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Assessment of suitability of guar gum with pH sensitive polymer matrix bases for colon specific delivery.

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ABSTRACT

Degradation of guar gum by colonic microorganisms has been extensively exploited to design colon specific drug delivery systems. Most of these designs suffer from the drawback of very high polymer proportion, need for enteric coating, use of other polysaccharides having better colon specific delivery tendency or compromised therapeutic efficacy due to variation in gastric transit time. In the present investigation, an attempt was made to first understand and rationalize important findings of earlier reports and then identify mechanisms that can be better suited for colonic delivery systems. It was concluded that a pH and transit time dependent mechanism of release preceding colonic microorganism mediated trigger for drug release will provide systems that are having biphasic release profile ideal for colonic delivery. In this study, controlled release colon specific formulations of indomethacin were designed with pH and transit time dependent release profile using combination of responsive polymers Eudragit (L100 or S100) and guar gum in matrix bases. In vitro drug release studies indicated a high burst release from matrix tablets with only guar gum as release retarding polymer, thereby confirming non-suitability of such matrix for colon specific release. The inclusion of pH responsive polymers Eudragit (L100 and S100) in the matrix base significantly reduced the drug released in the initial phase (0-6 h) followed with controlled release upto 14-16 h. A sigmoidal release pattern was observed from the designed formulations suitable for colonic delivery. This formulation design is expected to show a combined pH and swelling controlled release behavior with potential of a better performance when compared to coated systems and/ only guar gum based matrix tablets.

Keywords: Colon specific delivery, controlled release, pH sensitive polymers, matrix, guar gum

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INTRODUCTION

Colorectal cancer, inspite of the well-characterized molecular events in the adenoma-to-carcinoma sequence is the second largest cause of cancer related death in industrialized countries [1, 2]. The main course of treatment for cancer of the colon and rectum are surgery, radiation therapy, and chemotherapy [3]. In case of chemotherapy, the oral route is the most preferred and an appropriately designed targeted drug delivery system to the colon would ensure direct delivery of anticancer agent at the disease site, lower dosing and a reduction in systemic side effects.

For an ideal colon targeted system, drug release should be biphasic and characterized by minimal drug release in the initial phase (during transit through upper gastro intestinal (GI) tract) followed by complete drug release in colon. This has been achieved by the use of three major approaches - pH dependent systems, time based and bacterial enzyme based systems [4]. Several colonic drug delivery systems are reported such as cross-linked alginate-chitosan blend gel beads for pulsatile release of piroxicam into colon [5] and enteric-coated microgranules for pH dependent delivery of. ornidazole to the colon. [6].

Indomethacin, a non-steroidal anti-inflammatory drug, has shown anti cancer potential in the treatment of colorectal cancer [7]. Amongst the several mechanisms proposed for its tumor inhibition potential include induction of apoptosis, reduction in proliferation rates of HT-29 colon cancer cells, and down regulation of survivin (an apoptosis inhibitor) [8, 9]. A conventional oral formulation of indomethacin would cause unwanted systemic and local upper gastrointestinal side effects. However, a colon-specific formulation of indomethacin with negligible to low drug release till the formulation reaches the colon would achieve high local concentrations of drug in the colon and reduce its systemic absorption.

A polymeric matrix comprising of a combination of swelling controlled and pH dependent polymers can achieve a pH and transit time dependent system that releases the drug in a biphasic sigmoidal fashion that is characterized by negligible to low initial drug release for 4-6 h followed with controlled release for 8-10 h.

Guar gum, a naturally occurring galactomannan polysaccharide, derived from the seeds of *Cyamopsis tetragonolobus*, of the Leguminosae family, is degraded by gut microorganisms therefore various researchers have attempted this polymer as a trigger for colon specific delivery. Various reports on use of guar gum as excipient in the form of a matrix base or compression coat over tablets for selective drug release in the colon is summarized in Table 1. When used alone, very high percentage of guar gum is required in the matrix base [10,11] or compression coat [12,13,14] to achieve the desired retardation in the initial phase. Alternately, it has to be used in combination with other polymers [15,16] or suitably coated with enteric polymers [17,18] For some of the other reported guar gum based formulations, the drug release is slow and controlled by microbial degradation in the colon [15,19] Such systems may be unsuitable for patients whose colon transit time is low (in the range of 10-12 h) such as in case of diarrheal symptoms. Further, when antibiotics and antibacterials are co-administered, the microflora of the colon is disturbed and this may affect the degradation of guar gum matrix [20]. Drug release would be incomplete or irregular in such cases.

One way of protecting the formulation made of natural polymers from high initial release in acidic to weakly acidic environment is by enteric coating with pH sensitive polymers like

TABLE 1 Use of guar gum as matrix base and/or compression coat for colonic delivery

Drug	Technique employed	Important findings	Reference
Matrix base			
Indomethacin	Guar gum as carrier	Guar gum found suitable for colon targeting.	49
Albendazole	Matrix tablets with 20% guar gum; influence of metronidazole and tinidazole on drug release.	Drug release was found to decrease with increase in proportion of metronidazole and tinidazole	20
Mebendazole	Matrix tablets prepared with 20% or 30% guar gum	Tablets were suitable for selective drug delivery to colon with 50% drug release from tablets with 30% guar gum in simulated colonic fluids.	47
Celecoxib	Matrix tablets prepared with 20% or 30% guar gum	Tablets were suitable for selective drug delivery to colon.	39
Indomethacin	Matrix tablets prepared using guar gum/ xanthan gum/chitosan/ Eudragit E as binders	Tablets prepared using guar gum as binder were unable to protect drug release for initial 4-5 h.	23
Sennosides	Matrix tablets prepared with 30%, 40% and 50% guar gum	50% guar gum tablets were suitable for colon targeting. Lower proportions were not suitable.	11
Mesalazine	Matrix tablets prepared by slugging method	Selective drug release in colon.	50
Indomethacin	Matrix tablets prepared using combination of guar gum with xanthan gum	Guar gum alone not suitable for colon targeting. Guar gum and xanthan gum were a better combination.	15
Rofecoxib	Matrix tablets prepared with 40%, 50%, 60% and 70% guar gum.	57% drug release in simulated colonic fluid for tablets prepared with 70% guar gum.	10
Ondansetron	Guar gum/ sodium alginate matrix tablets prepared by direct compression method	Both types of formulations were found suitable for colonic delivery.	41
Metronidazole	Matrix tablets prepared with guar gum grafted with methacrylic acid	Upto 70% drug release during initial 4-5 h. Reduced significantly after enteric coating.	17
Diltiazem HCl	Guar gum / chitosan matrix tablets coated with shellac or inulin	Chitosan found to be more suitable for colon targeting. Enteric-coated tablets were better.	18
Compression coat			
5- Amino salicylic acid	Compression coat comprising of 150 mg of guar gum	Drug release was less than 2% in simulated gastric and intestinal fluids and about 93% of 5-ASA in pH 6.8 with rat caecal contents.	46
Tinidazole	Compression coat comprising of 55%, 65% and 75% of guar gum	Tablets coated with 55% guar gum gave 99% release in 24 h. Tablets with 65% and 75% guar gum released only 67% and 20% of drug.	12
5-Fluorouracil	Compression coat comprising of 60%, 70% and 80% of guar gum	80% guar gum in compression coat was found suitable for colon targeting. Lower proportions were not suitable.	13
Metronidazole	Compression coated tablets containing 275 or 350 mg of guar gum were compared matrix and multilayered tablets.	Matrix and multilayered tablets failed to retard drug release (43-52% and 25-44% in stomach and upper GI tract) while compression coated tablets gave suitable colon specific release.	14
Ornidazole	Compression coat comprising of 65%, 75% and 85% of guar gum	Tablets coated with 65% and 75% guar gum completed drug release in 24 h in the presence of rat caecal contents.	48

5-Fluorouracil	Compression coat comprising of guar gum and xanthan gum in different proportions	Tablets coated with xanthan: guar gum (10:20) released around 67.2% and 80.3% in presence of 2% and 4% caecal contents respectively after 19 h of incubation.	16

Eudragit L100 and/or Eudragit S100. However, variable in vivo drug release has been reported from systems coated with pH responsive polymers [21, 22].

Therefore the present investigation was aimed at designing pH and transit time dependent system based on pH sensitive polymers in guar gum matrix base.

It was envisaged that a matrix system of this nature can overcome the drawbacks of some of the guar gum based formulations reported previously. Such a design shall release the drug at predetermined rate and not depend solely on degradation by colonic enzymes, and thereby initiate and complete drug release within a smaller time period, suited for patients with faster colon transit times.

Indomethacin tablets for colon specific release have been prepared using guar gum as a binder [23] as a compression coat alone [24] and film coating base with Eudragit FS30D [25]. Indomethacin formulations for colon targeting purpose have also been attempted by coating with pH sensitive polymers Eudragit L100 and Eudragit S100 [26, 27] and compression coating with pectin / chitosan mixtures [28].

The effect of employing pH responsive polymers- Eudragit (L100 or S100) in matrix base either with each other or with ethyl cellulose [29] with polycarbophil and carbopol [30], xanthan gum [31]; and with ethyl cellulose in a microsphere matrix [32] on indomethacin release has already been reported by us. In the present study, the effect of varying polymer proportions of guar gum alone and in combination with EL100 or ES100 was studied in a medium of simulated GI fluid pH (without enzymes). The effect of storage on the stability and release profile of selected formulations was also investigated. The stored batches were also evaluated for the absence of physical and chemical interaction between drug and polymeric excipients.

MATERIALS AND METHODS

Indomethacin and Guar gum were obtained as gift sample and purchased from Ajanta Pharma Ltd, Aurangabad, India,and Signet Chem, Mumbai, India, respectively. Eudragit polymers were obtained from Rohm Pharma, Germany. All other chemicals and reagents used were either of analytical or pharmaceutical grades.

Analytical Method

Indomethacin in pure form and designed formulation was analyzed using in- house developed and validated UV-Visible spectrophotometric method using Jasco V-570 double beam UV-Visible spectrophotometer (Jasco Corporation, Tokyo, Japan) accompanied with Spectra Manager software. The method involved analysis of the drug at 320 nm in phosphate buffer 7.4 pH in the range of 5- 50 µg/ml using 1 cm matched quartz cells.

TABLE 2 Composition, physical characterization and release rate kinetics of guar gum (GG) based formulations

Batches	Composition#			Physical Characterization					Power law correlation						
	GG (mg)	EL100 (mg)	ES100 (mg)	Drug content [a] (mg/ tablet)	Weight variation [b] (%)	Crushing strength[c] (kg)	Friability[d] (%)	Thickness [e] (mm)	Correlation time span	r[f]	MSSR[g]	K[h] (%/h^n)	n [i]	t $_{10\%}$ [j]	t $_{90\%}$ [k]
Effect of GG only															
IGG5	3.75	-	-	72.5±0.3	±4.1	4.5 (±0.1)	0.4%	1.77 (±0.01)	1-4 h	0.9923	1.23×10^{-3}	15.041	1.34	0.4	3.8
IGG10	7.5	-	-	74.2±0.2	±1.2	4.5 (±0.2)	0.1%	2.02 (±0.02)	1-4 h	0.9748	1.13×10^{-3}	17.104	1.22	0.6	3.9
IGG20	15	-	-	73.2±0.4	±2.5	4.7(±0.3)	0.2%	2.04 (±0.01)	1-6 h	0.9886	1.24×10^{-3}	22.218	0.93	0.3	4.5
Effect of EL100/ES100 on indomethacin matrix															
IEL20	-	15	-	73.0±0.1	±1.5	4.9 (±0.2)	0.3%	2.10 (±0.02)	2-12 h	0.9130	3.75×10^{-2}	2.659	1.48	2.1	10.8
IES20	-	-	15	72.6±0.2	±2.3	4.5 (±0.1)	0.2%	2.02 (±0.01)	2-14 h	0.9750	1.16×10^{-3}	1.137	1.40	3.5	26.7
Effect of EL100/ES100 on GG matrix															
IGG5EL5	3.75	3.75	-	76.2±0.2	±3.6	4.6(±0.1)	0.3%	1.88 (±0.02)	2-10 h	0.9927	2.04×10^{-4}	3.760	1.22	2.2	13.5
IGG5EL10	3.75	7.5	-	73.8±0.2	±2.3	4.5 (±0.1)	0.1%	1.90 (±0.02)	2-12 h	0.9759	2.12×10^{-3}	1.149	1.87	3.2	10.3
IGG5EL20	3.75	15	-	75.0±0.2	±1.8	4.7 (±0.3)	0.2%	2.11 (±0.01)	2-12 h	0.9838	2.87×10^{-4}	1.494	1.75	3.0	10.4
IGG5EL40	3.75	30	-	75.4±0.3	±2.3	4.9 (±0.1)	0.4%	2.10 (±0.02)	2-14 h	0.9885	1.25×10^{-4}	0.901	1.74	3.2	14.1
IGG10EL10	7.5	7.5	-	74.2±0.2	±2.8	4.5 (±0.2)	0.5%	2.05 (±0.02)	4-12 h	0.9863	2.13×10^{-3}	1.336	1.73	3.5	11.4
IGG10EL20	7.5	15	-	73.2±0.2	±0.5	4.7 (±0.2)	0.4%	2.07(±0.01)	4-12 h	0.9947	3.54×10^{-4}	1.370	1.59	3.2	13.9
IGG5ES5	3.75	-	3.75	74.7±0.1	±0.9	5.0 (±0.0)	0.5%	1.87 (±0.01)	1-4 h	0.9821	8.42×10^{-4}	19.598	1.12	0.5	3.9
IGG5ES10	3.75	-	7.5	74.2±0.1	±2.3	4.8 (±0.2)	0.4%	1.92 (±0.03)	1-4 h	0.9433	4.67×10^{-3}	17.527	1.14	1.2	4.2
IGG5ES20	3.75	-	15	72.2±0.2	±1.2	4.8 (±0.2)	0.4%	2.12 (±0.03)	2-10 h	0.9851	1.50×10^{-3}	2.687	1.59	2.6	9.1
IGG5ES40	3.75	-	30	72.3±0.3	±1.3	4.7 (±0.2)	0.3%	2.15 (±0.01)	2-14 h	0.9859	2.53×10^{-3}	11.343	0.82	3.9	12.5
IGG10ES10	7.5	-	7.5	72.4±0.2	±1.9	4.6 (±0.2)	0.5%	1.93 (±0.01)	2-6 h	0.9889	1.63×10^{-3}	5.352	1.59	1.6	5.9
IGG10ES20	7.5	-	15	74.2±0.2	±4.9	4.6 (±0.1)	0.3%	2.13 (±0.03)	2-14 h	0.9937	1.89×10^{-3}	0.493	1.87	3.8	16.2

 # Each tablet contains 75 mg of indomethacin. Also contains 1% w/w talc and 0.5% w/w magnesium stearate as formulation additives. [a] Mean ± SD (n = 10) [b] SD from the mean value (n = 20) [c] mean ± SD (n = 10) [d] mean of 10 tablets [e] mean ± SD (n = 5).[f]Correlation coefficient [g] Mean sum of squared residuals [h]Release rate constant [i]Diffusional exponent indicative of the release mechanism [j] Time for 10% of the drug release (in h) [k] the predicted or calculated time for 90% of the drug release (in h from eq.2). The diameter of the tablets was 0.70 ± 0.01 cm.

Tablet manufacturing

Matrix embedded non-disintegrating tablets (each containing 75 mg of indomethacin) using GG alone or in combination with EL100/ ES100 were prepared by wet granulation technique. Batch quantities of drug and polymer(s) pre-sieved through #120 mesh (ASTM) and dried at 55°C were mixed. The dry blend was granulated with ethyl alcohol (q.s.) and passed through #40 mesh and dried at 55°C on a tray drier. The dried granules were passed through #60 mesh and the passings blended with 1% w/w talc and 0.5% w/w magnesium stearate and compressed using 7mm punches on a 16 station rotary tablet compression machine (Cadmach, Ahmedabad, India). Three batches of tablets were prepared for each formulation. Formulae of prepared matrix embedded tablets containing GG alone and in combination with EL100/ES100 are presented in Table 2 respectively. Tablets comprised of indomethacin alone were also prepared by a similar technique and used as a control in dissolution study.

Physical characterization of designed tablets

The designed formulations were studied for their physical properties like weight variation, thickness, crushing strength, friability and drug content uniformity. For estimating weight variation, 20 tablets of each formulation were weighed using a Mettler Toledo balance (AG135, Mettler Toledo, GMBH, Greifensee, Switzerland). The crushing strength of 10 tablets was measured using Monsanto (standard type) tablet hardness tester. Friability was determined on 10 tablets in a Campbell Electronic Friabilator for 4 mins at 25 rpm. For estimation of drug content, 10 tablets were crushed and the aliquot of powder equivalent to 10 mg of drug was extracted in methanol: phosphate buffer pH 7.4 (1:9), suitably diluted using phosphate buffer pH 7.4 and analyzed spectrophotometrically at 320 nm.

In vitro release studies

In vitro dissolution studies were carried out using USP Type II (paddle method) apparatus (Electrolab TDT-08L with autosampling unit, Mumbai, India) at 75 rpm. The dissolution was carried out for the first 2 h in distilled water (500 ml; pH 6.8-7.0). Then, 200 ml of phosphate buffer concentrate (4.75 g of KH_2PO_4 and 1.07 g of NaOH in distilled water) was added to raise the total media volume to 700 ml and the pH to 7.4 for the remaining period. At predetermined time intervals, a 10 ml sample was withdrawn and replaced with fresh dissolution media. The samples were filtered, suitably diluted using phosphate buffer pH 7.4 and analyzed spectrophotometrically at 320 nm. The release studies were conducted in duplicate per batch for three batches and the mean values from three batches along with the SD were plotted against time (Figure 1 to 3) and used for all further calculations. The release profiles from GG matrices were compared against pure indomethacin tablet (compact) which served as control (shown in Figure 1). Effect of Eudragit on drug release from GG matrix is compared against an indomethacin matrix formulation prepared with only EL100 or ES100 (20% w/w of drug) as a control (shown in Figure 2 and 3).

Effect of simulated GI fluid pH (without enzymes) on release

Selected formulations from previous study were studied in a medium of changing pH. The initial condition was 350 ml of 0.1N HCl (pH 1.2) for 0-2 h. From 2-4 h, the pH of the media was raised to 4.5 (for simulation of duodenum) with total dissolution media volume of 600 ml. From 4^{th} h onwards, the pH was raised to 7.4 by adding 300 ml phosphate buffer concentrate (2.18 g of KH_2PO_4 and 1.46 g of NaOH in distilled water) to get dissolution volume of 900 ml. The study was further continued till the end in 900 ml volume. At predetermined time intervals, a 10 ml sample was withdrawn and replaced with fresh dissolution media. After appropriate dilutions, the samples were analyzed by the UV method discussed in previous section. The corresponding release profiles are presented in Figure 4.

Characterization of sigmoidal release profiles by power law equation

In order to understand the mechanism of drug release from these formulations, the cumulative percentage drug release data (post 2 h) was fitted into the power law equation given by Korsemeyer et al. [33] and Ritger and Peppas [34]

$$M_t / M_\infty = Kt^n \qquad \ldots\ldots\ldots \quad (1)$$

Where, M_t / M_∞ is percentage of drug released at any time 't'; '*K*' is release rate constant incorporating the structural and geometric characteristics of the polymeric system and the drug; 'n' is the diffusion exponent indicative of the release mechanism of the drug. The value of n for a cylinder is < 0.45 for Fickian release (diffusion controlled), > 0.45 & < 0.89 for non- Fickian release (diffusion and polymer relaxation), 0.89 for case II release (only relaxation and swelling) and > 0.89 for super case -II release (relaxation and erosion) for swellable systems. For cylindrical systems like tablets, the n values of 0.45 and 0.89 represents pure diffusion or erosion controlled release respectively. The values of the coefficient were calculated using linear regression analysis between $\log M_t / M_\infty$ and $\log t$ data obtained from drug release studies on MS Office Excel 2003 software.

The values of correlation time span, K, n, $t_{10\%}$ and $t_{90\%}$, 'r' (correlation coefficient of the regression analysis) and MSSR (Mean sum of squared residuals) as obtained from the dissolution data of designed formulations are given in Table 2. The correlation time span is the period of drug release phase taken for calculation of release kinetics. Using the calculated values of K and n, the release profiles were predicted beyond 14 h till 24 h for each formulation and are shown as dotted trend line(s) in Figures 1 to 3.

The dissolution profiles of selected formulations in changing pH medium (without enzymes) were compared with the target dissolution profile (negligible to no release in first 6 h followed by controlled release up to 14-16 h) using f_1 (dissimilarity) and f_2 (similarity) factor [35] as shown below.

$$f_1 = \left\{ \left[\sum_{t=1}^{n} |R_t - T_t| \right] \Big/ \left[\sum_{t=1}^{n} R_t \right] \right\} \times 100 \qquad \ldots\ldots\ldots \quad (2)$$

$$f_2 = 50.log \left\{ \left[1 + (1/n) \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\} \qquad \ldots\ldots\ldots \quad (3)$$

Where *n* is number of sampling points, R_t and T_t is the drug release from reference and test sample at sampling point *t*, respectively. The corresponding data is presented in Table 3.

Batch reproducibility and stability on storage

Three batches of each formulation were prepared and the release studies were done using the same conditions for estimating batch reproducibility. In order to assess the long-term stability of the various formulations prepared, one selected formulation (IGG5ES20) from each batch was sealed in cellophane packets, placed in hermetically sealed vials and separately stored at ambient conditions (25°C/60% RH) and accelerated stability test conditions (40°C/ 75% RH) for 6 months. At the end of the study period, the formulations were observed for change in physical appearance, drug content and in vitro drug release characteristics. The initial (zero time) results were compared with post stability testing period results for statistical differences. The powdered

samples of indomethacin matrix tablets were also subjected to differential scanning calorimetry study and determination of IR spectrum.

a) *Differential scanning calorimetry (DSC)*
The possibility of any interaction between indomethacin and guar gum along with Eudragit polymers during tablet processing was assessed by carrying out thermal analysis on pure drug, guar gum, ES100, powdered samples of formulation matrix before and after storage using Differential Scanning Calorimeter (Shimadzu, Japan, Model- DSC-60). Pure drug and formulation (IGG5ES20) equal to 2.5 mg of drug were accurately weighed onto standard aluminium pans and then hermetically sealed. The DSC thermograms were obtained at a scanning rate of 10°C/min over a temperature range of 25–200°C under constant purge of nitrogen gas (flow rate of 30 ml per min). The thermograms of the samples were recorded and endothermic peak(s) were analyzed for melt temperature and enthalpy of fusion using TA-60WS software (version 1.51). Results are shown in Figure 5.

b) FTIR studies
FTIR study was also carried out for pure drug, physical mixture of drug and polymeric excipients as well as for batches stored at accelerated stability storage conditions to confirm absence of chemical interaction during storage. The samples were appropriately diluted with dried potassium bromide and IR spectra were acquired in the range of 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. The data was processed using Kubelka Munk method for baseline correction. Results for stored formulations at accelerated conditions are shown in Figure 6.

Data Analysis
The difference in the release data between the different formulations was compared using paired t-test for means and one-way analysis of variance (ANOVA) at 5% level of significance using Microsoft Office 2003, Excel package.

RESULTS AND DISCUSSION

Physical characteristics
Physical appearance, crushing strength, weight variation and drug content uniformity of different tablet formulations were found to be within satisfactory limits (Table 2). The crushing strength was found to vary between 4.5 – 5.0 kg. The percentage friability in all the formulations was observed to ≤ 0.5%. The manufactured tablets showed low weight variation (SD within ± 5% of the average weight of the tablet) and high degree of drug content uniformity (within ± 7% of the theoretical value) indicating that wet granulation is an acceptable method for good quality matrix tablets of indomethacin for GI fluid pH modulated delivery.

In vitro release of matrix tablets
Indomethacin, an indole acetic acid derivative with a pKa of 4.5, has been reported to have solubility of 0.01 mmol/L (3.66 µg/ml) in pH 1.2 and 5.52 mmol/L (1975 µg/ml) in pH 7.2 at 37°C [36]. Our studies reveal that the drug (present in a micronized form) has a solubility of ~ 53 µg/ml in distilled water at 25°C and ~ 80 µg/ml at 37°C and therefore, dissolution was carried out in distilled water for the first 2 h as saturation solubility was not achieved in distilled water even when 55-65% of the complete dose is released. The choice of distilled water as dissolution medium is supported by previous reports that state that as the patient consumes a tablet with a good quantity of water, dissolution of poorly water-soluble drugs can be done in distilled water for the initial period [37, 38]. Further, during preliminary studies it was observed that distilled water could discriminate well between the various formulations with and without EL100 / ES100

as both these polymers are insoluble in water (Figure 1 to 3). This was followed by testing in phosphate buffer pH (7.4) for the remaining period of study. This medium was considered as most suitable as the drug was freely soluble at this pH and it also mimics the alkaline environment of distal small intestine (pH > 6.8) and colon.

In case of the matrix bases comprised of guar gum in similar proportions (5, 10 and 20% w/w of drug), the in vitro drug release profiles against pure indomethacin tablets are shown in Figure 1. On exposure to the dissolution fluids, the gum becomes hydrated and forms a viscous gel layer that slows down further penetration of dissolution fluids towards the core tablets. On coming into contact with biological fluids, guar gum swells up and the drug release takes place by diffusion [39]. Mechanical erosion of the swollen guar gum layer then follows. Drug release from guar gum matrices is also reported to be controlled by water penetration, gelatinization, and diffusion [40]. This gelling retards the drug release from tablet dosage forms. In the present study, however, it was observed that guar gum proportions upto 20% w/w of drug in matrix base (IGG20) could not retard the drug release from the matrix. Formulations with 5% guar gum (IGG5) were found to give faster drug release (nearly 45% in 2 h) followed by complete release in 4 h. Formulations with 10% (IGG10) and 20% (IGG20) of the polymer were also found to swell rapidly with 30% drug release in the first 2 h followed by disintegration of the matrix resulting in complete release in 4-5 h. When one-way ANOVA test at 5% level was performed between mean cumulative percentage drug release values for IGG5, IGG10, and IGG20, the difference was statistically insignificant. When present in lower proportions in a matrix base, guar gum tends to swell and dissolve rapidly owing to its highly hydrophilic nature. This is the reason why drug release was faster for guar gum based formulations when compared to a pure drug tablet which is highly hydrophobic in nature and dissolved relatively slowly. This necessitates the use of large quantities of the polymer to generate relatively stable matrices. The calculated n values from the Peppas equation (Table 2) indicate the drug release mechanism for the various formulations to be super case – II type, implying drug release by erosion of matrix.

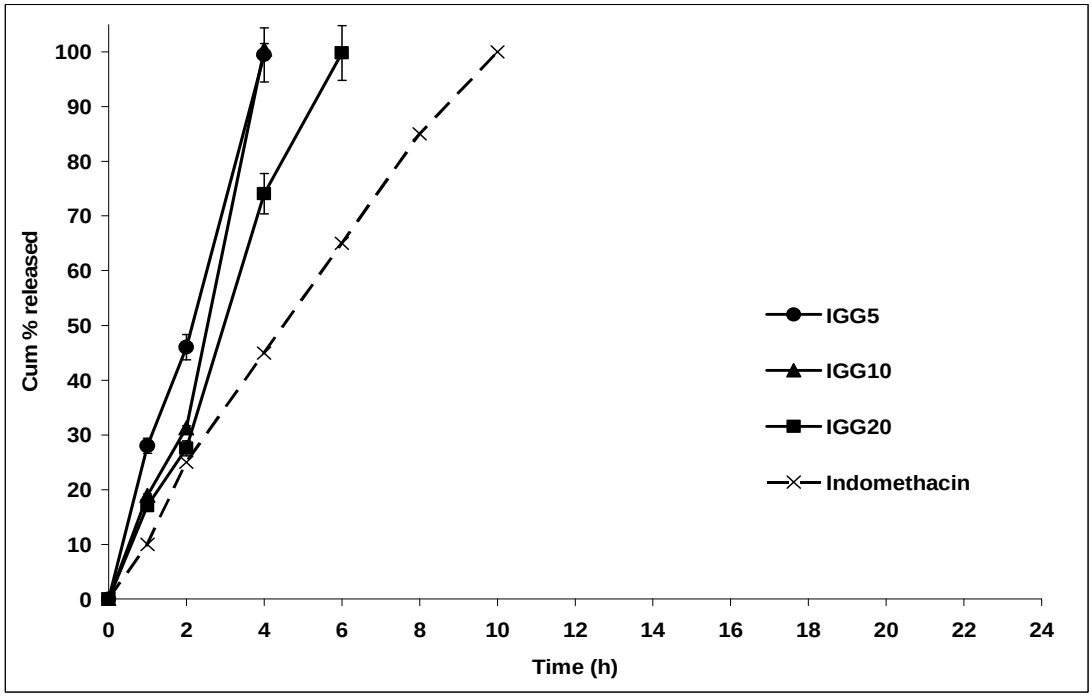


FIG. 1. Release profile of indomethacin from matrices containing varying proportions of guar gum. Each data point represents mean ± SD (*n* =6).

Effect of Eudragit L100 on guar gum matrices

For guar gum matrices, the formulations containing 5% guar gum with EL100 in varying proportions, (5, 10, 20 and 40% w/w of drug) against IEL20 (indomethacin with EL100), the in vitro drug release profiles are shown in Figure 2. It was found that on increasing the relative proportion of EL100 from 5% to 40% w/w of drug, there was significant retardation in the initial release rate (2-7% release in 2 h for all formulations) followed by increase in release rate post 2 h in phosphate buffer media. The $t_{10\%}$ extended from 2.2 h for IGG5EL5 to 3.2 h for IGG5EL40 (Table 2). The duration of drug release post 2 h was extended from 13.5 h for IGG5EL5 to 14.1 h for IGG5EL40. The release kinetics calculated for these formulations were significantly different when compared to the matrix base IGG5 (Figure 1). The release retardation in the initial phase was significant when compared to a previous study involving guar gum based indomethacin tablets wherein 21% of drug release occurred in the first 5 h [23]. However, the release profiles were not significantly different from IEL20, implying that inclusion of guar gum in 5% and 10% proportion could not contribute much to release retardation.

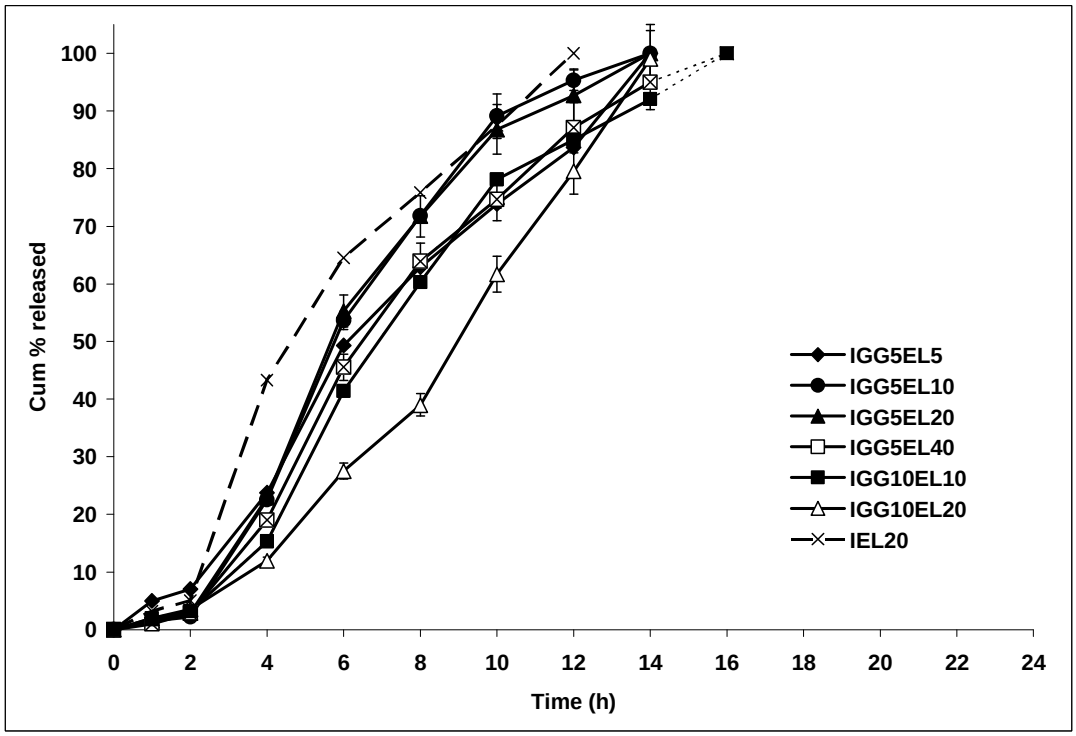


FIG. 2. Release profile of indomethacin from guar gum matrix showing effect of varying proportion of EL100. Each data point represents mean ± SD (*n* =6).

The effect of increasing the relative proportion of EL100 varied from 10 to 20% on drug release from formulations prepared using 5% guar gum (IGG5EL10 and IGG5EL20) and 10% guar gum (IGG10EL10 and IGG10EL20) was not significant. The change in the relative proportion of the two polymers did not exert a significant effect on drug release from the various formulations. There was no appreciable difference between the two formulations (IGG5EL10 and IGG5EL20) with respect to the drug release rates and dissolution profiles were almost similar for the two. However, a statistically significant difference was obtained between the dissolution profiles of IGG10EL10 and IGG10EL20 and drug release was extended from 11.4 h for IGG10EL10 to 13.9 h for IGG10EL20. This may be due to the presence of relatively higher proportion of EL100 in case of IGG10EL20. With increase in relative proportion of guar gum from 5% (IGG5EL10)

to 10% (IGG10EL10) in 10% EL100 matrix, drug release was extended from 10.3 h to 11.4 h (Table 2). However, in the case of IGG5EL20 and IGG10EL20, the release kinetics were almost similar with respect to the initial release for the two formulations while the duration release was slightly more extended in case of IGG10EL20 ($t_{90\%}$ of 13.9 h). Thus, increase in percentage of guar gum from 5% to 10% in 20% EL100 matrix did not affect the release rates of the formulations significantly.

In case of all these formulations, the drug release mechanism was inferred as being predominantly erosion based (n>1.0; super case II) due to the presence of EL100 (Table 2). As guar gum is non-ionic, its hydration should be unaffected by pH changes of the dissolution medium [41]. From the present study, it was observed that presence of EL100 in the guar gum matrix base could effectively control the initial swelling and impart pH dependent drug release kinetics. The explanation offered for this finding could be: during granulation, the granulating solvent (ethyl alcohol) dissolved a portion of EL100 which not only imparted the necessary adhesion between the matrix components but also formed a layer over the guar gum particles. This may have inhibited the swelling of the hydrophilic gum in water.

Secondly, EL100 in a polymeric base could impart a pH responsive drug release character. With increase in the pH of dissolution medium to 7.4, an increase in the drug release rate was observed on account of matrix erosion due to dissolution of EL100. The formation of a porous matrix then facilitated enhanced diffusion of the drug through the pores [42, 43].

TABLE 3 Release kinetics data for selected formulations in simulated GI fluid pH (without enzymes)

Batches	Power law correlation							Dissimilarity factor [#]	Similarity factor [#]
	Correlation time span	r[a]	MSSR[b]	k[c]	n[d]	$t_{10\%}$ [e]	$t_{90\%}$ [f]	f_1	f_2
IGG5EL20	4-14 h	0.9637	4.26×10^{-3}	1.023	1.86	4.5	11.1	2.03	51.0
IGG5ES20	4-14 h	0.9988	4.07×10^{-3}	1.007	1.67	5.7	14.6	3.95	53.8
IGG10ES20	4-14 h	0.9989	1.23×10^{-3}	1.107	1.47	5.9	19.7	14.9	34.5
IGG10EL20	4-14 h	0.9395	1.03×10^{-3}	2.601	1.39	4.1	12.8	19.4	21.3

[a] Correlation coefficient [b] Mean sum of squared residuals [c]Release rate constant [d] Diffusional exponent indicative of the release mechanism [e] Time for 10% of the drug release (in h) [f] Time for 90% of the drug release (in h).
[#] Comparison with theoretical target release profile. For similarity f_2 should be > 50 and f_1 < 15

Effect of Eudragit S100 on guar gum matrices

The effect of replacing EL100 with ES100 in similar ratios in guar gum matrices was not as pronounced as observed with the former (Figure 3). When compared to IES20 (indomethacin with ES100), guar gum actually enhanced the drug release rate. In the matrix containing 5% guar gum and equal proportion of ES100 (IGG5ES5), presence of ES100 could not control the drug release initially which is indicated by the low value of $t_{10\%}$ (0.5 h) and the duration of release could not be extended beyond 3.9 h. This implied that the relative proportion of ES100 was probably not enough to control the swelling of guar gum. On further increasing the relative proportion of ES100 from 5% to 40%, there was proportionate retardation in the release rate of the drug as indicated by a significant change in release kinetics (Table 2). With increase in the level of ES100 from 10% (IGG5ES10) to 20% (IGG5ES20), a corresponding retardation in the drug release rate was observed. The $t_{10\%}$ increased from 1.2 h for IGG5ES10 to 2.6 h for IGG5ES20 (Table 2). Similarly, the duration of drug release extended from 4.2 h (IGG5ES10) to 9.1 h (IGG5ES20). Subsequently, with increase in ES100 from 10% (IGG10ES10) to 20%

(IGG10ES20), a significant decrease in the initial and overall release rate was observed which resulted in relatively higher $t_{10\%}$ (3.8 h) and $t_{90\%}$ (16.2 h) values, implying a more suitable polymer proportion of ES100 (Figure 3). When the proportion of guar gum was increased from 5% (IGG5ES10) to 10% (IGG10ES10) on 10% ES100 matrix, the change was insignificant. When the percentage of guar gum was increased from 5% (IGG5ES20) to 10% (IGG10ES20) in 20% guar gum matrix, there was good retardation in the release rate to give higher $t_{10\%}$ (3.8 h) and $t_{90\%}$ (16.2 h) for IGG10ES20, implying retarding effect of guar gum on drug release. Therefore, the interaction between guar gum and ES100 was complex and unpredictable and could not be accounted for in the present investigation.

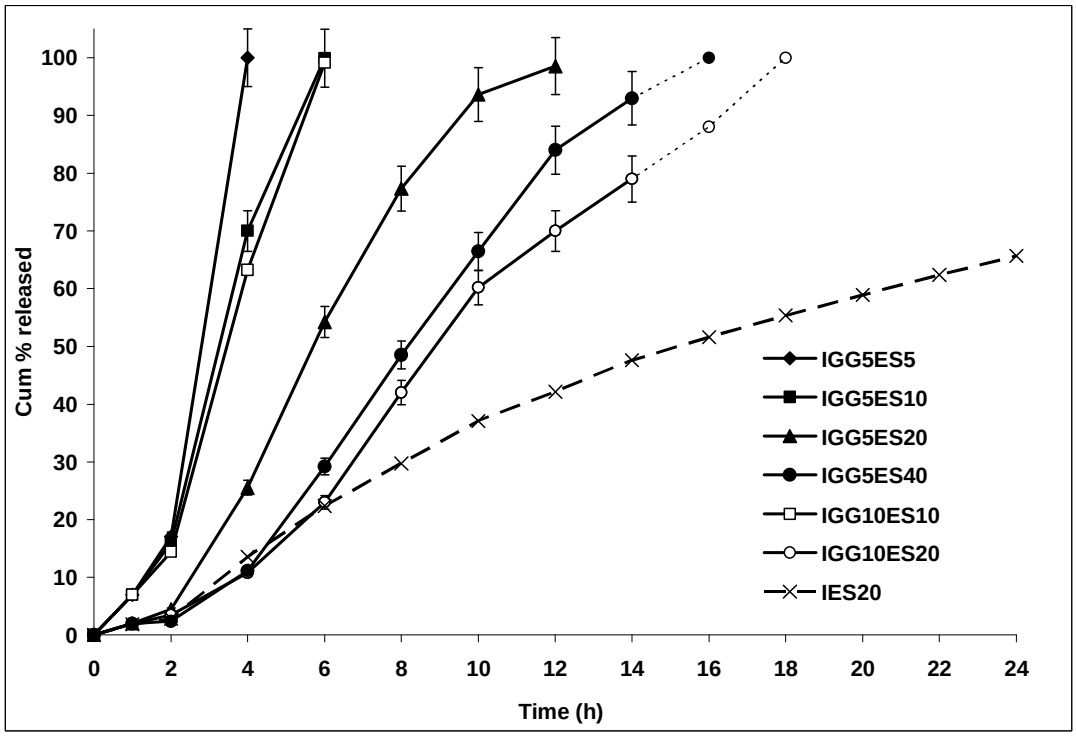


FIG. 3. Release profile of indomethacin from guar gum matrix showing effect of varying proportion of ES100. Each data point represents mean ± SD (*n* =6).

A good correlation of the dissolution data with the power law equation ('r' ranging from 0.9433 to 0.9947 and MSSR between 1.25 $x10^{-4}$ and 4.67 x 10^{-3}) for guar gum and Eudragit (both EL100 and ES100) matrices indicate the suitability of the Peppas model for calculation of release rate kinetics and understanding the mechanism of drug release (Table 2).

Effect of simulated GI fluid pH (without enzymes) on release

For any colon targeted drug delivery system, release of drugs must be completed within the residence time of the dosage form in the colon with an initial delay of 4-6 h (time taken for transit through stomach and small intestine). The colonic residence time is reported to be highly variable from 10 h [44] to 30-40 h [45]. For the present study, it was assumed that drug release profile with an initial time lag of 4-6 h subsequent complete release within 14-16 h would ensure that maximum drug release occurred in colon in controlled fashion even in cases when colonic transit time is on the lower side. This initial time lag would ensure the passage of the formulation intact upto the terminal ileum and ascending colon without appreciable drug loss. Based on these assumptions, a theoretical target release profile was defined as shown in Figure 4 as target profile.

The in vitro release studies conducted in the initial dissolution conditions were intended to characterize and understand the effect of Eudragit on hydrophilic matrix swelling and initial drug release (in distilled water medium for 2 h) and also to investigate the potential of the various formulations to complete drug release in the stipulated time- frame of 14-16 h in the alkaline environment of colon (pH 7.4 medium). The performance of selected designed formulations (IGG5EL20, IGG5ES20, IGG10EL20, and IGG10ES20) was also evaluated in a pH gradient system in order to investigate the suitability of formulations in real-time changing pH situation existing in GI tract (Figure 4). The choice of pH conditions was pH 1.2 for duration of 2 h (simulated gastric fluid), pH 4.5 for 2 h (simulated duodenum) followed by pH 7.4 (simulated distal ileum and colon) for the remaining period of study. The drug release from the various formulations was compared with the theoretical target values using f_1 (dissimilarity) and f_2 (similarity) factors (Table 3).

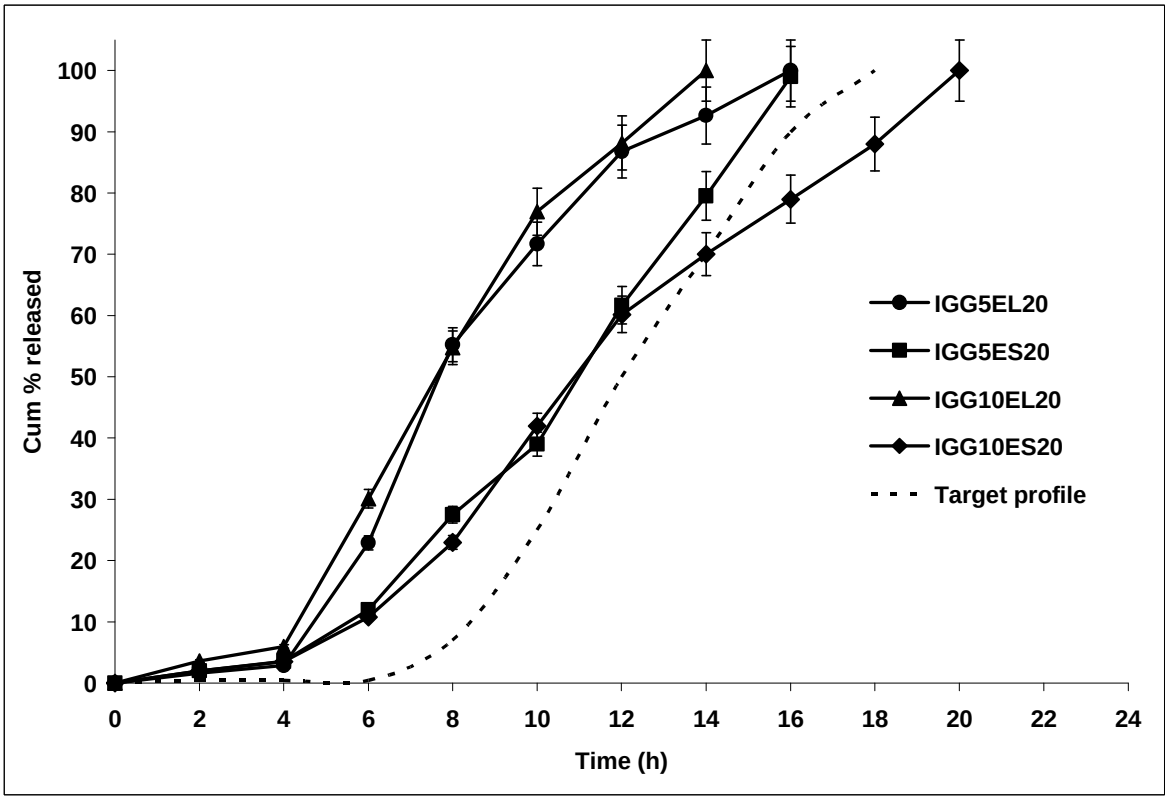


FIG. 4. Release profile of selected formulations in simulated GI fluid pH. Each data point represents mean ± SD (*n* =6).

It was observed that the $t_{10\%}$ and $t_{90\%}$ for different formulations were in the range of 4.1- 5.9 h and 11.1 to 19.7 h, thereby approaching close to target profile values. The release profiles of IGG5EL20 and IGG5ES20 showed good similarity with ideal target release with f_2>50 and f_1<15. It was observed that a significant pH and time dependent release pattern was observed for all the formulations implying suitability for colon specific release, even in the absence of enzymes. In a previous report, the use of graft copolymer of methacrylic acid with guar gum could not prevent drug release in upper GI tract conditions (drug release was 70% in the initial 4-5 h) [17]. However, in the present investigation, incorporating pH sensitive polymers in guar gum matrix base resulted in very low initial release corresponding to that observed after enteric coating [18, 23]. Thus, a separate coating step with Eudragit could be avoided and the uncertainty of drug release from a coated system could also be overcome. Since degradation of

guar gum is slow and requires prolonged colonic residence to initiate and complete drug release, formulations based either on the use of guar gum alone or microbial degradation may not be effective for patients experiencing diarrhoeal symptoms or whose colonic transit rate is fast. In such cases, the present design that works on the principle of a dual trigger mechanism – pH dependency and time-based release would ensure colon specificity in release.

In the present study, guar gum alone was incapable of retarding the drug release from the designed matrix tablets, as has been reported previously. In combination with Eudragit, it was possible to obtain desirable release kinetics from guar gum even when employed in very low polymer proportions of 5% and 10%, unlike in previous reports when higher proportions of guar gum were employed to generate stable matrices [12, 13, 14]. By utilizing a suitable blend of hydrophilic (GG) and slightly hydrophobic (EL100 or ES100) polymers, it was possible to regulate drug release from a matrix to achieve desirable release kinetics.

Batch reproducibility and stability on storage

No significant difference was observed in the release profile of different batches of each matrix formulation, indicating that the manufacturing process employed was reliable and reproducible. There was no change in the physical appearance of the different formulations at the end of the six-month storage period at 40°C/75% RH (data not shown). The formulations were also subjected to estimation of drug content, in vitro drug release and drug excipient interaction studies. There was no significant change in drug content. Further, in vitro release studies carried out on the formulations stored at accelerated test conditions indicated no statistically significant change in the drug release profiles when compared to formulations stored at ambient conditions (data not shown). These results imply good stability of product on long-term storage.

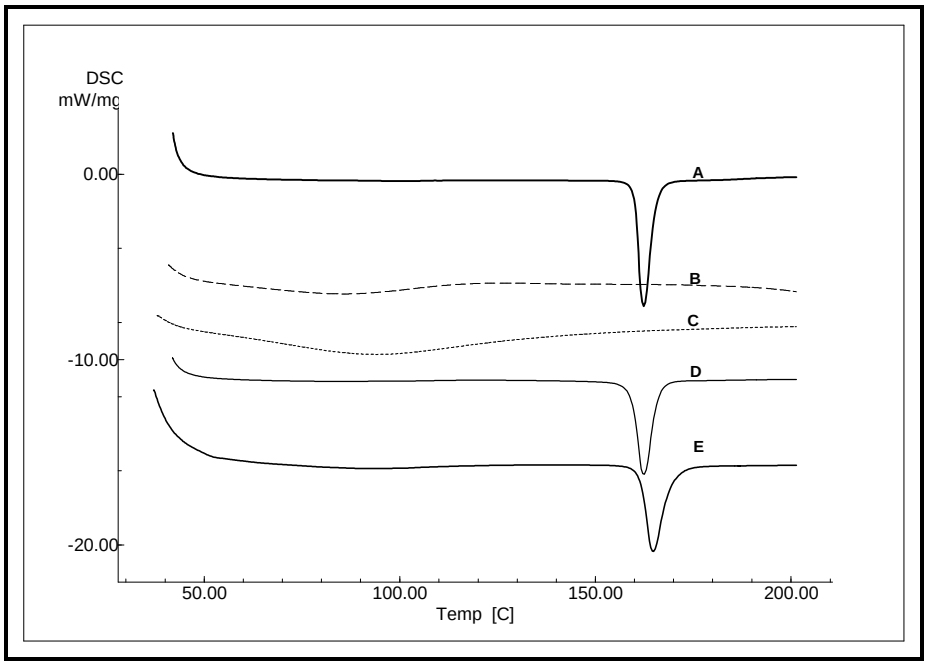


FIG. 5. DSC thermogram of A) pure drug) ES100 C) guar gum D) formulation IGG5ES20 (before storage) E) (after storage at 40°C/75% RH for six months).

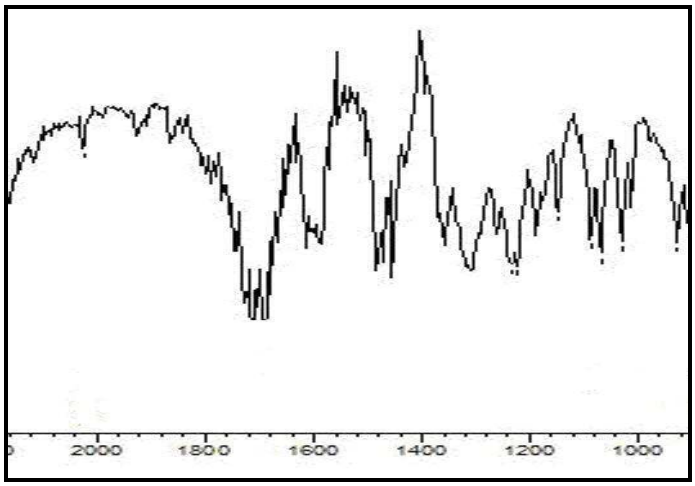
a) Differential scanning calorimetry (DSC)

DSC thermogram of indomethacin revealed a sharp melting endothermic peak of the drug at 161°C which corresponds with the melting point of pure drug (159-161°C) with an enthalpy value of -57.2 J/g. It may be observed from Figure 5 that there was little or no difference

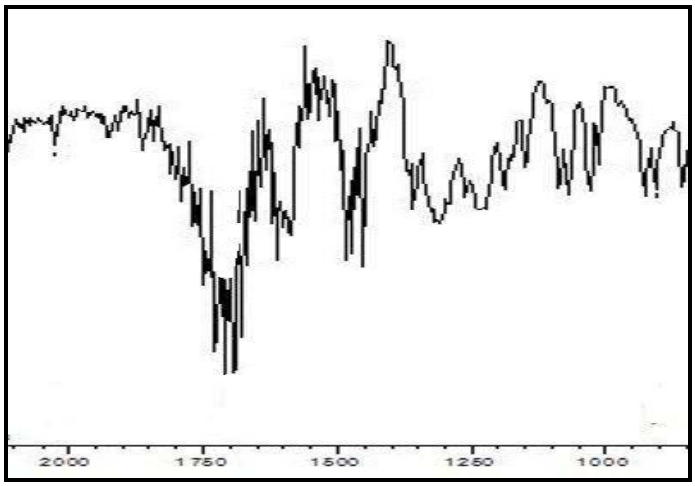
between the endothermic peak obtained for the pure drug and the formulation (IGG5ES20) before and after storage. There was no change in the enthalpy value of formulations after storage implying stability to tablet manufacturing process as well as compatibility with the polymeric excipients. A slight reduction in enthalpy value with little broadening of the endothermic peak was observed in the case of IGG5ES20 which might have occurred due to the mixing process that lowers the purity of the different components and also due to the presence of bound moisture present in guar gum [12]

b) FTIR studies

The IR spectrum of the drug revealed peak bands at 1060 cm^{-1} due to stretching of ether group (-C-O-). The peak corresponding to the tertiary amide (-CON-) was observed at 1650 cm^{-1} while carbonyl stretching of aliphatic COOH was observed at 1720 cm^{-1} (Figure 6 a). It was observed there was no change in spectrum of drug as is evident from the IR spectrum of the formulation IGG5ES20 (Figure 6 b) that all peaks due to the different functional groups of pure drug were well preserved even after storage at 40°C/75% RH for a period of six months implying absence of chemical interaction between the drug and the formulation excipients.



(a)



(b)

FIG. 6. IR spectrum of powdered sample of (a) pure drug (at zero time) (b) IGG5ES20 (after storage at 40°C/75% RH for six months).

CONCLUSION

Therefore, from the present study, it can be concluded that the use of pH based polymers in combination with hydrophilic polymer(s) like guar gum to form a polymeric matrix base controls the initial swelling of these polymers to a good extent which could prevent early drug loss from their matrices during upper GI transit. It also confers matrix strength and rigidity to the formulations, thereby enabling lower proportions of these polymers to be used in matrix bases. Therefore, mixed polymer matrix with pH modulated properties can serve as an alternative to coating technology which although has commercial feasibility, yet suffers from the drawback of inconsistent performance in vivo. A pH and time controlled matrix system can offer a suitable platform for colon targeting purpose with minimum drug loss during upper GI transit and maximum drug release in the colon.

Declaration of Interest

The authors also report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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