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Research Article

FORMULATION AND EVALUATION OF NOVEL CHROMENE DERIVATIVE AS AN ANTI-INFLAMMATORY AGENT USED FOR INFLAMMATORY BOWEL DISEASES

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ABSTRACT

Objective: To formulate and evaluate an extended-release (ER) tablet of a new molecule, 2-amino-4-(4-bromophenyl)-7-hydroxy-4H-chromene-3-carbonitrile using a combination of two polymers (hydroxypropyl methyl cellulose [HPMC] K100 and HPMC phthalate) which control the rate and degree of the drug release through 12 hrs period and protect the drug release from acidic pH.

Methods: Five batches of tablets (4HC1, 4HC2, 4HC3, 4HC4, 4HC5) were produced by direct compression method. Morphological evaluation of the powder blend was carried out by differential scanning calorimetry and Powered X-ray diffractometry. The evaluation studies such as flow properties, hardness, friability, drug content, and release study were conducted according to pharmacopoeial standards.

Results: The physicochemical characteristics of all the granules and tablets were generally satisfactory. The drug release followed zero order, Higuchi model kinetics with diffusion and dissolution mediated mechanism. Tablets were evaluated for physicochemical parameters and promising. Stability studies indicated the dosage form is stable for 3 months at accelerated conditions.

Conclusion: From the results received from all test, it was concluded that formulation 4HC4 are the most suitable choice for developing 12 hrs ER tablets. This finding reveals that a particular concentration of HPMC K100 was capable of producing ER.

Keywords: Chromene derivative, Extended-release, Hydroxypropyl methylcellulose phthalate, Hydroxypropyl methylcellulose K100.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic gastrointestinal (GI) disorder which includes ulcerative colitis and Crohns disease. The exact cause of disease is undefined; certain factors have been suggested to play a role, such as genetics, microbiome, environmental stress, and immune dysfunction. Current therapeutic agents are limited for use in IBDs as they are generally designed to achieve systemic delivery of therapeutics, which results in adverse effects and toxicity following distribution of drug around the body. Oral formulations achieving a localized effect are preferred in rational drug delivery design for IBD. Current therapeutic approaches specifically indicated for IBD rely on conventional dosage forms such as delayed or extended release (ER) mechanisms [1,2]. Conventional drug delivery systems are failed to treat such diseases because the drugs do not offer site-specific drug delivery. Hence, the site specific delivery of drugs is promising tool for such diseases whereby minimizing the side effects that occur because of unnecessary systemic absorption [3].

There are several methods available for site-specific drug delivery [4,5]:

- The use of carriers that degrade exclusively by intestinal bacteria
- Using pH dependent polymers
- Time-dependent dosage forms
- Prodrugs.

ER dosage forms are designed to achieve a prolonged therapeutic effect by continuously releasing the drug over an extended period after administration of a single dose [6,7]. The advantages of ER dosage forms over conventional forms include the less fluctuation in drug blood levels, frequency reduction in dosing, enhanced convenience, and compliance, reduction in adverse effects and reduction in overall health-care costs.

2-amino-4H-chromene derivatives with nitrile functionality have wide application in tumor necrosis factor α (TNF-α) mediated disease such as IBD, rheumatoid arthritis, and other inflammation mediated disease [8-10]. Chromene moiety represents a novel class of TNF-α inhibitor, one of the best examples in this class is 2-amino-4-(4-bromophenyl)-7-hydroxy-4H-chromene-3-carbonitrile is a novel chromene derivative (Fig. 1) [11]. The compound is synthesized by condensation reaction of malononitrile, substituted aldehyde, and activated phenol in the presence of an organic base (pyridine) for several [12,13]. The solubility of the synthesized compound enhanced by complexation technique using hydroxypropyl beta cyclodextrin.

The main objective of the present study to formulate site-specific drug delivery system containing a chromene derivative using polymers like hydroxypropyl methylcellulose (HPMC) phthalate and HPMC K100 and evaluates its physical parameters and *in vitro* drug release studies. The aim of present study was to formulate and evaluate an ER tablet of a new molecule 2-amino-4-(4-bromophenyl)-7-hydroxy-4H-chromene-3-carbonitrile using a combination of two polymers (HPMC K100 and HPMC phthalate). Which control the rate and degree of the drug release through 12 hrs period and protect the drug release from acidic pH. Site-specific drug delivery devices are provided with a pH-sensitive polymer. Many studies have indicated the considerable variation in pH gradient in the GI tract; therefore, completely depending on the pH sensitivity of the polymer. In this research work, the ER tablets were compressed with varying proportions of HPMC phthalate and HPMC K100 and studied *in vitro* for their release profile in various pH conditions. The drug release kinetics was studied by using various kinetic studies available, such as Higuchi, Korsemeyer–Peppas, zero order, and first order kinetics. The results of the overall study, release kinetics, and mechanism of drug release were elaborately discussed in this work.

METHODS

2-amino-4-(4-bromophenyl)-7-hydroxy-4H-chromene-3-carbonitrile is synthesized in our laboratory. HPMC K100, microcrystalline cellulose 102 (MCC 102), lactose, aerosil, and magnesium stearate were purchased from Yarrow chem products Mumbai. HPMC phthalate was purchased from Colorcon Asia Pvt. Ltd. Goa.

Calibration graph of synthesized drug

Weigh 10 mg of synthesized compound; add sufficient quantity of methanol to produce a stock solution of 100 µg/ml. From the standard stock solution, dilutions were made into the concentration of 10-50 µg/ml. Take the absorbance at 318 nm on ultraviolet spectrophotometer against blank.

Preparation of ER tablet

Direct compression method was used to prepare ER tablets of newly synthesized compound. Required amount of the, synthesized drug, MCC 102, lactose, polymers were taken and were sieved through #44. It was then mixed with magnesium stearate and aerosil which was previously sieved using #60. Five different set of tablets were prepared on rotary compression machine [14,15].

Evaluation studies

The granules were evaluated for various physicochemical parameters including angle of repose, bulk density, tap density, compressibility index and Hausner ratio. The prepared powder blend was characterized by differential scanning calorimetry (DSC) and Powered X-ray diffractometery (PXRD).

The compressed tablets were characterized by their physicochemical parameters. The hardness of the tablets was tested using Monsanto tablet hardness tester. The average weight was also determined as per (Indian Pharmacopoeia [IP]). 10 tablets from each batch of formulations were selected randomly, and thickness of tablets was measured using vernier caliper. The Friability was determined in roche friabilator. Friability was calculated as a percentage of weight loss after 100 rotations (IP).

Drug release study

In vitro dissolution studies were performed using the United States Pharmacopeia (USP) dissolution apparatus II. The volume of dissolution medium was 900 ml, and it was maintained at 37±0.5°C and stirred at paddle speed of 50 rpm. Simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 7.4), prepared according to standard monograph without enzymes were used as dissolution media. Dissolution test was run for 1 hr in simulated gastric fluid and subsequently on stimulated intestinal fluid. 5 ml of samples were collected at time intervals of 5, 10, 15, 20, 30, 45, 60 minutes and replaced by the equal amount of dissolution medium to maintain a constant volume. Samples were analyzed spectrophotometrically at 318 nm to determine the amount of drug released from which the percentage drug release was calculated.

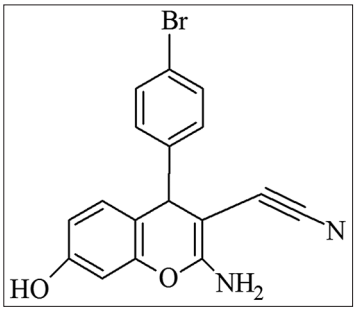

Fig. 1: 2-amino-4-(4-bromophenyl)-7-hydroxy-4H-chromene-3-carbonitrile

Table 1: Formula for 1 tablet with formulation code

Ingredients (mg)	4HC1	4HC2	4HC3	4HC4	4HC5
Drug	30	30	30	30	30
HPMC K100	15	30	45	53.136	60
MCC 102	64.05	25.92	25.92	25.92	31.784
Lactose	54.75	77.5	69.75	54.75	54.75
Hypromellose phthalate	52	52	52	52	52
Magnesium stearate	0.81	0.81	0.81	0.81	0.81
Aerosil	0.81	0.81	0.81	0.81	0.81

Drug: Synthesized drug-HPβCD complex, HPMC K100: Hydroxypropyl methylcellulose, MCC 102: Microcrystalline cellulose 102

Stability study

The stability studies were performed both at room temperature and accelerated stability study conditions. The prepared formulations were stored at 40±2°C and 75±5°C relative humidity in a stability chamber for 3 months and observed the physical changes occurred in each month.

RESULTS

Calibration curve

Fig. 2 indicates the standard calibration curve of synthesized compound. The absorbance of the different concentration of synthesized drug was measured using methanol as a blank (λ_{max}=318 nm), and the correlation coefficient (R^2) was found to be 0.994.

Evaluation of granules

All the data obtained were compared with the standard value mentioned in IP. Based on the criteria, it was found that the all the ER tablets have well flow properties and better compressibility. Table 2 represents the Physical characteristics of the prepared granules.

The bulk density of the granules ranged from 0.57 to 0.62 g/ml and tapped density from 0.58 to 0.67 g/ml. The angle of repose of the granules was in the range of 27.94-32.15°.

The compressibility (Carr's index) for all the formulations was ≤10% while Hausner ratio was in the range 1.00-1.11.

Physicochemical characteristics of prepared formulations

Table 3 indicates the results of the various physicochemical tests (hardness, friability, and drug content) performed on the tablet formulations.

The hardness of the prepared tablets was in the range 5.1-5.8 kg/cm^2. All the formulations showed <1% friability which is within the prescribed limit. Drug content was uniform within each batch and ranged from 97.22% to 98.54% of the theoretical value.

Characterization of ER tablet by DSC and PXRD

The thermal behavior of optimized formula and powder blend was studied using DSC. The thermogram of the powder blend shows a characteristic peak of excipients in Figs. 3 and 4.

PXRD

The PXRD pattern of optimized formulation displayed intense and sharp peaks at 12.40, 19.585° with high intensity. The PXRD were shown in Figs. 5 and 6. The lack of crystallinity in the formulation might be due to solubilization of synthesized molecule in inclusion complex which is absorbed into carrier. This suggested although some crystalline signals of physical mixture were still detectable in the optimized formulation.

In vitro drug release study

The *in vitro* drug release results for the 5 different formulations are shown in Fig. 7. From the dissolution study, it was observed that a formulation 4HC4 has the highest release compared to other batches in terms of both the rate and extent of the drug released.

It was concluded that HPMC K100 can be used as an effective extend release polymer to retard the release of drug. The best ER of tablet was obtained from the blend of drug and HPMC K100 in 1:1.8 ratios.

Table 2: Physical characteristics of powder mixtures

Formulations	Angle of repose (°)	Bulk density (g/ml)	Tapped density (g/ml)	Carr's index (%)	Hausner's ratio
4HC1	32.15±1.04	0.574±0.013	0.656±0.018	9.54±0.58	1.023±0.010
4HC2	29.16±1.06	0.577±0.010	0.612±0.017	9.07±0.54	1.034±0.012
4HC3	28.23±1.07	0.573±0.014	0.589±0.019	9.84±0.63	1.048±0.015
4HC4	27.45±1.08	0.611±0.015	0.627±0.013	10.44±0.52	1.037±0.011
4HC5	27.94±1.04	0.628±0.013	0.677±0.017	9.04±0.67	1.032±0.015

Table 3: Physicochemical characteristics of prepared ER tablets

Formulation code	Hardness (kg/cm^2)	Thickness (mm)	Friability (% loss)	Weight variation (mg)	Drug content (%)
4HC1	5.5±0.04	4.15±0.11	0.49±0.068	168.42±0.035	97.22±0.24
4HC2	5.8±0.35	4.27±0.10	0.45±0.0.016	167.54±0.741	98.54±0.32
4HC3	5.1±0.21	4.26±0.13	0.38±0.073	167.66±0.137	98.42±0.35
4HC4	5.7±0.37	4.23±0.13	0.55±0.01	167±0.212	97.68±0.47
4HC5	5.5±0.39	4.52±0.17	0.44±0.16	166±0.389	97.51±0.28

ER: Extended release

Table 4: *In vitro* drug release profile of prepared ER tablets

Time (hrs)	Cumulative amount of percentage drug release				
	4HC1	4HC2	4HC3	4HC4	4HC5
0 (minute)	0	0	0	0	0
5 (minutes)	2.02±0.416	1.97±0.720	1.55±0.08	3.80±0.006	4.001±0.271
10 (minutes)	6.74±0.423	4.18±0.201	3.99±0.672	9.23±0.334	8.63±0.298
15 (minutes)	9.65±0.296	6.11±0.03	9.17±0.543	18.34±0.543	16.72±0.373
30 (minutes)	15.25±0.33	8.911±0.85	15.63±0.55	21.10±0.339	20.11±0.127
45 (minutes)	18.43±0.21	14.23±0.8	18.076±0.1	23.65±0.432	23.66±1.763
1 (hr)	22.06±0.42	19.93±0.07	20.31±0.06	28.36±0.003	26.78±0.765
2 (hrs)	27.26±0.21	26.35±0.04	28.73±0.03	39.98±0.110	28.99±0.109
3 (hrs)	31.54±0.223	31.43±0.333	36.76±0.438	49.47±0.22	33.42±0.080
4 (hrs)	44.32±0.224	36.78±0.304	45.33±0.866	61.754±0.010	39.57±0.117
6 (hrs)	49.52±0.267	39.32±0.317	53.59±0.543	72.47±0.007	47.04±0.103
7 (hrs)	53.77±0.287	51.58±0.264	59.34±0.414	78.32±0.372	56.45±0.677
8 (hrs)	57.53±0.246	59.41±0.266	65.44±0.810	83.66±0.507	63.33±0.257
9 (hrs)	64.78±0.292	65.98±0.711	76.83±0.197	85.002±0.611	69.05±1.120
10 (hrs)	70.81±0.518	71.77±0.681	81.55±0.185	88.43±0.2469	78.11±0.9841
11 (hrs)	76.42±0.293	77.18±0.281	83.67±0.912	90.11±1.223	80.42±0.7935
12 (hrs)	83.28±0.237	81.72±0.623	80.39±0.864	90.88±0.267	76.02±0.4586

ER: Extended release

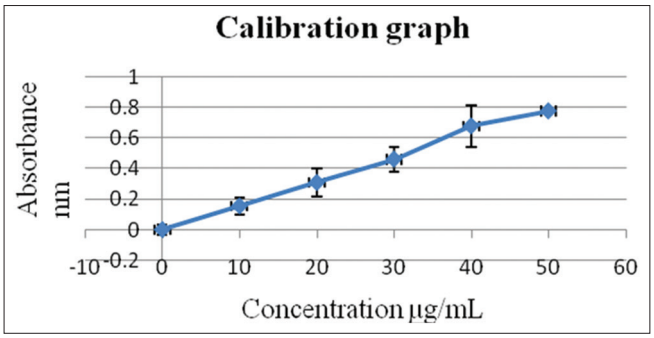

Fig. 2: Calibration graph of synthesized drug

Mathematical modeling of kinetics

The optimized formulation 4HC4 followed zero order kinetic mechanism. In addition, Higuchi as best fitted model, non-Fickian diffusion as the corresponding values of n is lower than or equal to the standard value of Fickian release behavior. Thus, the results point out the mechanistic diffusion and dissolution phenomena. Table 5 mentions kinetic modeling from the optimized formulation.

Stability study

The physical parameters of tablet kept for stability study were evaluated and compared with the initial values of any significant changes. Results which are shown in Table 5. Stability studies were done for 3 months, and it was noted that surface of tablet free from microbial or fungal growth or bad order. The tablets showed not <98.5% drug content and all the physical parameters within the specified limit there was no significance in the values.

DISCUSSION

In the presented study, ER tablets of synthesized compound were prepared for optimized drug release patterns during *in vitro* dissolution studies. Various formulations were prepared containing varying amount of polymer to see the effect of polymer concentration on drug release rates. The prepared mixed powder was physically evaluated with some parameters. The average compressibility index of the mixed powder of all the formulations was ≤10.44±0.52, and average angle of repose was in the range of 27.94-32.15° which indicates that the mixed powder was having good flow properties. The prepared ER tablets were evaluated for some physical tests. The average hardness of ER tablets was in the range 5.1-5.8 kg/cm^2, average friability was <1% and average thickness were in the range

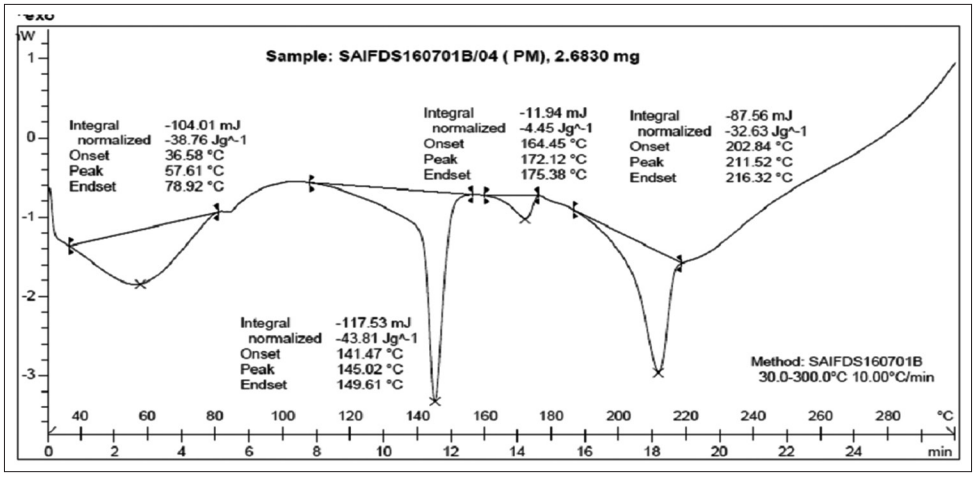

Fig. 3: Differential scanning calorimetry thermogram of physical mixture

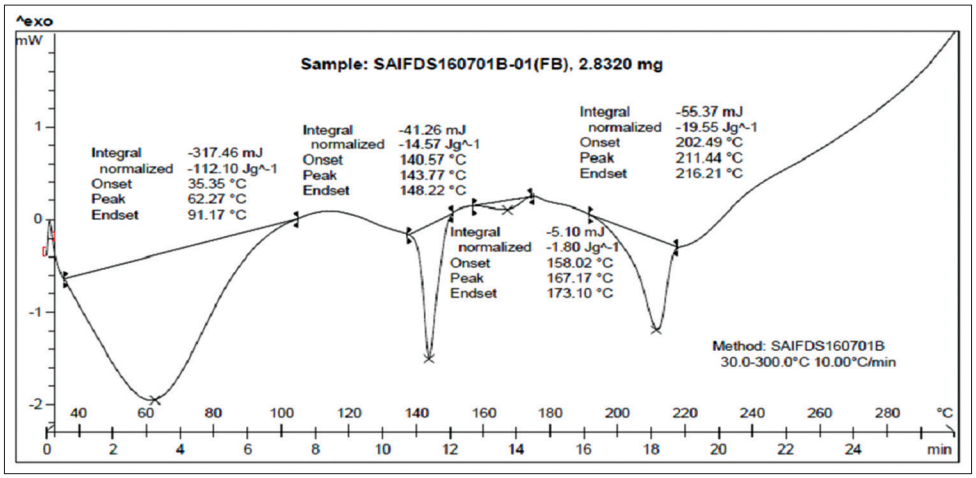

Fig. 4: Differential scanning calorimetry thermogram of optimized formulation

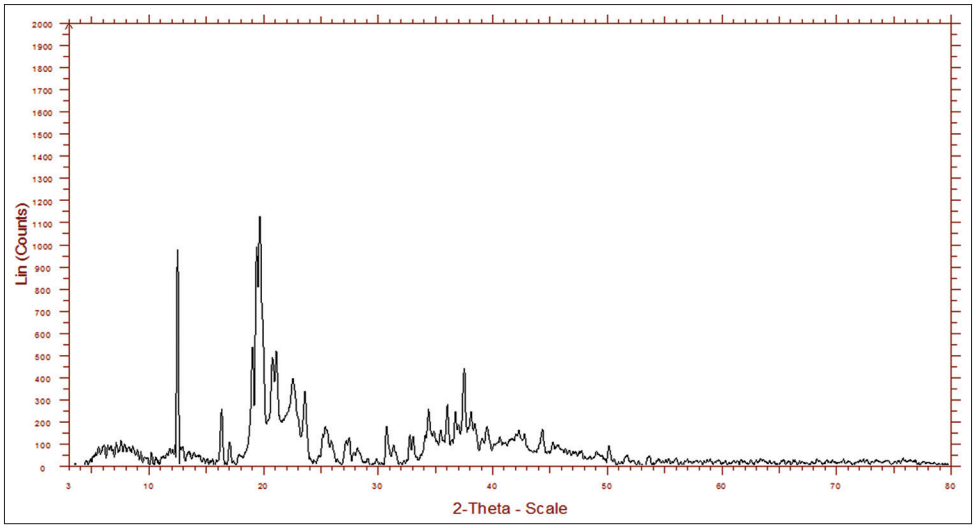

Fig. 5: Powered X-ray diffractometery of physical mixture

of 4.15±0.11-4.52±0.17 mm which was all in acceptable USP ranges. The release of drug from ER tablets prepared at 5 different D: P ratios and the affects of different coexcipients on drug release rates can be seen from the Fig. 7. It was observed that about 90% drug was released in 12 hrs from the preparations prepared at D: P ratio 1:1.8 (4HC4), while the most extended drug release profiles were observed from the formulation prepared at D: P ratio 1:1.8 (4HC4).

Different kinetic models were applied to the optimized formulation and observed that the 4HC$ followed zero order kinetic model. The best linearity was found in Higuchi model (where n=1.11 is the release exponent); applicability of data indicating non-Fickian diffusion.

The stability study for the selected formulation of 4HC4 was performed as per ICH guidelines. Stability studies were done for 3 months, and it

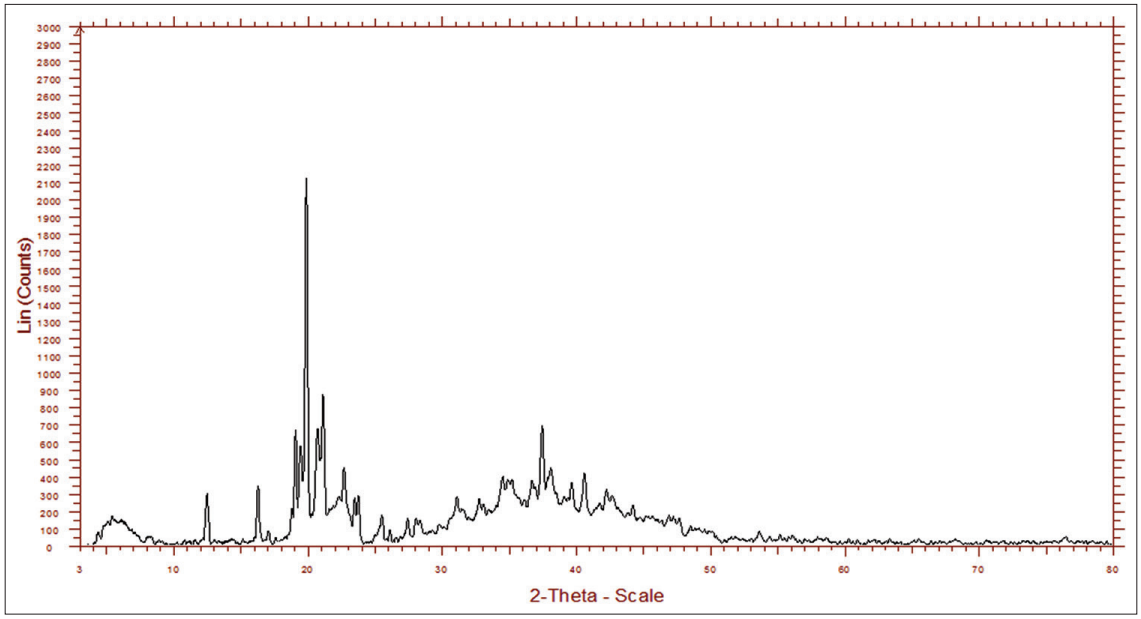

Fig. 6: Powered X-ray diffractometery of optimized formulation

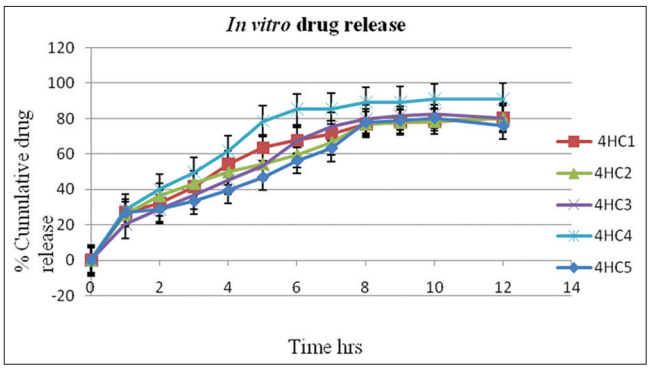

Fig. 7: *In vitro* dissolution data of extended release tablets

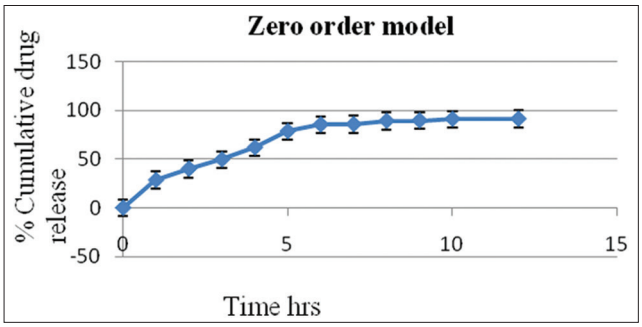

Fig. 8: Percentage cumulative drug release v/s time (zero order model) from the optimized formulation

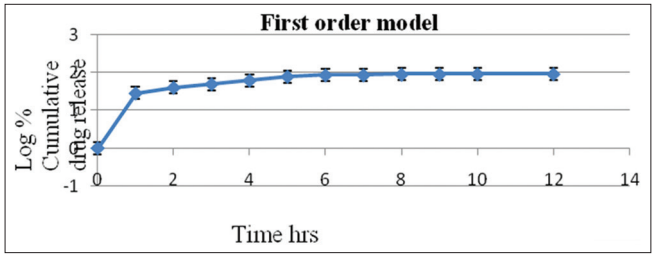

Fig. 9: Log cumulative drug release v/s time (first order model) from the optimized formulation

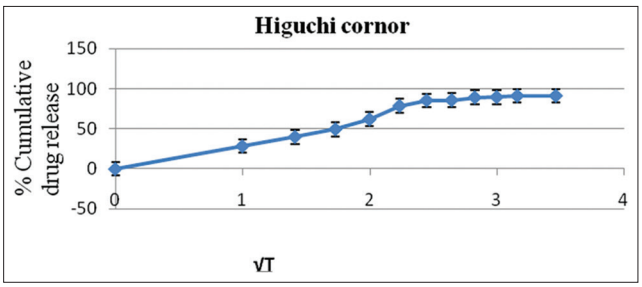

Fig. 10: Percentage cumulative drug release v/s square root time (Higuchi model) from the optimized formulation

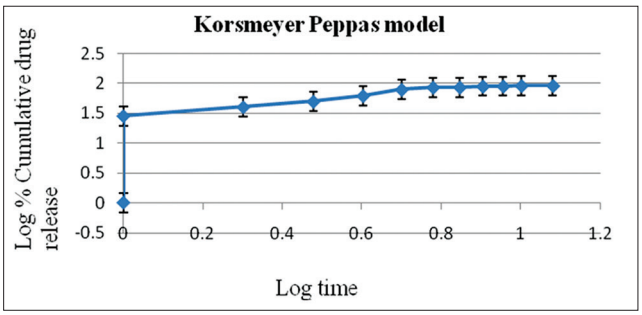

Fig. 11: Log cumulative drug release v/s log time (Korsmeyer–Peppas model) from the optimized formulation

was noted that surface of tablet free from microbial or fungal growth or bad order. The tablets showed not <98.5% drug content and all the physical parameters within the specified limit there was no significance in the values.

CONCLUSION

ER tablet containing chromene derivative was prepared successfully by direct compression method. All the tablets possessed good physical property. Among all the formulations, the formulation which prepared using HPMC K100 found to be effective in controlling the drug release up to 12 hrs. Stability studies revealed that there was no significant change in appearance, drug content and *in vitro* release of selected formulation (4HC4). It was observed that all batches gave the release by the diffusion-dissolution controlled mechanism. The dispersion of the

Table 5: Release parameters of optimized formulation

Code	Zero order	First order	Higuchi	Korsmeyer-peppas	
	R^2	R^2	R^2	n	R^2
4HC4	0.809	0.456	0.946	1.113	0.576

Table 6: Physical parameters of ER tablets (formula 4HC4 kept under stability study)

Months	Hardness (kg/cm^2)	Friability (% loss)	Weight variation (mg)	Drug content (%)	Physical appearance
Initial	3.1±0.341	0.402±0.12	167±0.40	98.33±1.26	White
After 2 week	3.1±0.21	0.48±0.17	167±0.30	98.57±0.30	White
After 1 month	3.2±0.35	0.46±0.20	166±0.22	98.57±0.36	White
After 2 months	3.3±0.32	0.46±0.18	165±0.30	98.54±0.30	White
After 3 months	3.4±0.31	0.46±0.18	167±0.40	98.54±0.30	White

ER: Extended release

drug in the polymer network altered its dissolution profile at pH 7.2, thus making it possible to obtain a gradual and prolonged release, and to modulate the release pattern.

Our preliminary findings on optimized ER tablets can be served for further investigation to assess the efficacy of the formulation by *in vitro* and *in vivo* IBD model.

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