when a delay in drug absorption is desired from a therapeutic point of view, such as treatment of diseases that have peak symptoms early in the morning like nocturnal asthma, angina or arthritis.[1] By definition, an oral colonic delivery system should retard drug release in the stomach and small intestine, but allow complete release in the colon. The fact that such a system will be exposed to a diverse range of gastrointestinal conditions on passage through the gut makes colonic delivery via the oral route a challenging proposition. Targeted drug delivery to the colon would therefore ensure direct treatment at the disease site, lower dosing, and fewer systemic side effects.[3]

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The inability of the GIT enzyme to digest certain plant polysaccharides is taken advantage of in developing a colon-specific drug delivery system. The biodegradable polymer matrix core embedded the drug by compressing the blend of active drug, a biodegradable polymer and additives. Various polysaccharides are being evaluated for colon targeting such as pectin, guar gum, gum ghatti, dextran, chitosan, and xylan. The bacterial enzymes of the colon degrade the carrier polymer in a well-defined manner and release the contents for localized colonic delivery or systemic absorption through the colon.[4]

The important bacteria present in the colon are *Bacteroides, Bifidobacterium, Eubacterium, Peptococcus, Lactobacillus,* and *Clostridium,* and they secrete a wide range of reductive and hydrolytic enzymes, such as, b-glucuronidase, b-xylosidase, b-galactosidase, a-arabinosidase, nitroreductase, azoreductase, deaminase, and urea hydroxylase. These enzymes are responsible for degradation of di-, tri-, and polysaccharides.

Quercetin is a flavonoid, and to be more specific, a flavonol. It is the aglycone form of a number of other flavonoid glycosides, such as rutin and quercitrin, found in citrus fruit and onions. Quercetin forms the glycosides quercitrin and rutin, together with rhamnose and rutinose, respectively.

Quercetin has many health promoting effects, including improvement of cardiovascular health, reducing risk for cancer, and anti-inflammatory and anti-allergic effects. All these activities are caused by the strong antioxidant action of quercetin. The anti-inflammatory action of quercetin is caused by the inhibition of enzymes, such as lipoxygenase, and the inhibition of the inflammatory mediators. Studies have shown that quercetin reduces the cancer risk of prostate, ovary, breast, gastric, and colon cells.[5]

In cancer, much of the recent research on quercetin has shown it to be an anticarcinogen to numerous cancer cell types, including breast, leukemia, colon, ovary, squamous cell, endometrial, gastric, and non-small-cell lung.[6]

Few studies exist on humans regarding quercetin absorption. It appears that only a small percentage of quercetin is absorbed after an oral dose, possibly only two percent, according to one study. A recent study on absorption in 'healthy' ileostomy patients has revealed absorption of 24 percent of pure aglycone and 52 percent of quercetin glycosides from onions. However, no intestinal permeability values have been obtained in this group, and thus the results may not be reliable. Absorbed quercetin is transported to the liver bound to albumin, where some may be converted via methylation, hydroxylation, or conjugation.[7]

MATERIALS AND METHODS

Selection and Collection

Guar Gum was selected as a carrier for colon targeting with active medicament quercetin. Quercetin and guar gum were purchased from National Chemical, Vadodara, Gujarat. Starch, Lactose, and Magnesium stearate were purchased from the Central Drug House (P) Ltd., New Delhi.

Characterization of Guar Gum

The guar gum was identified and characterized using the following parameters:

Particle characters, angle of repose, bulk density, tape density, Housner's ratio, loss on drying [Table 1].

Tablet formulation using guar gum with quercetin

Matrix tablets using guar gum were prepared using the wet granulation method. Lactose was used as a diluent and a mixture of talc–magnesium stearate (2:1) was used as a lubricant. Guar gum was included in the formulations in various proportions [Table 2]. In all the formulations, guar gum was sieved (< 250 μm) separately and mixed with the drug (< 150 μm) and lactose (< 250 μm). The powders were blended and granulated with 8% starch paste. The wet mass was passed through sieve No. 18 and the granules were dried at 500C for two hours. The dried granules were passed through sieve No. 22 and these granules were lubricated with a mixture of talc–magnesium stearate (2:1). The lubricated granules were on an eight station tabletting machine. A separate formula (F-3) was used to prepare tablets without guar gum, which acted as the control.

Table 1: Characterization of Guar Gum

Parameter	Obtained value	Result
Particle characters	Gum powder is a white to off-white powder with a nearly neutral flavor, odor, and color	Gum powder is a white to off white powder with a nearly neutral flavor, odor, and color
Angle of repose	35.5^0	Very poor flow
Bulk density	0.588 gm/cm^3	0.558 gm/cm^3
Tape density	0.769 gm/cm^3	0.769 gm/cm^3
% compressibility	23.5	Highly compressible
Hausner ratio	1.3 gm/cm^3	Very poor flow
Loss on drying	11.0%	Non-hygroscopic
pH	7.1 ± 0.2	Neutral
Total ash value	0.85%	Within the limit
Solubility	Gum powder is soluble in hot and cold water	Gum powder is soluble in hot and cold water

Table 2: Quantity of different ingredients of different matrix tablet formulation

Ingredient	Qty. taken		
	F_1	F_2	F_3
Quercetin	10%	10%	10%
Guar Gum	40%	50%	-
Lactose	45%	35%	85%
Starch Paste (8%)	Qs	Qs	Qs
Magnesium Stearate	1%	1%	1%
Talc	2%	2%	2%

Tablet evaluation

Tablet evaluation includes parameters such as, weight variation, hardness, friability, and content uniformity.[8]

In vitro drug release studies

The ability of matrix tablets of quercetin to remain intact in the physiological environment of stomach and small intestine was assessed by conducting drug release studies under conditions mimicking mouth to colon transit.

Drug release studies were carried out using the USP dissolution rate test apparatus (Apparatus 1, 100 rpm, 37^0C) for two hours in 0.1N HCl (900 ml). Then the dissolution medium was replaced with pH 7.4 phosphate buffer (900 ml) and tested for drug release, for three hours. At the end of the time period, 5 ml of the sample was taken without a pre-filter. The dissolution samples were taken without a pre-filter to include drug particles that might erode from the outer layer of the swollen matrix tablets. Following that the dissolution medium was replaced with

pH 6.8 phosphate buffer (900 ml) and the experiment was continued for 24 hours. At different time intervals, 5 ml of the sample was withdrawn without a pre-filter and replaced with 5 ml of fresh phosphate buffer. One milliliter of the liquid was suitably diluted, filtered, and analyzed for percentage drug release at 256 nm, for quercetin, with the help of the UV method, using the double beam UV-spectrophotometer.[1,9,10] The percentage drug release was calculated by using the standard curve of quercetin, which was prepared in different dissolution mediums, such as, 0.1N HCL, pH 7.4 phosphate buffer, and the pH 6.8 phosphate buffer [Figure 1].

Drug release studies in the presence of rat cecal contents

To assess the vulnerability of guar gum being acted upon by colonic bacteria, drug release studies were carried out in the presence of rat (white albino rats) cecal contents, because of its similarity with human intestinal microflora, in order to induce the enzyme that specifically acts on guar gum in the cecum. Male albino rats were maintained on a normal diet with administering 4 ml, 1% dispersion of guar gum in water for seven days. Thirty minutes before the commencement of the drug release studies, three rats were killed by spinal traction. The abdomen was opened and isolated, ligated at both ends, cut loose, and transferred into pH 6.8 phosphate buffer, previously bubbled with CO_2. The rat cecal bags were opened, their contents were weighed and 4% w/v solution of rat cecal contents was prepared in pH 6.8 phosphate buffer.

The drug release studies were carried out using the USP dissolution rate test apparatus (Apparatus 1, 100 rpm, 37^{0}C) with slight modifications. After five hours, the experiment was carried out using 100 ml of 4% w/v rat cecal content medium in pH 6.8 phosphate buffer, in a 150 ml beaker. At different time intervals, 5 ml of the sample was withdrawn without a pre-filter and replaced with 5 ml of fresh phosphate buffer. One milliliter of the liquid was suitably diluted, filtered, and analyzed for percentage drug release at 256 nm for quercetin by the UV method, using a double beam UV-spectrophotometer.[1,9,10]

RESULTS AND DISCUSSION

The aim of the present study was to develop a polymer-based matrix tablet with sufficient mechanical strength and promising *in vitro* mouth-to-colon release profile, for targeting quercetin to the colon, to treat the diseases associated with colon or more specifically to treat colon cancer.

The colon is a site where both local and systemic drug delivery can take place.[11,12] A local means of drug delivery could allow topical treatment of inflammatory bowel disease, for example, ulcerative colitis or Crohn's disease. Site-specific means of drug delivery could also allow oral administration of peptide and protein drugs, which normally become inactivated in the upper parts of the gastrointestinal tract.

Three formulations of quercetin were prepared using guar gum as a polymer. The evaluation of the formulation was done and the results obtained are presented in Table 3.

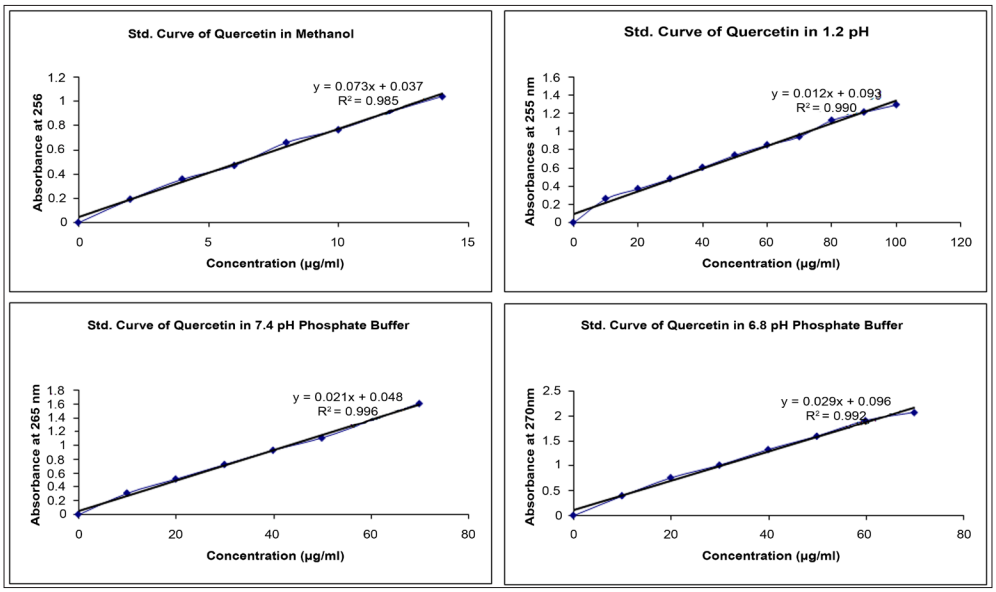

Figure 1: Standard curve of quercetin in different dissolution mediums

Drug release studies were carried out using the USP dissolution rate test apparatus (Apparatus 1, 100 rpm, 370C) for two hours in 0.1N HCl (900 ml). When the quercetin tablet was formulated without guar gum (F-3) 49.25% drug release was observed after five hours (up to the small intestine) in the *in vitro* drug release study. When it was formulated with guar gum, only 15.4 and 12.4% drug release (for 40 and 50% guar gum containing formulation, respectively) was observed in the upper part of the GIT. This indicated the capability of guar gum to protect the active medicaments to release in the upper part of the gastrointestinal tract. Forty percent guar gum-containing formulation (F-1) showed better drug release, that is, 94.27%, at the end of the twenty-fourth hour of dissolution study in the presence of rat cecal contents, in comparison to 50% guar gum-containing formulation (F-2), which released 82.13% [Tables 4 and 5] [Figures 2 and 3].

Quercetin is used to treat colon cancer, but they have very poor absorption in the upper part of GIT, so by the help of this study, it can be seen that quercetin can be positively delivered to the colon, for effective colon cancer treatment, using guar gum, and the bioavailability can be increased effectively. Guar gum is a natural polymer that has been used frequently in colon targeting, as a carrier. Guar gum has highly branched galactomannans and the cleavage of the branched galactomannans by enzymatic or acid hydrolysis does not reduce the viscosity to the same extent as the cleavage of more linear galactomannans. The highly branched molecular structure of guar gum resists the enzymatic breakdown in the digestive tract. Also *in vitro* evaluation of the tablet prepared using guar gum, shows that guar gum is capable of protecting the drug from being released in the physiological environment of the stomach and small intestine. The presence of rat cecal content in the dissolution medium results in improved drug release at different time periods when compared to the control. The result of *in vitro* evaluation demonstrates that guar gum is susceptible to the enzymatic action of cecal contents that cause better drug release, and this has been found, up to 91.1%, in the formulation containing 40% Guar Gum in the presence of rat cecal contents, rather than in that without rat cecal contents, which is 70.03%. Hence, data reveals that guar gum may be used as a potential carrier for colon-specific drug delivery.

Table 3: Evaluation of different formulation

Formulation Code	Weight (mg)	Hardness (kg/cm²)	Friability (%)	Content Uniformity (%)
F-1	511	5.8	0.790	98
F-2	503	5.7	0.750	98.9
F-3	506	4.6	0.92	98.93

Table 4: Cumulative % drug release of different formulations without rat cecal contents

Time (hr)	Cumulative % drug release		
	F-1	F-2	F-3
2	12.5	10.3	32.11
5	15.4	12.4	49.25
6	33.3	26.8	-
7	42.2	31.5	-
9	49.6	57.81	-
18	66.3	59.0	-
24	70.04	63	-

Table 5: Cumulative % drug release of different formulations with rat cecal contents

Time (hr)	Cumulative % drug release	
	F-1	F-2
2	12.5	10.3
5	27.4	18.3
6	36.47	27.2
7	40.24	36.6
9	58.63	47.3
18	78.29	76.5
24	94.27	82.13

F-1 Formulation of quercetin with 40% guar gum, F-2 Formulation of quercetin with 50% guar gum, F-3 Formulation of quercetin without guar gum (Control)

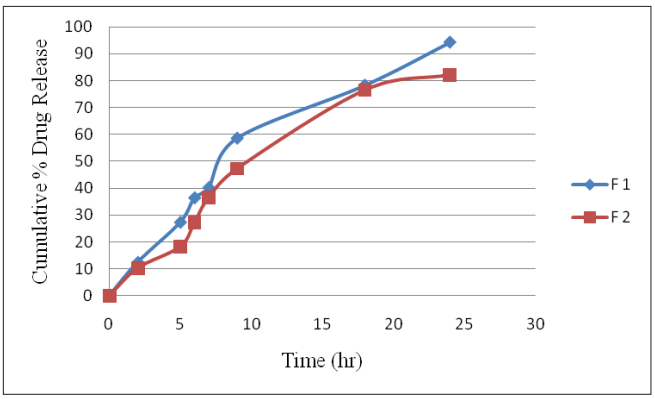

Figure 2: Cumulative % Drug Release of different matrix tablet formulation with Rat Cecal Contents

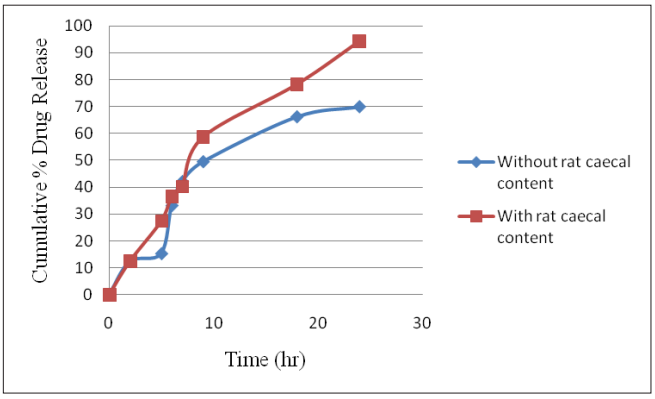

Figure 3: Cumulative % Drug Release of matrix tablet formulation (F1) without and with Rat Cecal Contents

CONCLUSION

The present study concludes that guar gum has the capability of protecting the release of active drug quercetin in the upper part of the GIT and allowing the release of the drug content in the colon. By using this approach absorption of quercetin may be increased, which leads to better bioactivity in lesser doses, and by this approach we can get both local and systemic action. Therefore, via approaching this method of drug targeting, quercetin can be used in the treatment of colon cancer and the oral dose of quercetin may be decreased, but still the bioavailability can be increased.

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