Improved Analgesic Effect and Alleviated Gastric Problems of Diclofenac Sodium Through a Gastric Floating in-Situ Gelling System

Mohammed Jafar[1*], Mohammed Saifuddin Khalid[2], Hajer Saleh Ali Alghamdi[1], Eman Ashoor Al-Qurain[1], Zahra Hussain Alalwan[1], Suhaila Amer Alshawwaf[1] , Zainab Abdul Khaliq Al Mahfoudh[1], Abrar Najeeb Al-Darwiesh[1]

[1] Department of Pharmaceutics, College of Clinical Pharmacy, Imam Abdulrahman Bin Faisal University, P.O. Box 1982, Dammam-31441, Saudi Arabia.

[2] Department of Pharmacology, College of Clinical Pharmacy, Imam Abdulrahman Bin Faisal University, P.O. Box 1982, Dammam-31441, Saudi Arabia.

ABSTRACT

The present investigation was aimed to develop sustained release gastric floating in-situ gels of diclofenac sodium mainly to improve the analgesic activity, and alleviate the gastric problems of diclofenac sodium. Fifteen gastric floating in-situ gel formulations were prepared and optimized using a Box-Behnken design. The independent variables (concentration of gellan gum, calcium carbonate and diclofenac sodium respectively) were improved in order to obtain the required responses. The design expert software was applied to analyze the probable interaction existing between the independent variables. The results revealed the optimized gastric floating in-situ gel with short floating lag time (6.2 minutes); low viscosity (112.1 cps); and the high in-vitro drug release at 24th hr (39.18 %) was obtained using an optimized combination of CaCO3 (0.6 %w/v); gellan gum (0.9 %w/v) and diclofenac sodium (0.5 %w/v); respectively. The analgesic effect of the optimized formulation was found to be better than that of pure diclofenac sodium (8.14±0.43) at 4th hour. Ulcerogenic study results showed that the optimized formulation produced less damage (almost 43% less) to the gastric mucosa than the severe gastric injury exhibited by free diclofenac sodium. Thus, the existing and established NSAID's delivery systems can be replaced by this study's promising formulation of diclofenac sodium.

Key Words: *NSAID, Raft System, In-Vivo Activity, Box-Behnken Design, In-Vitro Drug Release.*

eIJPPR 2018; 8(4):43-52

HOW TO CITE THIS ARTICLE: Mohammed Jafar, Mohammed Saifuddin Khalid, Hajer Saleh Ali Alghamdi, Eman Ashoor Al-Qurain, Zahra Hussain Alalwan, Suhaila Amer Alshawwaf and et al. (2018). "Improved Analgesic Effect and Alleviated Gastric Problems of Diclofenac Sodium Through a Gastric Floating in-Situ Gelling System", International Journal of Pharmaceutical and Phytopharmacological Research, 8(4), pp.42-54.

INTRODUCTION

Oral administration has been the most appropriate, flexible, and frequently used route of drug delivery for local and systemic actions. These days, sustained release drug delivery systems have been the most commonly prescribed dosage forms because they have many advantages over the conventional dosage forms including a better patient compliance owing to less frequent drug administration, the decreased fluctuation in steady-state drug levels, the maximal usance of the drug, the slash in the healthcare costs through better therapy and shorter period of treatment. Certainly, for sustained release systems, oral route of administration has gained much recognition and advancement, since the gastrointestinal physiology provides more versatility in delivery system design than the alternative routes. The progress of an efficient oral sustained release drug delivery system requires an understanding of three important features: [1] GI anatomy and physiology [2] the physiochemical attributes of the drug and [3] the dosage of the factors [1, 2]. Till today, the number of oral sustained drug delivery systems such as tablets, capsules, suspension, emulsions,

Corresponding author: Mohammed Jafar
Address: Assistant Professor, Department of Pharmaceutics, College of Clinical Pharmacy, Imam Abdulrahman Bin Faisal University, P.O. Box 1982, Dammam-31441, Saudi Arabia .
E-mail: ✉ mjomar @ iau .edu . sa
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest .
Received: 05 July 2018; **Revised:** 23 August 2018; **Accepted:** 25 August 2018

etc. have been developed to retard the drug release. For drugs with a limited absorption in the intestine or acting locally in the stomach or better absorbed from upper GIT but irritant to the gastric mucosa due to their direct contact with the said mucosal membrane, the challenging task is not only to prolong the drug release but also the safe retention of the dosage form in the stomach and upper part of the small intestine. Oral solution formulations have offered several advantages over the other oral pharmaceutical dosage forms, but they have often suffered from the abrupt gastrointestinal transit. This could be a serious problem for the drugs which are having absorption window in the stomach in particular. The gastric retention of the oral solutions containing these drugs could be favorably achieved through a novel approach of the liquid in-situgelling system. These systems are polymeric gel formulations responding to physical or chemical signals, including pH, ionic factor, metabolite, or temperature [3-5].Various polymers such as pectin, chitosan, gellan gum, xyloglucan, and xanthan gum have been investigated for this purpose. Gellan gum is a bacterial anionic deacetylated polysaccharide produced by pseudomonas elodea. It undergoes gel formation due to the temperature change or because of the presence of cations (E.g. Na^{+}, K^{+}, Ca^{+2}). It is a water soluble polysaccharide. It produces a gel via formation of double helices, followed by their ionic cross linking. When the appropriate quantities of gas forming substance like calcium carbonate, get incorporated to the aqueous solutions of the above polymers, they could get floated on the surface of the gastric fluid [6]. This floating property of the gels could help in minimizing the gastric irritant effect of weakly acidic drugs (which are better absorbed at upper GIT) by preventing direct contact with the stomach mucosa [7]. Moreover, gastric floating in-situ gels could improve the oral bioavailability of many drugs by preventing their rapid gastric transition and promoting the sustained release of the drug in the stomach for the absorption through gastric mucosa [8].

Diclofenac sodium, is a pioneer molecule of the phenyl acetic acid class of NSAIDs. It is the most widely prescribed drug for the treatment of rheumatoid arthritis, osteoarthritis, acute gout, migraine, muscle and other body pains. It has a short biological half-life (around 2h), therefore frequent administration is necessary to maintain its concentration within the therapeutic level [9, 10]. Moreover, almost all NSAIDs including diclofenac sodium on oral administration, exhibit variable oral bioavailability and also cause severe GI adverse effects like abdominal pain, indigestion, GI bleeding and peptic ulceration [11, 12]; this risk may become much higher for the people who are older in age [13], have poor health, or consume a large quantity of alcohol [14].

Over the years, the substantial work has been mainly related to different oral prolonged release of diclofenac formulations such as controlled release tablets [15], and liquid filled soft gelatin capsules [16] have been reported. However, there has been a lack of evidence in the literature about the diclofenac sodium gastric floating in situ gelling solution, even though this has been considered to be very beneficial from the pharmaceutical and therapeutic point of view.

Therefore an attempt was made to develop a sustained release gastric floating in-situ gelling system of diclofenac sodium using a gellan gum, floating agent and the other excipients. Box-Behnken design [17], which confers limited trial runs and takes very little time, and consequently offers a far more effective strategy than the traditional methods of statistical optimization of a pharmaceutical formulation [18], was used in the design of floating in-situ gelling solutions of diclofenac sodium. The optimized diclofenac sodium gastric floating in-situ gelling solution would provide the benefit of ease of administration, as being a solution, and also be better patient compliant.

MATERIALS AND METHODS

Materials

Diclofenac sodium was purchased from UFC Biotechnology New York (USA). Calcium carbonate, Sodium citrate, and gellan gums were purchased from SD Fine chemicals Pvt. Ltd. India. All the other chemicals used were of the analytical research grade.

Preparation of gastric floating in-situ gels

The gastric floating in-situ gels of diclofenac sodium were prepared employing Box-Behnken design as per the method reported by Shendge et al., [19]. Gellan gum at different concentrations was dissolved in half of the total volume of distilled water containing sodium citrate (0.25% w/v), and a low level of cations present in the solution was sufficient to hold the molecular chains together and inhibit the hydration. The gellan gum solution was heated to 90^{0}C with continuous stirring. After cooling the room temperature, solutions containing various concentrations of calcium carbonate and diclofenac sodium prepared in the remaining half of the distilled water were added and mixed well. The resulting in-situ gelling liquids of diclofenac sodium were stored in a cool place until further use.

In-Vitro evaluation of gastric floating in-situ gels of diclofenac sodium

Appearance

The color and the clarity of the gastric floating in-situ gels of diclofenac sodium were evaluated by the visual

inspection of the solutions against a dark illuminating background.

pH measurement

The pH of gastric floating in-situ gels of diclofenac sodium was measured by a digital pH meter (HI-2214 logging pH bench meter, UK) at room temperature using 30 ml of the sample.

Viscosity measurement

The viscosity of gastric floating in-situ gels of diclofenac sodium was determined by a viscometer (SV-10 Japan) at room temperature using 30 ml of the sample.

In-Vitro gelation study

In-vitro gelation study was conducted on gastric floating in-situ gels of diclofenac sodium as per the method described in the literature [20]. In-vitro gelation study was carried out on freshly prepared gastric floating in-situ gels of diclofenac sodium as reported in the literature. 2 ml of the sample was transferred to 100 ml of 0.1N HCL (pH 1.2) in a beaker without much turbulence to prevent shattering of the formed gel. Gelling was observed in the beaker by the visual inspection and the formulations based on their gelling consistency were given with different grades.

In-Vitro floating study

In-vitro floating study of gastric floating in-situ gels of diclofenac sodium was conducted as per the method reported in the literature [21]. 500 ml of the dissolution medium (pH 1.2) was taken in a dissolution flask (USP Type-II) and the temperature of the dissolution medium was maintained at $37^0C \pm 0.5^0C$. 10 ml of the gelling solution was carefully transferred to the bottom of a dissolution flask without much disturbance in the flask. The amount of time taken by the in-situ gel to come up to the surface of the medium (floating lag time), and also the amount of time, the gel incessantly floated on the surface of the medium (duration of floating) were recorded.

In-Vitro drug release study

In-vitro release of diclofenac sodium from the floating in-situ gels was determined in a USP XXIV rotating paddle apparatus (8 basket Dissolution Test Station, Electrolab, India) at 37^0C using the paddle method at 50rpm/min. The dissolution medium used was 500mL of 0.1N Hydrochloric acid (pH 1.2). The in-situ gel was carefully transferred to the bottom of the dissolution basket without much disturbance. 5mL samples were withdrawn at fixed time intervals, and analyzed at 275 nm using UV-Visible Spectrophotometer (Shimadzu). After each withdrawal, an immediate replacement of 5mL fresh dissolution medium was done to maintain a sink condition. Each determination was performed in triplicate till 24 hours.

Optimization

The three independent variables or factors (diclofenac sodium, calcium carbonate and gellan gum) influencing the performance of the gastric floating in-situ gels of diclofenac sodium were selected based on the factor screening study. The Box-Behnken experimental design was employed to optimize the floating in-situ gels wherein the concentrations of Calcium carbonate (A), Gellan gum (B), and diclofenac sodium (C) were selected as independent variables or factors. Each factor was kept at low, medium and high levels. Floating lag time, Viscosity, and percent cumulative drug release at 24^{th} hour were taken as dependent variables or responses (Table 1).

Table 1: Variables in a Box-Behnken design for the gastric floating in-situ gels of diclofenac sodium

Factors	Level used, actual coded		
Independent variables	Low (-1)	Medium (0)	High (+1)
A= Calcium Carbonate (%)	0.3	0.6	0.9
B= Gellan gum (%)	0.3	0.6	0.9
C= Diclofenac sodium (%)	0.5	0.75	1
Dependent variables	**Goals**		
Y_1= Floating lag time (Sec)	Shorten		
Y_2 = Viscosity (cps)	Decrease		
Y_3 = Cumulative drug release (%)	Sustain		

The effect of factors on the observed responses was analyzed employing Design expert version 11.0.3. (Stat-Ease, Inc, USA) software. The responses were statistically analyzed by ANOVA test method. The best gastric floating in-situ gel of diclofenac sodium was chosen by the quantitative optimization process utilizing the profitability function. For assessing the impact of all factors on the observed responses, the polynomial coefficients for in-situ gelling solutions were ascertained. The polynomial equation created by the Box-Behnkan experimental design was as follows:

$$Y = b_0 + b_1A + b_2B + b_3C + b_{12}AB + b_{13}AC + b_{23}BC + b_{11}A^2 + b_{22}B^2 + b_{33}C^2$$

Where Y is the response; b0 is the intercept; b_1–b_{33} are the regression coefficients calculated from the observed experimental values; and A, B, and C are the coded levels of the factors. The terms A, B, and C_i^2 (i = 1, 2 or 3) constitute the interaction and quadratic terms; respectively.

In-Vivo studies

Male albino Wistar rats weighing 70-120g were used for the study. They were obtained from the Institute for Research and Medical Consultation (IRMC) of Imam Abdulrahman Bin Faisal University. Dammam. Saudi Arabia.

The rats were maintained at 22 ± 1°C on a 12h light- dark cycle , and given standard laboratory diet and water ad libitum. Three groups of rats (*n* = 7 rats per group) were used. The allocation of animals to all groups was randomized. *In-vivo* experimental protocols had the

approval of the Institutional Review Board (IRB**)** IRB-UGS-2018-05-023; dated: 11-01-2018.

Before initiating the investigation, the rats were placed individually in wire mesh in standard plastic cages to stop coprophagy under the controlled environment conditions. Food was taken away for 12hr , but water was given ad libitum [22].

Analgesic activity

Hot plate test in rats: The hot plate latency assay was based on the method of Eddy *et al.* (1950) [23]. Using this method, the rats in the experimental groups (II & III) were given 70mg per kg, orally (p.o) diclofenac sodium as depicted in Table 2 after 12 hours of fasting [24, 25]. The rats in group I (control group) had 10ml per kg normal saline orally. The second group served as the standard.

Table 2: In-Vivo activity experimental design

Groups (n= 7)	Treatment	Dose (mg/kg; p.o)
A	Control (Saline)	--
B	Diclofenac sodium Pure Sample	70
C	Gastric floating in-situ gel formulation of diclofenac sodium	70

The hot plate consisted of an electrically heated surface (Socrel DS-35, Ugo Basile, Comerio, VA, Italy) kept at the constant temperature of 54±0.4^{0}C. For each rat, the intermissions for paw licking or jumping were noted. Then, 30 minutes after applying the drug which was the formulation or saline, the animals were put on the hot plate, and the reaction time, which was the time spent by the animal to begin licking the paw or jumping from the hot plate, was considered as Hot Plate Latency (HPL). The test was carried out at the beginning of the experiment (baseline) and at 1st hour, 2nd hour, 4th hour and 6th hour after the administration. In order to prevent tissue damage, no animal was kept on the hot plate for more than 45 sec. The mean HPL for each group was measured. The analgesic effectiveness of the drug was determined as a percentage of the maximum possible effect (%MPE), based on the formula (TL-BL)/ (45-BL) X 100, where TL = test latency, BL = baseline latency (0hr), 45= cut off time, in seconds [26].

Data were expressed as mean ± SEM. To identify the significance of the difference between the control group and the rats treated with the test compounds, Student's *t*-test was administered. When $P<0.01$, the differences observed in the results were regarded significant [27].

Ulcerogenicity study

Acute ulcerogenesis test was carried out as per Cioli et al and Al-Ghamdi et al [28, 29]. After the analgesic studies, the rats were continued to fast for another 12hr .The rats were anaesthetized with ether, sacrificed, the stomach was removed and opened along the greater curvature, cleaned gently by dipping in saline. The mucosal damage examined grossly under magnifying lens. The severity of the mucosal damage was assessed by the modification of a previously reported rating scale [30].

Observation	Score
No lesions	0.0
Punctiform lesion (lesions less than 1mm)	0.5
Five or more punctiform lesions	1.0
More than five small ulcers or one large ulcer (2-4 mm)	3.0
More than one large ulcer (greater than 4 mm)	4.0

Considering the severity of the mucosal damage, the specimen was assigned on the ordinal score according to the scoring scheme. For instance, a specimen which had five punctiform lesions, two small ulcers, and one large ulcer was given the score of 3.0. The formation of lesions or ulcers was not observed in the control specimens, and accordingly, the score of 0 was assigned to them. The scores were averaged [31], the ulcer incidence [30] and Ulcer length (mm) [32] were tabulated, where the mean ulcer score [31], the percentage of ulcer incidence [31], the cumulative ulcer length (mm) [31] and the ulcer index [33] were calculated.

All data for severity of ulcer were presented as mean ± standard error mean (SEM) of (n=7). The differences between groups were evaluated with one-way ANOVA followed by student's't' test. The difference were considered statistically significant when the *-p <0.05 [26].

To calculate the Ulcer index [33]:

the number of ulcers was counted by using the magnifying glass. The severity scores included: normal coloration as 0, red coloration 0.5, spot ulcer 1.0, hemorrhagic stress 1.5, deep ulcer 2.0 and perforations as 3.0.

Ulcer index = (UN + US+ UP) x 10 rise to power -1

UN = Average of number of ulcer per animal

US = Average of severity score

UP = Percentage of animal with ulcer.

RESULTS AND DISCUSSION

Optimization

Fifteen formulations got from the design with 3 middle points with their observed and predicted responses have been depicted in Table 3.

Table 3: The observed response in Box-Behnken design for gastric floating in-situ gels of diclofenac sodium

Run No.	A*	B*	C*	Floating lag time (Minutes)	Viscosity (cps)	Cumulative Drug Release (%)
1	0.9	0.6	1	6	121	15.69
2	0.3	0.9	0.75	11.2	101.1	26.19
3	0.6	0.6	0.75	2.8	119.5	13.1
4	0.6	0.3	0.5	6	52.7	34.46
5	0.6	0.9	1	7.2	210	16.41
6	0.3	0.6	0.5	8.6	119.3	34.78
7	0.6	0.6	0.75	2.8	119.5	13.1
8	0.6	0.6	0.75	2.8	119.5	13.1
9	0.9	0.9	0.75	6.32	125.31	23.42
10	0.9	0.3	0.75	4	49.2	20.81
11	0.6	0.9	0.5	6.22	112.13	39.18
12	0.3	0.3	0.75	4.53	59.73	25.97
13	0.3	0.6	1	7.1	185	16.26
14	0.6	0.3	1	4.75	76.16	14.53
15	0.9	0.6	0.5	4	108	31.22

***A**, Calcium carbonate; ***B**, Gellan gum; ***C**, diclofenac sodium*

The arithmetical correlations were set up, and the coefficients of the second order polynomial equations were derived employing the multiple linear regression study for a floating lag time, viscosity and drug release. The equations obtained for the above parameters were found to be quadratic in nature with the interaction terms. The coefficients of the polynomials fitted well to the data, with the values of R^2 ranging between 0.4218 and 0.9885 ($p < 0.05$). The three dependent values ranged from 2.8 minutes to 11.2 minutes, 49.2cps to 210cps and 13.1% to 39.18% in floating lag time, viscosity and drug release correspondingly. A definite value in the polynomial equations was relative to the sequel that benefited the optimization, whereas an opposing value represented a converse alliance between the factor and the response. The polynomial equations derived by the statistical interpretation of the outcome have been depicted in Table 4.

Table 4: Model summary statistics given by a Box-Behnken design

Model	R^2	Adjusted R^2	Predicted R^2	SD	% CV
F.L.T*=+2.80-1.39A+1.46B+0.0288C1.09AB+0.8750AC+0.5575BC+2.05A²+1.67B²+1.58C²					
Linear	0.4218	0.2641	0.0702	2.01	17.93
2FI	0.5393	0.1937	0.1562	2.10	
Quadratic	0.9340	0.8151	0.0565	1.01	
Viscosity=+119.50-7.70A+38.84B+25.00C+8.69AB-13.18AC+18.60BC-7.54A²28.12B²+21.37C²					
Linear	0.6493	0.5536	0.2553	29.35	17.58
2FI	0.7374	0.5404	0.3717	29.79	
Quadratic	0.9284	0.7995	0.1455	19.67	
CDR*= +13.10-1.51A+1.18B-9.59C+0.5975AB+0.7475AC-0.7100BC+4.67A²+6.33B²+6.72C²					
Linear	0.6785	0.5908	0.5472	5.74	7.13
2FI	0.6835	0.4461	0.3060	6.68	
Quadratic	0.9885	0.9679	0.8166	1.61	

* ***F.L.T:** Floating lag time, *CDR: Cumulative drug release, **A**: Calcium carbonate, **B**: Gellan gum, **C**: diclofenac sodium.*

All the responses studied for fifteen in-situ gel formulations were collectively fitted to various models using Design expert version 11.0.3. (Stat-Ease, Inc, USA). The best-fitted model of the three factors and their comparative values of R^2 predicted R^2, adjusted R^2, SD and % CV have been given in Table 4. The ''predicted R^2'' was more or less in accordance with the ''adjusted R^2'' values (Figure 1).

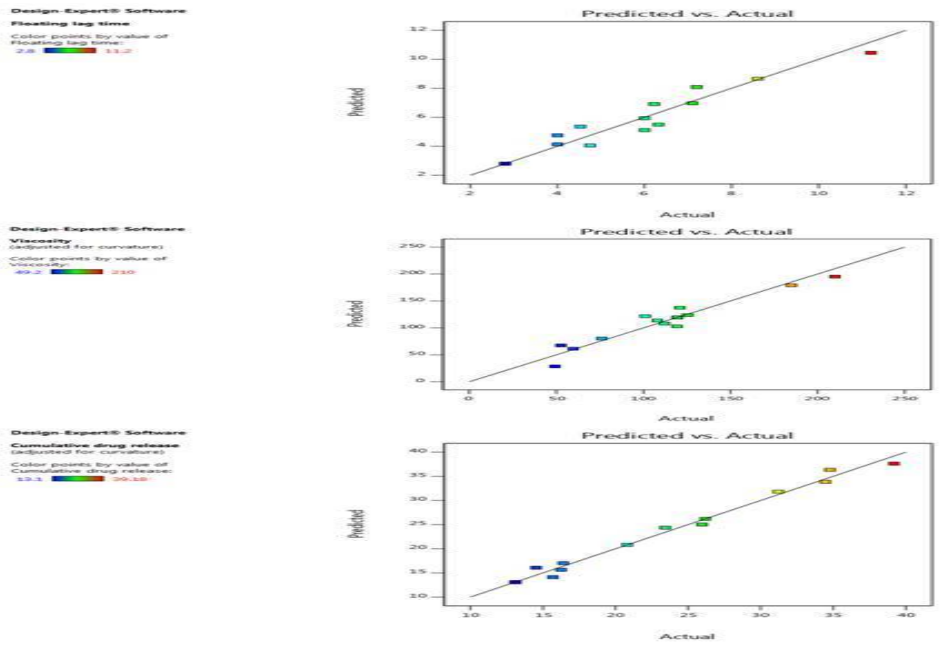

Fig. 1: Predicted Vs Actual responses

The 3D response surface graphs presenting the interaction effects of the factors on the responses have been illustrated in Figure 2.

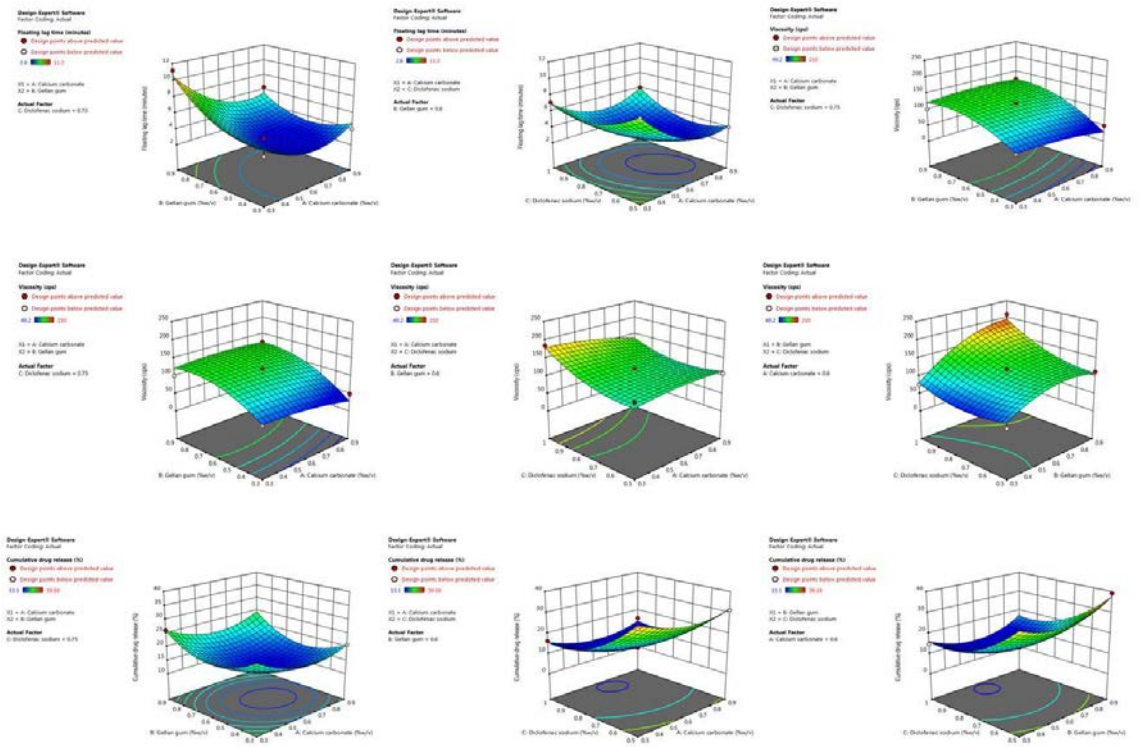

Fig. 2: 3D Graphs of independent variables

The fitting outcomes showed that the optimized in-situ gels of diclofenac sodium with short floating lag time (6.2 minutes), low viscosity (112.1cps) and high drug release at 24th hour (39.18%) were obtained using an optimized combination of calcium carbonate (0.6%w/v), gellan gum (0.9%w/v) and diclofenac sodium (0.5%w/v), respectively. All the response surfaces were best fitted with the quadratic polynomial models, and were capable of predicting the interaction effects, as well. Finally, the model was analyzed for ANOVA ($p < 0.05$), which disclosed that the

model terms for the main effects and the interaction effects were highly significant.

In-Vitro evaluation of floating in-situ gels

All gastric floating in-situ gels of diclofenac sodium were clear, viscous, and colorless. The pH of the in-situ gels was in the range of 8.52 to 9.61 indicating the high stability of the formulations in the upper GIT. Moreover, all the formulations exhibited the desired gelation property and showed long duration of floating on the surface of an acidic medium (pH1.2). These effects could be due to the free availability of a sufficient number of calcium ions and carbon dioxide content from the calcium carbonate in an acidic medium. The obtained results were in agreement with the results reported previously [5].

By raising the quantity of gellan gum, the viscosity was increased significantly in-situ gels, which was occurred because an increasing the chain interaction by gellan gum concentration. Similarly, an increase in the amount of calcium carbonate also increased the viscosity of the in-situ gels at all three gellan gum percentages. It could be due to the high concentration of finely dispersed particles of calcium carbonate in the gelling liquid. On the other hand, the concentration of diclofenac sodium did not contribute much in increasing the viscosity of in-situ gels as it was in its freely soluble form.

A marked decline in the rate and extent of in-vitro drug release was noted with the increase in the gellan gum concentration in in-situ gels (Figure 3). It was attributed to the high density of the

System, and it was also due to the increase in the drug's diffusional path length. In order to study the drug release mechanism, the in-vitro release data obtained for the formulations were fitted to the various kinetic equations. The release model of the optimized in-situ gel formulation followed Matrix (Higuchi matrix) kinetics.

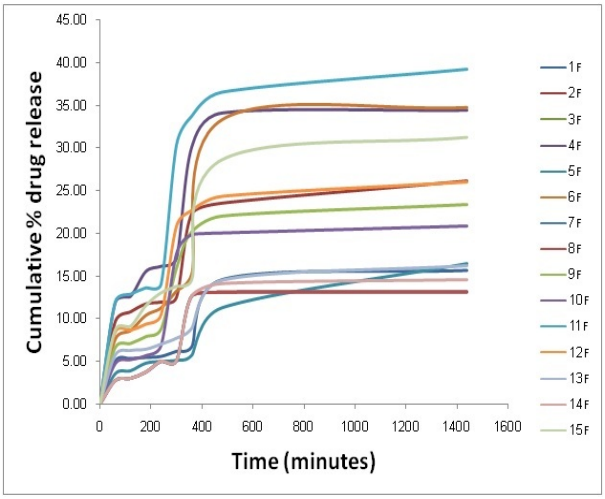

Fig. 3: In vitro drug release profile of floating in situ gels of diclofenac sodium

In-Vivo activity

The analgesic activity of the optimized formulation was studied using thermal test (hot plate test) in rats. Hot plate model was chosen because the test was sensitive to the strong and long acting analgesics and limited tissue damage, which was because of the cut off point that was usually used to restrict the amount of time the animal spends on the hot plate. In this test, gastric floating in-situ gel of diclofenac sodium (optimized formulation) at 70mg/kg displayed a prolonged anti nociceptive activity with the hot plate latency of 9.0±0.61 at 18th hour. The analgesic effect of the formulation was found to be better than that of diclofenac sodium pure (8.14±0.86) at 4th hour (Figure 4).

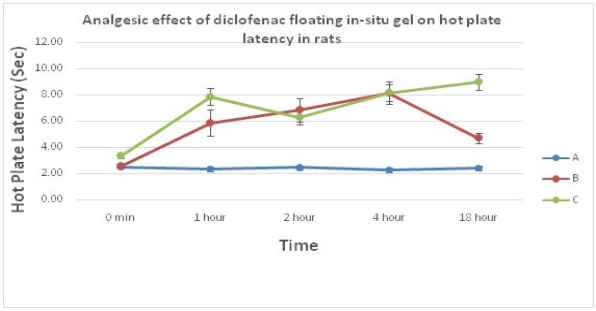

Fig. 4: Analgesic effect of gastric floating in-situ gel formulation of diclofenac sodium and pure diclofenac on hot plate latency in rats.

**Data are expressed as mean ± standard error mean (S.EM) of n=7.*

** A; Control, B; Diclofenac sodium pure sample, C; Gastric floating in-situ gel of diclofenac sodium.*

While this was the maximum possible effect (MPE) for diclofenac sodium floating in-situ gel formulation (13.58 %) at 18th hour (Figure 5), it corresponded to potent synthetic opioid analgesics. In addition, the anti nociceptive effect of diclofenac sodium gastric floating in-situ gel formulation was produced without CNS depression.

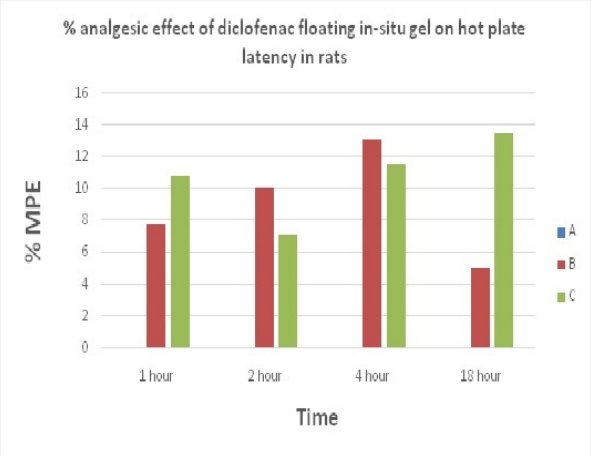

Fig. 5: Percentage of Analgesic effect of gastric floating in-situ gel formulation of diclofenac sodium and pure diclofenac on hot plate latency in rats.

** A; Control, B; Diclofenac sodium pure sample, C; Gastric floating in-situ gel of diclofenac sodium*

The formulation, which showed the analgesic activity comparable to that of the standard drug diclofenac, was screened for their ulcerogenic activity. Pure diclofenac sodium 70mg/kg alone produced a 100% ulcer incidence with the greatest degree of gastric ulcer observed in this study. From the results obtained, it was clear that, using pure diclofenac sodium in a dose of (70mg/kg) caused significant gastric damage. The ulcer index, the mean ulcer score, and the cumulative ulcer length per rat as well as the ulcer incidence were larger compared with diclofenac sodium gastric floating in-situ gel formulation. The floating gel of diclofenac sodium formulation showed ulcerogenic activity of 4.56±0.51, whereas the standard drug diclofenac sodium showed high severity index of 12.09±0.11

After the administration of a single high dose (70mg/kg) of diclofenac sodium gastric floating in-situ gel formulation, there was a significant reduction of 43% in ulcerogenicity as compared to pure diclofenac sodium in regard to the occurred ulcer index of both groups.

The macroscopic examination pictures of rat stomach (treated with standard diclofenac sodium and diclofenac sodium gastric floating in-situ gel) have been presented in Figure 6a, b, c.

From Figure 6a, it is obvious that, the gross study of gastric lumina of the control group showed normal gastric mucosa and normal mucous covering layer.

Figure 6b depicts the picture of rat stomach treated with pure diclofenac sodium having pin point haemorrhagic streaks as indicated by the red spots which are blood clots. The rat stomach treated with gastric floating in-situ gel of diclofenac sodium formulation showed a normal gastric mucosa with a small area of congestion along with very small red spots, as shown in Figure 6c.

The integrity of the gastric mucosa was determined based on the balance between the aggressive (HCl, pepsin) and protective factors (mucus and HCO_3^- secretion, prostaglandins, mucosal blood flow, nitric oxide) [34]. The treatment was effective depending not only on the blockade of the acid secretion, but also on the increased production of factors responsible for keeping the gastric mucosa, therefore preventing damage to the epithelium [35]. The inhibition of prostaglandin synthesis has been well identified as the central mechanism that cause the gastrointestinal injury [36]. This was the outcome of inhibition of cyclooxygenase enzyme which changed the unsaturated fatty acids (which were released during the cell injury) for example arachidonic acid to prostaglandins. In the stomach, prostaglandin synthesis was protective because of the increased mucosal blood flow, and the stimulation of mucous and bicarbonate secretion [37].

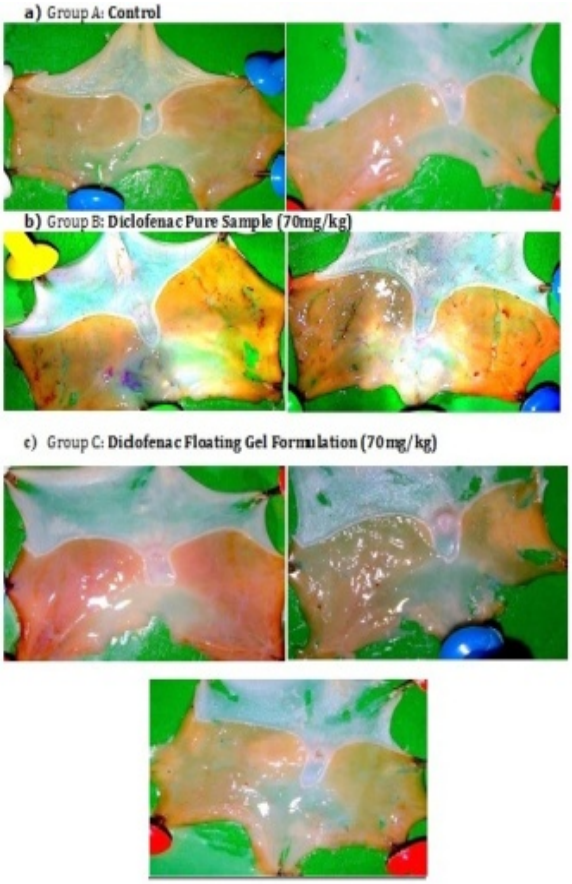

Fig. 6: Illustration of the mucosal injury in rats following single high dose (equivalent to 70mg/kg BW) oral administration of Diclofenac pure drug and Diclofenac floating gel formulation

In the current study, the remarkable increase in ulcer index following the oral administration of pure diclofenac

sodium in the rats might be due to either the free radicals formation or the inhibition of prostaglandin synthesis. The decreased prostaglandin level has been attributed to the impaired gastro-protection and increased gastric acid secretion which were important events in the etiology of mucosal ulceration. The current study agreed with the reports of Bech *et.al* [38]. Biplab *et al* [39], and Muhammed *et al* [40], where diclofenac sodium was reported to have caused alterations in the gastric secretions of rats.

From this study, it was obvious that diclofenac sodium gastric floating in-situ gel formulation has produced long lasting analgesic effect; and the primary objective of the present investigation was to determine whether this gastric floating in-situ gel formulation would provide protection in comparison to the pure diclofenac sodium -induced damage to gastric mucosa while inhibiting prostaglandin synthesis. The results revealed that floating gel formulation in the study produced less damage, almost 43% less, to the gastric mucosa than the severe gastric injury exhibited by free diclofenac (pure drug). This might be due to the reason that the gel kept away diclofenac sodium in contact with gastric mucosa (local effect) or diclofenac sodium in floating gel formulation might not disrupt prostaglandin induced protective barrier with mucous and bicarbonate secretion.

CONCLUSION

Box-Behnken design provided an optimized composition of the gastric floating in-situ gels of diclofenac sodium. The optimized gastric floating in-situ gel of diclofenac sodium demonstrated a desired gelling and floating property with prolonged in-vitro drug release in an acidic medium. From the results, it could also be said that the optimized floating gel formulation of diclofenac sodium exhibited a good analgesic activity as well as playing an important role in protecting the GIT from hemorrhage and ulceration generally induced by NSAID's. The proposed formulation of diclofenac sodium showed better protection of the stomach as compared to the pure diclofenac sodium. Further, the pharmacokinetic and histopathological studies are required to understand the prolongation of the analgesic activity and mechanism of ulcer protection; respectively. Thus, the existing and established NSAID's delivery systems can be replaced by gastric floating in situ gelling systems.

Abbreviations

ANOVA – Analysis of variance
CDR – Cumulative drug release
FDA – Food and Drug Administration
GIT – Gastro intestinal tract
HCL – Hydrochloric acid
HPL – Hot plate latency
MPE – Maximum possible effect
NSAIDs – Non Steroidal Anti-inflammatory drugs
SEM – Standard error of mean

Declaration of interest

None

ACKNOWLEDGEMENTS

The authors would like to express their thanks to Prof.Mastour Al-Ghamdi, Dean, College of Clinical Pharmacy, Imam Abdulrahman Bin Faisal University for his encouragement, guidance and support in this project work.

REFERENCES

[1] Vyas SP, Khar RK, 2012. Controlled drug delivery; concepts and advances. 2nd ed. Vallabh Prakashan India, pp: 196-217.
[2] Kumar R, Patil S, Patil MB, Patil SR, Paschapur MS, 2009. Design and In-Vitro evaluation of oral floating matrix tablets of aceclofenac. Int J Chem Tech Res, 1(4), pp. 815-825.
[3] Miyazaki S, Kawasaki N, Kubo W, Endo K, Attwood D, 2001. Comparison of in situ gelling formulations for the oral delivery of cimetidine. Int J Pharm, 220(1-2), pp. 161-168.
[4] Kubo W, Miyazaki S, Attwood D, 2003. Oral sustained delivery of paracetamol from in situ-gelling gellan and sodium alginate formulations. Int J Pharm, 258, pp.55–64.
[5] Prabaharan M, Mano JF, 2006. Stimuli-responsive hydrogels based on polysaccharides incorporated with thermo-responsive polymers as novel biomaterials. Macromol Bio sci, 6(12), pp.91–1008.
[6] Rajinikanth PS, Balasubramaniam J, Mishra B, 2007. Development and evaluation of a novel floating in situ gelling system of amoxicillin for eradication of Helicobacter pylori. Int J Pharm, 335(1-2), pp.114-22.
[7] Thanoo BC, Sunny MC, Jayakrishnan A, 1993. Oral Sustained-release Drug Delivery Systems using Polycarbonate Microspheres Capable of Floating on the Gastric Fluid. J Pharm Pharmacol, 45(1), pp.21-24.
[8] Lopes CM, Bettencourt C, Rossi A, Buttini F, Barata P, 2016. Overview on gastro retentive drug delivery systems for improving drug bioavailability. Int J Pharm, 510, pp.144-158.
[9] Katzung B, Masters S, Trevor A, 2009. Basic & clinical pharmacology, 1st edition, McGraw-Hill Medical, New York: Edition, pp: 638-641.

[10] Altman R, Bosch B, Brune K, Patrignani P, Young C, 2015. Advances in NSAID Development: Evolution of Diclofenac Products Using Pharmaceutical Technology. Drugs, 75(8), pp.859-877.

[11] Wallace J L, 2000. How do NSAIDs cause ulcer disease? Baillieres Best Pract Res Clin Gastroenterol,14(1), pp.147-59.

[12] Sostres C, Gargello CJ, Arroyo MT, Lanas A, 2010. Adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs, aspirin and coxibs) on upper gastrointestinal tract. Best Practice & Research Clinical Gastroenterology, 24(2), pp.121-132.

[13] Shih SC, Chang CW, 2007. Non-steroidal anti-inflammatory drug related bleeding in the elderly. International Journal of Gerontology, 1(1), pp.40-45.

[14] Neutel CI, Appel WC, 2000. The effect of alcohol abuse on the risk of NSAID-related gastrointestinal events. Ann Epidemiol, 10(4), pp. 246-50.

[15] Bravo SA, Lamas MC, Salomon CJ, 2002. In-vitro studies of diclofenac sodium controlled-release from biopolymeric hydrophilic matrices. J Pharm Pharm Sci, 5(3), pp. 213–9.

[16] Center for Drug Evaluation and Research CR, 2007. Zipsor (diclofenac potassium soft gelatin capsules) NDA, pp. 22-202. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/ 2009/022202s000chemr.pdf.

[17] Box GEP, Wilson KB, 1951. On the experimental attainment of multifactorial conditions. R Stat Soc, 13, pp.1–12.

[18] Alam S, Aslam M, Khan A, Imam SS, Aqil M, Sultana Y, Ali A, 2016. Nanostructured lipid carriers of pioglitazone for transdermal application: from experimental design to bioactivity detail. Drug Deliv, 23(2), pp.601-610.

[19] Shendge RS, Jamdhade AA, Pande VV, 2014. Novel Strategy in Controlled Gastro retentive Drug Delivery: In-Situ Floating Gel. Int J Drug delivery, 6, pp. 230-243.

[20] Sharma A, Sharma J, Kaur R, Saini V, 2014. Development and Characterization of in-situ oral Gel of Spiramycin. Biomed Research International, pp.1-7.

[21] Rajinikanth PS, Mishra B, 2009. Stomach-site specific drug delivery system of clarithromycin for eradication of Helicobacter pylori". Chem .Pharm .Bull, 57(10), pp. 1068—1075.

[22] El-shitany N, 2006. Mechanism of omeprazole induced gastric protection against ethanol induced gastric injury in rats; Role of mucosal nitric oxide and apoptotic cell death. Proceeding of 1st international Egyptian-Jordanian conference on biotechnology and sustainable development: current status& future scenarios, Medical & Pharmaceutical, 2, pp.183-193.

[23] Eddy N.B, Touchberry C.F and Lieberman I.E, 1950. Synthetic analgesics: A methadone isomers and derivatives. J. Pharmacol. Exp. Therapeutics, 98, pp. 121-137.

[24] Ibironke G.F and Ajiboye K.I, 2007. Studies on the anti-Inflammatory and Analgesic Properties of CheopodiumAmbrosioides Leaf Extract in rats. International Journal of Pharmacology, 3 (1), pp. 111-115.

[25] Al-Ghamdi M.S, 2001. The anti-inflammatory, analgesic and antipyretic activity of Nigella sativa. Journal of Ethanopharmacology, 76, pp. 45-48.

[26] Luigi A. Pini, Maurizio Sandrini B, Giovanni V, 1996. The anti nociceptive action of paracetamol is associated with changes in the serotonergic system in the rat brain. European Journal of Pharmacology, 308, pp. 31-40.

[27] Mohd Amir, Kumar Shikha, 2004. Synthesis and anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation activities of some new 2-[(2,6-dichloroanilino) phenyl]acetic acid derivatives. European Journal of Medicinal Chemistry, 39, pp. 535–545.

[28] Al-Ghamdi M.S, Dissanayake A.S, Cader A.A, Jain S, 1991. Tenoxicam-induced Gastropathy in the rat: a Comparison with Piroxicam and Diclofenac Sodium, and the Inhibitory Effects of Ranitidine and Sucralfate. The Journal of International Medical Research, 19, pp. 242-248.

[29] Cioli V, Putzolu S, Rossi V, Sorza Barcellona P, Corradino C, 1979. Toxicol. Appl. Pharmacol, 50, pp. 283–289.

[30] Dalal P.S and Narurkar M.M, 1991. In vitro and in vivo evaluation of sustained release suspensions of ibuprofen. Int.J.Pharm, 73, pp. 157-162.

[31] Glavin G. B, Sitar D. S, 1986. The effect of sulindac and its metabolites on stress-induced gastric ulcers in rats. Toxicol. Appl. Pharmacol, 83, pp. 386-389.

[32] Bozkurt A, Yuksel M, Haklar G, Kurtel H, Yegen BC, Alican I, 1998. Adenosine protect against indomethacin induced damage in rats. Drug. Dis. Sci, 43, pp. 1258-63.

[33] How to calculate the ulcer index (UI) in the stomach?. Available from: https://www.researchgate.net/post/How_to_calculate_the_ulcer_index_UI_in_the_stomach [accessed May 27, 2017].

[34] Lam E, Tai E, Koo M, Wong H, 2007. Enhancement of gastric mucosal integrity by

Lactobacillus rhamnosus. G. G. Life Sci, 80, pp. 2128-2136.

[35] Moraes M, Kushima H, Moleiro C, Santos C, 2009. Effects of limonene and essential oil from Citrus aurantiumon gastric mucosa, role of prostaglandins and gastric mucus secretion. Chem. Bio. Int, 180, pp. 499-505.

[36] Vane R, 1971. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat New Biol, 231, pp. 232-235.

[37] Hawkey J, 2002. Cyclooxygenase inhibition, between the devil and the deep blue sea. Gut, 50, pp. 25-30.

[38] Biplab A, Sudhir KY, Kshama R, Sandip KB, Subrata C, 2011. Black tea and the aflavins assist healing of indomethacin-induced gastric ulceration in mice by anti-oxidative action. Evid Based Complem Alt. Med, 11, pp. 11-22.

[39] Bech PL, Xavier R, Lu N, Nanda NN, Dinauer M, Podolsky DK, 2000. Mechanisms of NSAID-induced gastrointestinal injury defined using mutant mice. Gastroenterology, 119(3), pp. 699-705.

[40] Muhammed A, Thamotharan G, Sengottuvelu S, Haja-Sherief S, Sivakumar T, 2012. Evaluation of antiulcer activity of Ficuspumila L. leaf extract in albino rats. Glob. J. Res. Med. Plants Indig. Med, 1(8), pp. 340-351.