

Formulation and Assessment of Celecoxib Floating Beads in Capsule using 2³ Full Factorial Design

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Abstract

Floating beads have been formed to create a prolonged drug release in the stomach and to reduce the number of frequencies, thereby overcoming its side effects. The current study is concerned with the design and evaluation of stomach-specific oil embedded floating beads of celecoxib in a capsule. Hard gelatin capsules (size 1) were filled with celecoxib oil entrapped beads. A 2³ factorial design was used to investigate the effects of different weight masses of HPMC K4M, Sodium alginate, and maize starch on drug encapsulation efficiency (EE) of beads and percent of cumulative drug release at 6 hours (R_{6h}) from capsules. The optimization results show an increase in EE in the oil entrapped beads and a decrease in R_{6h} from capsules with increases in the weights of HPMC K4M, Sodium alginate, and maize starch. The optimized formulation (F6) had EE of 91.80±0.20% and R_{6h} of 37.88±1.39%. The capsules floated over 6 h and released the drug in gastric pH (1.2) during the first two hours then in phosphate buffer pH (6.8) for another four hours. An X-ray imaging in vivo study of optimised capsules containing CXB oil embedded floating beads in rabbits indicated stomach-specific gastro retention throughout a long time. An in vivo anti-inflammatory efficacy of optimized CXB floating beads showed sustained drug release and better inhibition of rats' hind paw edema.

Keywords

Celecoxib, Floating beads, Gastric residence

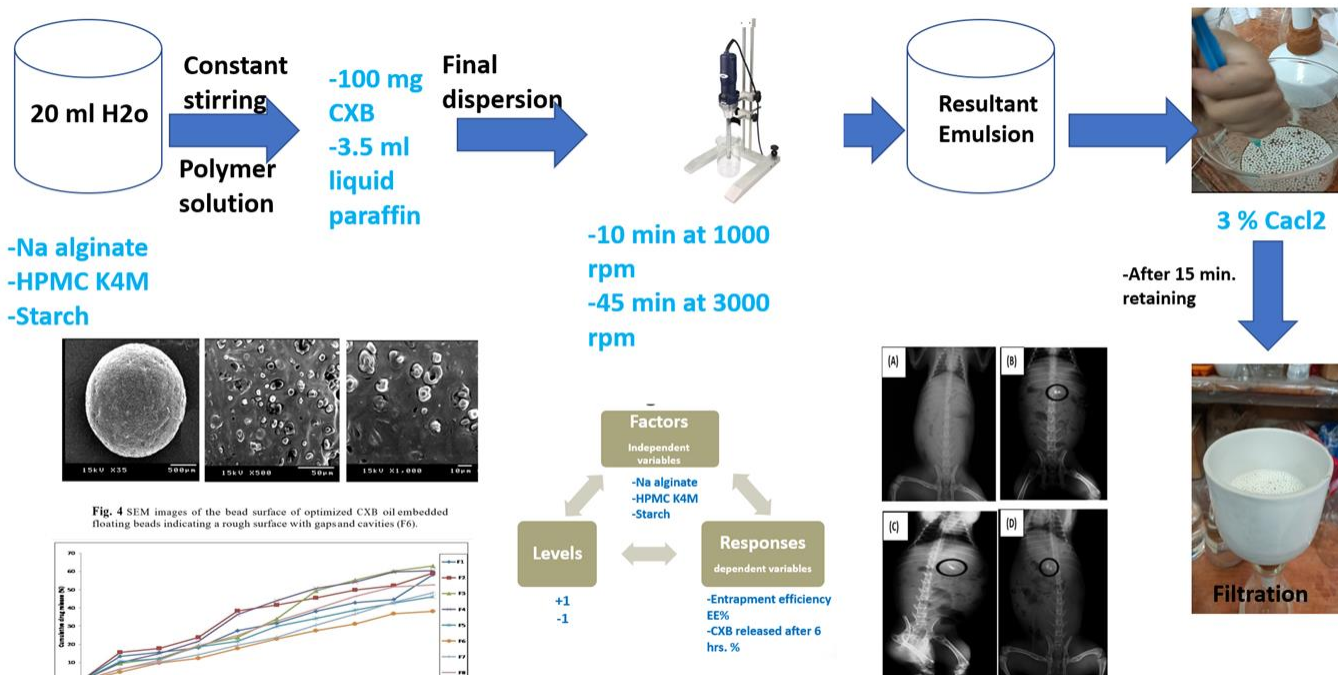


Fig. 4 SEM images of the bead surface of optimized CXB oil embedded floating beads indicating a rough surface with gaps and cavities (F6).

Fig. 3 The in vitro drug release profile of celecoxib oil entrapped floating beads in capsules in simulated gastric fluids (pH=1.2 for the first 2 hours then pH=6.8 for the next 4 hours).

Fig. 8 In vivo X-ray images of optimized capsules of celecoxib oil embedded floating beads in rabbits' gastric area: (A) prior to administration, (B) after 1 hour, (C) after 2 hours, and (D) after 6 hours.

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1. Introduction

several oral drug delivery systems have been developed and evaluated over the last few decades to behave as drug stores whereby drug molecules can be released in a sustained way over a prolonged period of time [1]. These long-acting oral dosage forms have numerous benefits, including specific drug delivery to the target area, trying to limit fluctuations inside the therapeutic window, reducing adverse effects, decreasing frequency of administration, and improving patient satisfaction [2]. The problem in developing these systems is really to maintain the release of the drug and moreover extending the residence of dosage forms in the stomach till the drug is completely delivered at the required time [3]. Extended stomach residency improves bioavailability while prolonging the drug release and reducing drug wasting [4,5]. Extended stomach residency of dosage forms has pharmacokinetic benefits, such as the stability of therapeutic levels throughout a long time subsequently, a decrease in therapeutic level fluctuations. Floatation [6,8], bio or mucoadhesion [9-11], sedimentation [12], expandable or swelling systems [13], are some oral long-acting gastro-retentive techniques.

Floating dosage forms are one of the leading patterns in improving drug retention in the stomach, and numerous techniques have been utilized to promote dosage formulations floating in the gastric contents [14-19]. The key aim for all approaches: is to achieve a density less than the gastric content, allowing medications which act in the anterior gastrointestinal tract locally, are unstable in the inferior of the gastrointestinal tract, or are badly absorbed in the gastrointestinal system can be administered [20]. Numerous oil embedded floating beads have recently been established [21]. On the other hand, the oral handling of multi-unit systems carries the risk of drug mono-dose determination at administration time. As a result, in this study, we tried to develop only a one-unit floating dosage form with oil embedded multi-unit systems allowing drug extended release. Sodium alginate, hydroxyl propyl methylcellulose (HPMC K4M), and maize starch were used to produce these oil embedded multi-unit systems. Sodium alginate, HPMC K4M, and maize starch have the potential to retard the drug release [1,22]. Embedded liquid paraffin which is of low density, and HPMC K4M, on the other hand, can create buoyancy [21-23].

Celecoxib (CXB), is a nonsteroidal anti-inflammatory drug, a selective COX-2 inhibitor, that is being used to treat a variety of inflammatory and painful conditions [24]. For now, the only CXB dosage forms available are oral. CXB oral absorption, on the other hand, is restricted with a bioavailability varying from 22 to 40% [25]. The Biopharmaceutics Classification System (BCS) classifies CXB as a Class II drug because it is poorly insoluble at pH conditions in the gastrointestinal system [26]. CXB's extremely low water solubility causes multiple formulation issues and reduces therapeutic efficacy by retarding its absorption and onset of action [27].

In humans, it has a distribution volume which is 455 ± 166 L and is evenly distributed in vivo. Its lipophilic nature, as well as its poor water solubility and high distribution volume, may contribute to its limited bioavailability. It is insoluble at physiological pH owing to its pKa value of about 11 [28].

Celecoxib has a pKa of 11.1 [29], with a low aqueous solubility of 3-7 $\mu\text{g/mL}$ [25]. With a pKa of 11.1, celecoxib is unionized at physiological pH. It is not surprising that celecoxib is well-absorbed from the GI tract considering its log P of 2.82 between buffer and octanol at pH 7.4 and room temperature [29,30].

Celecoxib, in addition to its main use in relieving pain, it has shown promise as a chemotherapeutic agent for the management of breast, lung, and other cancer types [31]. Because COX-2 inhibition and tumor growth reduction are linked to distinct structural areas of the molecule, derivatives might hypothetically reduce tumor development without impacting COX-2 [31]. That might be advantageous because long-term use of COX-2 inhibitors has been linked to an increased risk of cardiovascular diseases [32].

Ordinarily, pharmaceutical producers create different formulations via changing each factor at a time, which really consumed time and being an exhausted technique [34]. As a result, it is crucial to know the effect of formulation factors on the efficiency of the formula with a limited number of experiments, then select formulation factors to obtain an optimized formulation [35]. Factorial designs have been thought to be a much more powerful statistical optimization method for assessing the influences of multiple formulation factors including the least number of experiments and time, as these variables were always investigated with all possible permutations through studying the impact of independent variables as well as their own relationships with the least number of experiments [36]. The optimization model, based on the factorial designs, involves the development of model equations for examined results throughout the research study to investigate the factor value settings in order to attain optimal formulation [37]. Using a 2^3 factorial design based on computer optimization, the influence of HPMC K4M, sodium alginate, and maize starch weights as three different factors on drug encapsulation efficiency of oil embedded multi-unit floating beads with celecoxib as well as celecoxib start releasing from stomach-specific capsules with celecoxib oil embedded floating beads were investigated in this research.

2. Materials and Methods

2.1 Materials

Free sample of celecoxib from Borg Pharmaceutical Industries (Egypt). Sodium alginate (The general chemical and Pharmaceutical Co., Ltd., England), calcium chloride (Alpha chemika, India), hydroxyl propyl methylcellulose (HPMC-K4M), Sodium Chloride (Isochem Co. Ltd., Egypt), maize Starch and liquid Paraffin (El Gomhouria Co., Egypt) and Hydrochloric Acid (Alpha Chemicals, Egypt).

2.2 Celecoxib oil entrapped floating beads preparation

The ionotropical emulsion-gelation method was used to create oil (liquid paraffin) entrapped beads containing celecoxib. Simply, in 20 ml distilled water, sufficient quantities of HPMC K4M, sodium alginate, and maize starch were dissolved while being constantly stirred. After that, 100 mg of celecoxib and 3.50 ml of liquid paraffin were then transferred to the polymer mixture while it was constantly stirred. The finished dispersions of HPMC K4M, sodium alginate, maize starch, and liquid paraffin that included celecoxib have been homogenized for 10 minutes at 1000 rpm with a homogenizer (BL232, BIO-LAB Instruments Mfg., Co., Korea) and stirred continuously at 3000 rpm for 45 minutes till a stable emulsion was produced. Those emulsions have been injected through a 23G needle into a 100 ml of calcium chloride solution 3% (w/v) at a constant rate (9-11) drops/min, to complete a curing reaction; the extruded droplets are then kept for 30 minutes in the CaCl_2 solution. Before being dried at room temperature for 24 hours, The produced beads were filtered and

washed multiple times with distilled water. Dried celecoxib beads were stored inside desiccators till they were utilized.

2.3 Study design

The design of this study to optimize CXB oil embedded beads is a 2^3 (3 factors and 2 levels) factorial design, in which distinct weights of HPMC K4M (A), sodium alginate (B), and maize starch(C) as three chosen independent factors differed at two levels, that is low (-1), and high (+1). According to the trial proposal of 2^3 factorial design, various trial formulations of CXB oil entrapped beads were developed. The drug encapsulation efficiency (EE) and percent of cumulative drug release after 6 hours (R_{6h}) (in simulated gastric fluid pH 1.2 for the first 2 hours then shifting pH to 6.8 for the next 4 hours) were used as dependent variables (responses). For the development and evaluation of the statistical experimental design, statistical Minitab Software (Minitab 17.3.1 Software) was used. Table 1 shows the design matrix, which includes analyzed factors and responses.

Within this study, the impacts of independent variables on responses could be represented for optimization utilizing polynomial equations regarding independent factors and their relationships for multiple observed responses. The influence of different independent factors on measured responses was mathematically represented by a 2^3 factorial design for optimization:

$$Y = b_0 + b_1 A + b_2 B + b_3 C + b_{12} AB + b_{13} AC + b_{23} BC + b_{123} ABC$$

The dependent variable is Y, while the intercept is b_0 , the regression coefficients are b_1 - b_2 - b_3 , and the independent variables are A, B, and C. The effect of a single factor is represented by a single coefficient, whereas the interaction of many factors is represented by multiple coefficients. After assessing the value of the coefficient and also the mathematical sign it bears, polynomial equations can be used to make conclusions. A positive sign in front of the terms clearly shows that the factors have a synergistic effect, whereas a negative sign clearly shows that the factors have an antagonistic effect [38]. A one-way ANOVA test was used to determine the model's significance ($p < 0.05$) and various response parameters.

2.4 Bead size determination

Slide callipers (SMEC, China) were used to measure the diameters of dried beads by incorporating the beads between 2 metal panels and measuring the diameter of the obtained beads. The diameter of 20 beads from each batch was then measured to determine the average size [39].

2.5 Determination of drug encapsulation efficiency (EE)

From each batch, 100 mg of dried beads were accurately weighed and crushed in a mortar. 100 ml of Phosphate buffer (pH 6.8) was added to the crushed particles. A magnetic stirrer was used to stir the mixture for 24 hours. Filtering with Whatman® filter paper (No. 40) excluded the polymer debris produced after the fragmentation of the beads. A UV-vis spectrophotometer (Jenway 6305 Ltd., Fesltd, Dunmow, U.K.) was used to determine the drug content in the filtrate samples by measuring absorbance at 254 nm. The following equation was used to calculate the EE (%) of beads [40]:

$$(\%) \text{ DEE} = \frac{\text{(Drug content in beads (actual))}}{\text{Drug content in beads (theoretical)}} \times 100$$

2.6 Invitro drug release studies

The drug's in-vitro release from produced oil embedded floating beads in capsules was studied. In vitro release studies were performed on capsules containing CXB oil embedded floating beads (equal to 100 mg drug) utilizing USP rotating paddle apparatus (Erweka DT-D6, Heusenstamm, Germany) with 50 rpm stirring speed at $37 \pm 0.5^\circ\text{C}$ for 6 hours. Two release media with pH 1.2 and 6.8 were used successively to simulate the pH changes along with the GIT. At first, 250 ml of 0.1 N hydrochloric acid (pH 1.2) (as the release medium) was used for 2 hours, then removed, and 150 ml of 0.1 M tribasic sodium phosphate dodecahydrate (TBS) then added within the next 4 hours [41]. Samples were taken at regular intervals of 0.25, 0.5, 0.75, 1, 2, 4, 5, and 6 h and compensated with the same amount

Table 1: 2^3 factorial design and the observed responses of the oil entrapped floating bead of CXB.

Formulation	HPMC K4M (mg)(A)	Sodium Alginate (mg)(B)	Maize Starch(mg)(C)	Responses	
				DEE (%)	R_{6h} (%)
F1	25(-1)	650(-1)	0(-1)	22.85±0.35	58.06±0.59
F2	125(+1)	650(-1)	0(-1)	43.04±0.163	59.03±0.41
F3	25(-1)	750(+1)	0(-1)	35.93±0.37	62.31±0.72
F4	125(+1)	750(+1)	0(-1)	77.45±0.79	60.31±2.25
F5	25(-1)	650(-1)	100(+1)	39.31±0.30	46.01±6.10
F6	125(+1)	650(-1)	100(+1)	91.80±0.20	37.88±1.39
F7	25(-1)	750(+1)	100(+1)	45.25±0.04	48.10±0.65
F8	125(+1)	750(+1)	100(+1)	85.27±0.29	52.52±0.79

EE =Encapsulation Efficiency of CXB (%), R_{6h} (%) =cumulative amount of the drug released from capsule after 6 hours.

Mean± S.D, A, B, and C constitute essential factors; Higher level (+1) and lower level (-1).

of fresh dissolution media to preserve sink conditions throughout the experiment. The collected samples have been analysed by using a UV-vis spectrophotometer (Jenway 6305 Ltd., FesLtd, Dunmow, U.K) by measuring absorbance $\lambda_{\max}=254$ nm.

2.7 kinetics of in vitro drug release

For estimating and identifying the release rate of celecoxib from prepared oil embedded floating beads in simulated gastric fluid, it must be fitted into an appropriate mathematical model. Kinetically, the in vitro drug release values were studied [5]:

$Q = kt + Q_0$	zero order
$Q = Q_0 e^{kt}$	First-order
$Q^{1/3} = kt + Q_0^{1/3}$	Hixson–Crowell
$Q = kt^{0.5}$	Higuchi diffusion
$Q = ktn$	Korsmeyer – Peppas

where Q indicates the amount of drug released at time t , k is the rate constant, Q_0 is the initial value of Q , and n is the release exponent.

These models' efficiency and predictive power are analysed for the calculation of correlation coefficient (r).

Once more, the model of Korsmeyer–Peppas was used to differentiate between multiple competing release mechanisms in an in vitro drug release behavior analysis of different pharmaceutical preparations: Fickian diffusion release, anomalous non-Fickian transport, and case-II transport. If n is 0.5 it suggests Fickian diffusion and if it is 1.0 suggests case-II transport. n values within 0.5 and 1.0 can be used to predict both behaviors (anomalous transport). Only slab geometry, accepts maximum values for n , 0.5, and 1.0, different values have been derived for spheres and cylinders [5]. For all these capsules, a cylindrical geometry is taken into account when determining the values of n .

2.8 Scanning Electron Microscopy analysis

Celecoxib beads' surface morphology was studied using scanning electron microscopy (SEM). SEM studies are performed to check and describe the shape, morphology, and size of the particles. Use the measurement procedures of SEM adhesive double-sided tape to attach the developed formulation to the metal stub. The formulation was vacuum dried before even being sputter-coated with a 10 nm thick gold layer and viewed in SEM of high resolution. (Joel Instrument 5400, USA) [42].

2.9 Fourier transform-infrared(FTIR) spectroscopy

Using an FTIR spectrophotometer (IR-470, Shimadzu, Japan), samples (CXB, optimized CXB oil entrapped floating beads, and blank oil embedded floating beads (without CXB)) were crushed to powder and examined as KBr pellets. The sample holder was loaded with the pellet. In the wavelength range of 4000 and 400 cm^{-1} , Spectral scanning was carried out [43].

2.10 Differential scanning calorimetric (DSC) studies

Analysis of DSC were performed on the selected samples (CXB, HPMC K4M, Sodium (Na) alginate, Starch, optimized oil entrapped beads containing CXB (F6), physical mixture (PM) of CXB, and all polymers were carried on the DSC-50 Shimadzu instrument. The operation conditions of DSC were carried out under purging of nitrogen at a rate of 40-50 ml/min, and heating at a rate of 10 °C/min. Aluminum pans were used, the weight of the sample was (4-5mg) and the temperature range was from room temperature to 300 °C [44].

2.11 Filling hard gelatin capsules with celecoxib oil entrapped beads

For celecoxib oil embedded beads of a known equivalent amount, were blended for a further 3 minutes with magnesium stearate (5%, w/w) as a lubricant before being manually loaded into empty hard gelatin capsules (size 1). To retain uniformity of content and weight, care has been taken to fulfill the content completely.

2.12 Determination of swelling index of oil entrapped CXB beads

The swelling rate of the optimized CXB oil entrapped beads (F6) was measured as a function of pH, known weight (100mg) of floating beads was incubated in phosphate buffer (pH 6.8) and 0.1N HCL (pH 1.2) solutions (using dissolution apparatus) at 37 ± 5 °C. After eliminating the excess water with filter papers the beads were removed and weighed at various time intervals. Their changes in weight were measured during swelling. The swelling index was determined using the equation [45]:

$$(\%) \text{ Swelling index} = [(W_t - W_o) / W_o] \times 100$$

W_t is the weight of the bead at every time and W_o is the beginning weight of the bead prior to swelling (dry bead). Every measurement was carried out three times.

2.13 Determination of in vitro floating properties

In a beaker containing 100ml 0.1N HCL, twenty beads from each formulation were put. The number of beads that remained buoyant was counted at predetermined time intervals. The buoyancy percentage was calculated using the following equation [46]:

$$(\%) \text{ Buoyancy} = \frac{\text{Number of floating beads}}{\text{Total number of beads}} \times 100$$

2.14 X-ray radio imaging in vivo study

An in vivo performance of the optimized formulation was assessed using an X-ray radio imaging study [47]. The in vivo X-ray radio imaging investigation used adult male New Zealand white rabbits having weight between 2500 and 2800 g. The rabbits have been introduced to their unfamiliar surroundings and supplied with a regular laboratory regimen. Prior to such a study, the rabbits were fasted overnight and placed in individual cages at constant room temperature. To minimize circadian influence, all tests have been done between the hours of 8 AM and 12 PM. The animal ethics guidelines of Asyut University, Egypt were

applied to the experimental protocol. Every effort was undertaken to reduce both suffering of animals and the number of animals involved. The capsules were prepared with an optimized formulation of oil embedded beads (F6) and 50 mg of barium sulfate for each capsule. Those capsules were given via plastic tubing, which was then flushed with distilled water (25–30 ml). The rabbits only had free access to water throughout the investigation. At 1, 2, and 6 hours, X-ray images of the gastric area were captured.

2.15 In-vivo anti-inflammatory effects of CXB floating beads

The paw edema test in rats was utilized to evaluate the anti-inflammatory effect of the selected formulation (F6). According to the animal ethics guidelines of Asyut University, Egypt, experiments were performed. Four groups of male albino rats weighing 200 ± 50 g were formed (6 per group). Rats fasted for 24 hours and had unlimited access to water before the test. The animals of group 1 (control) obtained placebo beads (floating beads without drug), group 2 obtained commercial capsules Indomethacin® (capsule content suspended in 2 ml water), group 3 obtained commercial capsules Celebrog® (capsule content suspended in 2 ml water) and group 4 obtained CXB floating beads (F6) (equivalent amount of beads suspended in 2 ml water), respectively. In order to induce edema, a dose of 0.1ml of 1.0% (w/v) carrageenan suspension in 0.9% saline solution was injected into the sub plantar area of the rat's left hind paw [48]. Before carrageenan injection, instantly after carrageenan injection (at time 0), then every hour for up to six hours, the thickness of each rat's left paw was determined using a Vernier Caliper (SMEC, China). The following equation was used to calculate the percentage of inhibition of paw edema:

$$(\%) \text{ Inhibition} = \frac{(V_t - V_0) \text{ control group} - (V_t - V_0) \text{ treated group}}{(V_t - V_0) \text{ control group}} \times 100$$

V_t and V_0 are the post-treatment and pretreatment average volumes for each group, respectively.

2.16 Statistical analysis

Multiple regression analysis would be used to validate the results into the factorial model using Statistical Minitab software, version 17 (Minitab Inc., Pennsylvania, USA). For each factor, regression coefficients were estimated and found to be significant at ($p < 0.05$). All the studies were done three times, and the outcomes were provided as mean \pm SD.

3. Results and Discussion:

3.1 Analysis of factorial design

The 2^3 factorial design recommended a total of 8 trial formulations for 3 independent factors: quantities of HPMC K4M (A), sodium alginate (B), and maize starch (C), all of which differed at low level (-1) and high level (+1). In the current study, the influences of those independent factors on EE and R6h were studied as response variables. Various celecoxib trial formulations were prepared using a 2^3 factorial design. The experimental trial and measured responses are summarized in table 1. As shown in Table 2, the ANOVA findings showed that the studied models seem to be significant for all response variables.

After fitting this data, the Statistical Minitab software (Minitab 17.3.1 Software) developed appropriate polynomial model equations including individual variables and interaction variables.

Table 2: ANOVA Summary of responses

Source	Sum of square	DF	Mean square	F-value	P-value
EE(%)					
Model	13969.7	7	1995.67	4651.1	0.000(S)
A	8920	1	8919.97	20788.87	0.000(S)
B	825	1	824.98	1922.70	0.000(S)
C	2544.2	1	2544.23	5929.57	0.000(S)
AB	29.4	1	29.42	68.57	0.000(S)
AC	355.7	1	355.66	828.91	0.000(S)
BC	867	1	867.05	2020.74	0.000(S)
ABC	428.4	1	428.38	998.37	0.000(S)
Error	6.9	16			
Total	13976.5	23			
R6					
Model	1542.13	7	220.30	38.30	0.000(S)
A	10.40	1	10.40	1.81	0.198(N)
B	194.88	1	194.88	33.88	0.000(S)
C	1164.95	1	1164.95	202.55	0.000(S)
AB	30.65	1	30.65	5.33	0.035(S)
AC	1.72	1	1.72	0.30	0.592(N)
BC	42.74	1	42.74	7.43	0.015(S)
ABC	96.78	1	96.78	16.83	0.001(S)
Error	92.02	16			
Total	1634.15	23			

EE = Encapsulation Efficiency (%) of CXB oil entrapped floating beads. R_{6h} = (%) cumulative drug release from CXB oil entrapped floating beads in capsules after 6 hours; A, B, and C indicate factors-Quantities of HPMC K4M, sod. Alginate and starch (in mg), respectively; S and NS indicate significant and non-significant, respectively. D.F= degree of freedom.

The following equation for EE as a response became:

$$DEE\% = 32.58 - 1.1844 A + 0.07751 B - 0.0010 C + 0.002133 AB + 0.025199 AC + 0.000131 BC - 0.000034 ABC$$

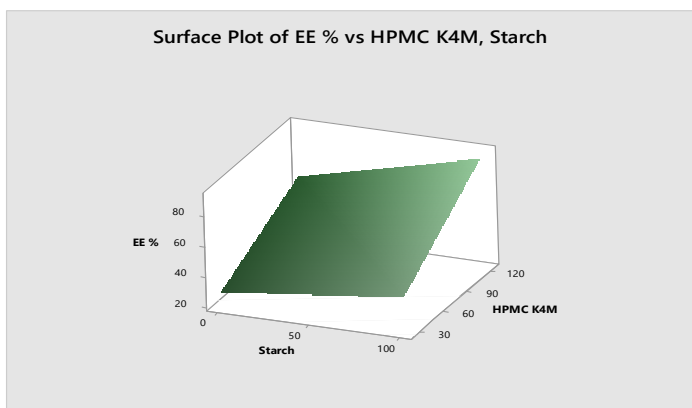
The following equation for R_{6h} as a response became:

$$R6h\% = 21 + 0.238 A - 0.0566 B + 0.338 C - 0.000351 AB - 0.01135 AC - 0.000671 BC + 0.000016 ABC$$

The impacts of main effects (factors) on responses (here, EE and R_{6h}) were investigated using the response surface technique. It is commonly used in pharmaceutical technology for drug delivery system design and formulation optimization [11, 7].

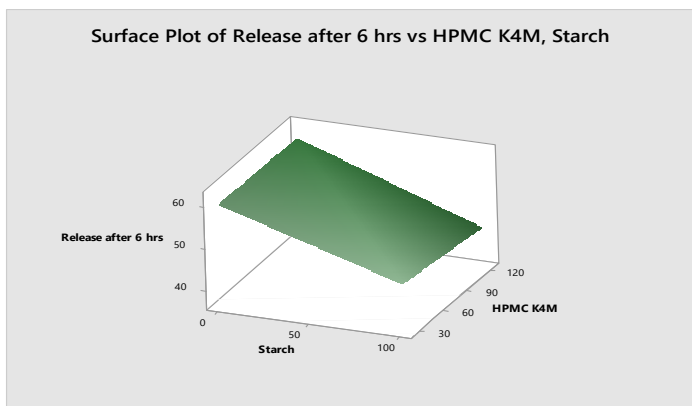
The response surface graph (three-dimensional) is extremely beneficial for knowing the main and interaction impacts of the independent variables (factors), while the contour plot (two-dimensional) provides a visual representation of response estimates [7].

The response surface graph (three-dimensional) regards EE (Fig. 1a) shows that the increase in the amount of HPMC K4M (A) and maize starch (C) in the prepared celecoxib oil entrapped floating beads increases EE

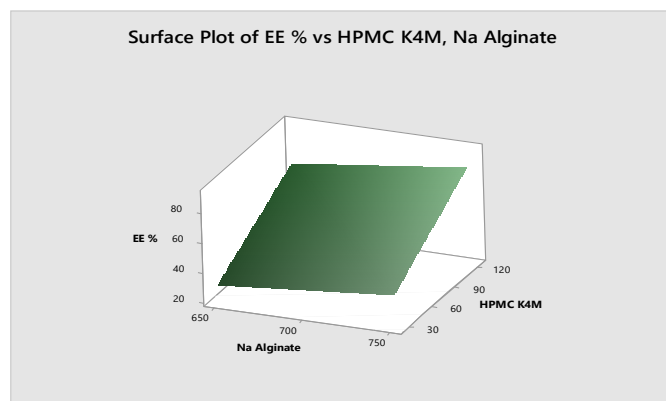


(Fig. 1a)

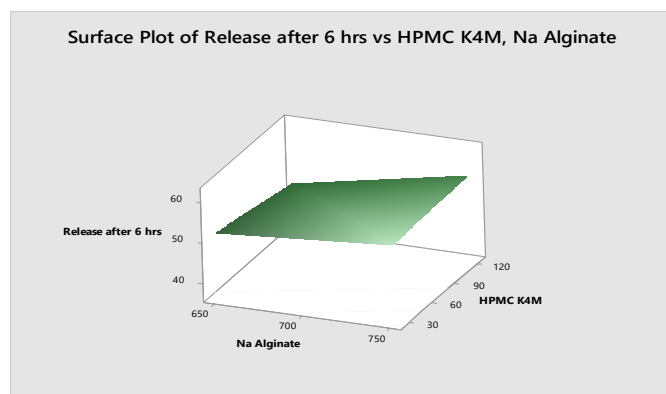
The response surface graph (three-dimensional) regards R_{6h} on the other hand, shows a decrease in R_{6h} values as the amount of HPMC K4M (A) and maize starch (C) increases.



(Fig. 1b)



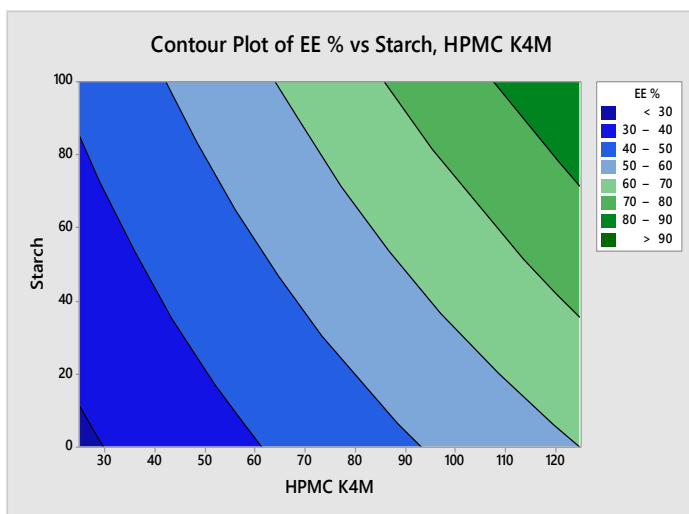
(Fig. 1c)



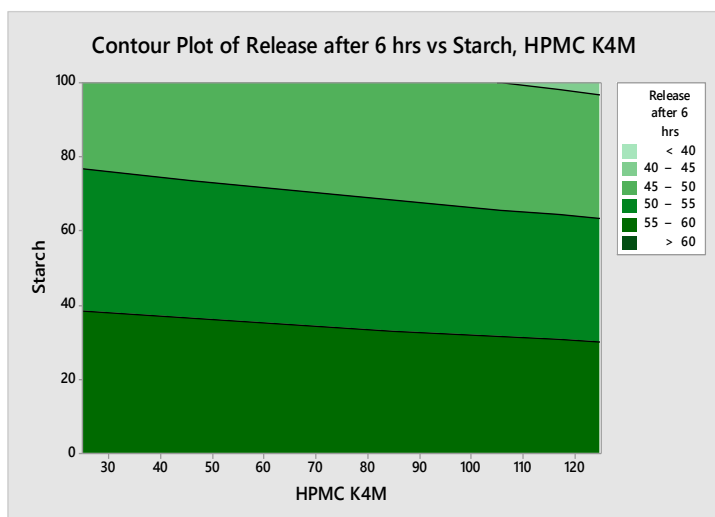
(Fig. 1d)

Figures (1 a, b, c, d): The effect of HPMC K4M, starch, and Na Alginate weight masses on DEF (%) and R_{6h} was demonstrated by 3-dimensional response surface plots.

In all observed responses analyzed in this research, as shown in Fig. 2 (a and b), all contour plots exhibit nonlinear correlations between two independent factors (here, HPMC K4M quantity and maize starch quantity).



(Fig. 2a)



(Fig. 2b)

Figures (2 a, b): Two-dimensional contour plots of the influences of HPMC K4M and starch weights on (a)= EE (%) and (b)= R6h (%).

3.2 Determination of Bead size

As shown in Table 3, the dried beads had an average diameter of 1.46 ± 0.13 to 1.76 ± 0.27 mm. Increased amounts of HPMC K4M, sodium alginate and maize starch resulted in larger beads' size. Increasing the overall number of polymers involved in the production of CXB oil entrapped floating beads increases bead size, once more. This may be responsible for the increase in emulsion viscosity as the proportion of total polymers in the emulsion increases, resulting in a larger droplet size throughout adding emulsion to the cross-linking solution.

Table 3: Average bead diameter of numerous oil entrapped beads containing celecoxib.

Formulation	Avg. Bead diameters
F1	1.46 ± 0.13
F2	1.50 ± 0.18
F3	1.53 ± 0.17
F4	1.70 ± 0.23
F5	1.56 ± 0.17
F6	1.63 ± 0.19
F7	1.73 ± 0.25
F8	1.76 ± 0.27

Mean \pm S.D

3.3 Formulation Optimization

When the experimental design was carried out, an optimized formulation was chosen based on the effect of each factor on the responses (EE, R6h). Since CXB has low aqueous solubility according to the USP classification system, the most critical parameter to optimize was maximum EE.

Furthermore, selecting the second response, which was the percentage of CXB released, was difficult. Provided that the initial goal of the beads was to attain gastric retention for a particular period of time and then release the drug contents into the stomach throughout that period of time. As a result, the minimum percentage released after 6 hours was chosen.

The software selects an optimum formulation with maximum EE and minimum release after 6 hours based on the previously mentioned factors.

The optimal formulation for further research was chosen because the ratios in this improved formula match those in F6.

This could be due to the presence of HPMC, which is a known release inhibitor. When hydrated, this polymer can form a viscous environment, resulting in a decrease in the release rate [49].

3.4 Drug Encapsulation Efficiency (EE)

The EE of formulated CXB oil embedded floating beads ranged from $22.85 \pm 0.35\%$ to $91.80 \pm 0.20\%$ as indicated in Table 1. The EE of these beads increased as the quantities of HPMC K4M, sodium alginate and maize starch increased. An increase in emulsion viscosity with raising the quantities of HPMC K4M, sodium alginate, and maize starch could have prevented drug from leaking into the cross-linking solution, resulting in higher EE. Furthermore, higher drug encapsulation with increased polymer content in these oil embedded beads could be attributed to the tangling of greater drug quantity within the polymeric gel matrix.

3.5 In vitro drug release studies

As shown in Figure 3, all of these capsules of celecoxib oil entrapped floating beads demonstrated 6 hours of sustained drug release (in simulated gastric fluid, pH 1.2 for the first 2 hours then shifting to 6.8 for the next 4 hours).

According to the response surface analysis, increasing the amounts of HPMC K4M, sodium alginate, and maize starch resulted in a decrease in R6h values. The hydrophilic polymers utilized to make these oil entrapped beads may entangle well with an aqueous medium forming a viscous gel structure, which could explain why celecoxib release from CXB oil entrapped beads in capsules is delayed. This could clog pores on the beads' surface, limiting the drug release rate. Another possibility for the long-term release of drugs from such capsules seems to be that the majority of drugs stay saturated and distributed in the oil cavities of the capsules containing oil embedded floating beads, resulting in a drug-oil distributed network. The drug may be transported from those capsules containing beads to the dissolving media in two stages [35].

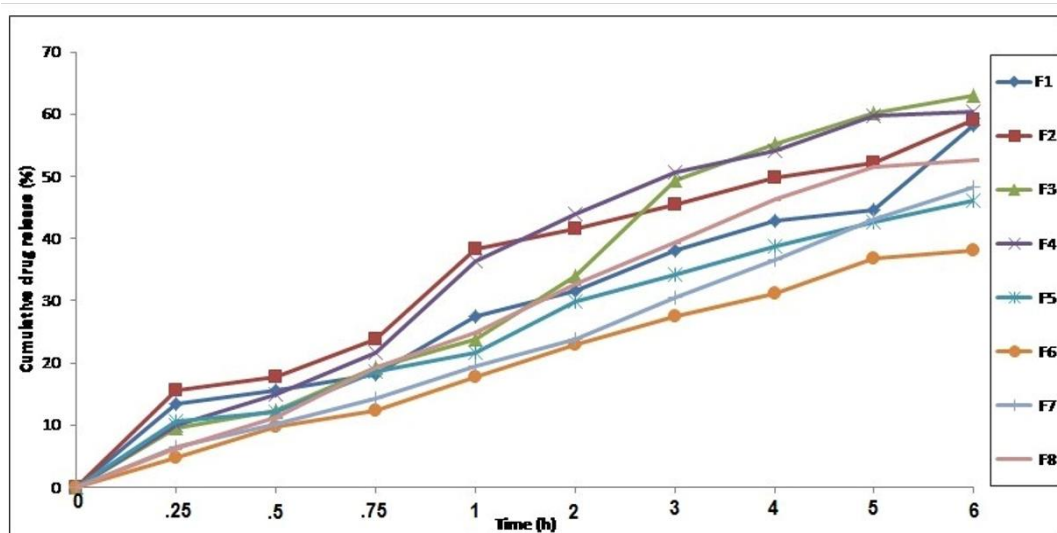


Fig. 3 The in vitro drug release profile of celecoxib oil entrapped floating beads in capsules in simulated gastric fluids (pH=1.2 for the first 2 hours then pH=6.8 for the next 4 hours).

The drug could first permeate from oil cavities into the bead network. Second, it has the potential to diffuse into the dissolution media from the matrix. Furthermore, magnesium stearate's hydrophobic nature might prevent hydration of the prepared capsules [8] consequently, maintaining celecoxib's drug release profile.

Multiple mathematical models were used to investigate the kinetics of in vitro drug release from different celecoxib oil embedded floating beads in capsules including zero-order, first-order, Higuchi diffusion, Hixson-Crowell, and Korsmeyer-Peppas. Table 4 shows the outputs of the curve fitting into the previously indicated mathematical models. When the drug

release correlation coefficients were compared, It was discovered that it followed the Korsmeyer-Peppas model ($r = 0.994-0.964$) during a course time of 6 hours. The value of the release exponent (n) calculated from in vitro celecoxib release data of numerous capsules of celecoxib oil embedded floating beads varied from 0.424 to 0.646, suggesting that the release of the drug from capsules (F1) to (F4) obeyed diffusion-controlled release (Fickian diffusion) but from (F5) to (F6) obeyed the anomalous transport (non-fickian diffusion) mechanism controlled by both diffusion and polymeric chain enlargement or relaxation mechanisms due to presence of starch.

Table 4: Curve fitting results for in vitro celecoxib release information from multiple capsules of celecoxib oil embedded floating beads.

Formulations	Zero-order	First-order	Higuchi-diffusion	Hixson-Crowell	Korsmeyer-Peppas	
	R	R	R	R	r	N
F1	0.973	-0.974	0.981	0.976	0.983	0.453
F2	0.923	-0.951	0.961	0.943	0.964	0.424
F3	0.972	-0.988	0.993	0.984	0.994	0.532
F4	0.918	-0.952	0.965	0.941	0.967	0.573
F5	0.970	-0.984	0.991	0.980	0.994	0.682
F6	0.968	-0.979	0.984	0.976	0.993	0.609
F7	0.988	-0.995	0.995	0.994	0.994	0.608
F8	0.958	-0.978	0.978	0.972	0.990	0.646

r = correlation coefficient; n= the release exponent.

3.6 Scanning electron microscopy analysis

SEM photographs of the surface of the bead of optimized CXB oil entrapped beads (F6) noticed a rough surface with small gaps cavities as shown in Figure 4. Corrugations gave the surface an “orange peel” look. On the bead surface, there were no large crystals of the drug, revealing that the drug molecules were finely distributed in the polymeric matrix.

3.7 FTIR spectroscopy

Fig. 5 illustrates the FTIR spectra of (A) celecoxib, (B) optimized CXB oil embedded beads, and (C) blank oil embedded beads (without celecoxib). Celecoxib's FTIR spectrum indicated distinctive peaks at 3340.56 cm^{-1} and 3235.02 cm^{-1} for the drug's -NH symmetric and asymmetric stretching vibrations of the primary amine group, 2926.64 cm^{-1} for C-H stretching, and 1347.76 cm^{-1} for S=O (sulfonamide group) stretching. The FTIR spectrum of optimized CXB oil entrapped beads (F6), showed several characteristic peaks of CXB with no distinct shifting. Furthermore, the distinctive peaks noticeable in the FTIR spectrum of blank oil embedded beads were also noticeable in the FTIR spectrum of optimized CXB oil embedded beads (F6).

According to the FTIR analyses, there was no interaction between celecoxib and the additives utilized in the bead preparation. The FTIR analysis revealed celecoxib's compatibility with the additives used to formulate CXB oil embedded beads.

3.8 DSC studies

The DSC thermograms of pure celecoxib, a physical mixture (PM), oil entrapped CXB beads (F6), HPMC K4M, starch, and sodium alginate, as shown in Figure 6. Celecoxib shows an endothermic peak at 164°C. The thermograph of HPMC K4M has an endothermic peak at 49°C, a Starch endothermic peak at 94°C (due to evaporation of water), and a sodium alginate endothermic peak at 107°C (due to evaporation of water). Towards the physical mixture, there is a characteristic peak at 163°C, which indicates the involvement of CXB. In the thermogram of formulation (F6), the characteristic peak of the drug disappeared completely, indicating the complete inclusion of the celecoxib within the polymer matrix forming the beads.

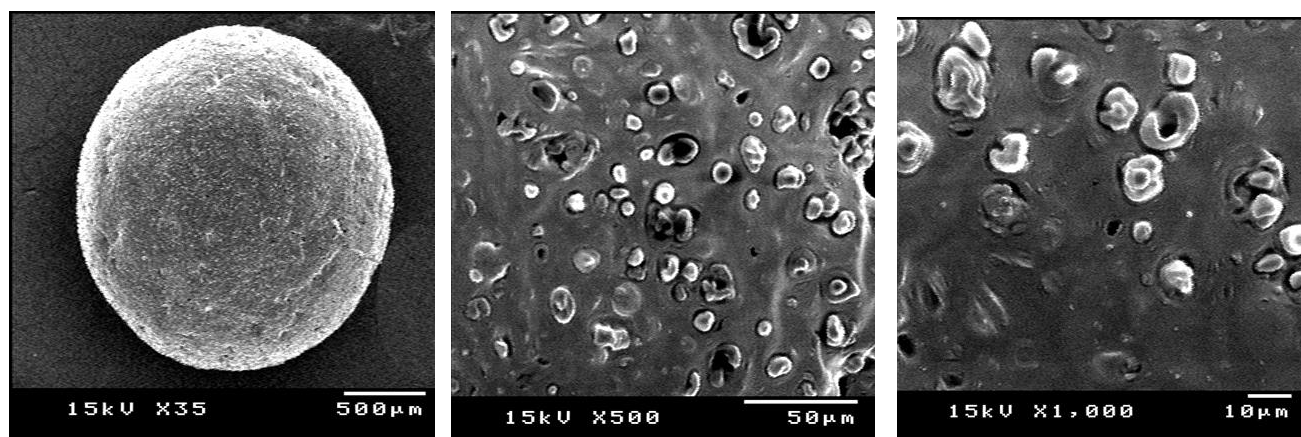


Fig. 4 SEM images of the bead surface of optimized CXB oil embedded floating beads indicating a rough surface with gaps and cavities (F6).

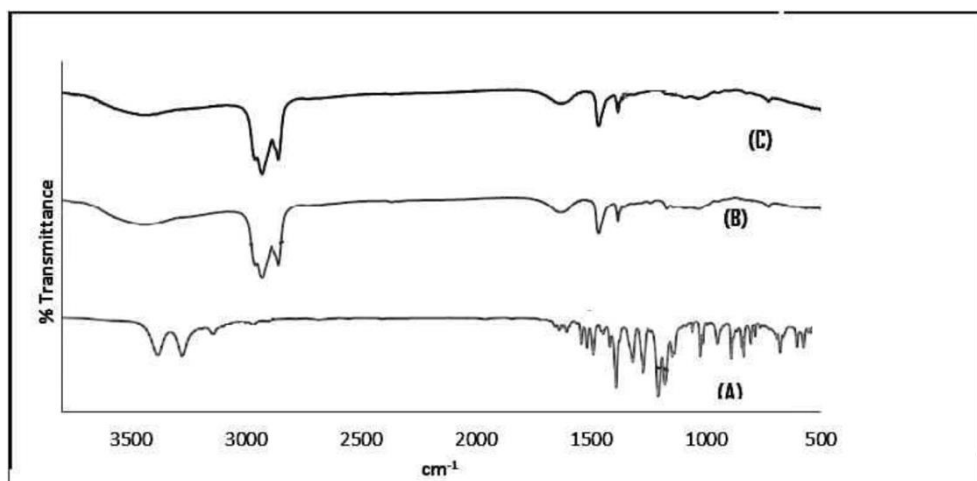


Fig. 5 The FTIR spectra of celecoxib (A), optimized CXB oil embedded beads (B), and blank oil embedded beads (without celecoxib) (C).

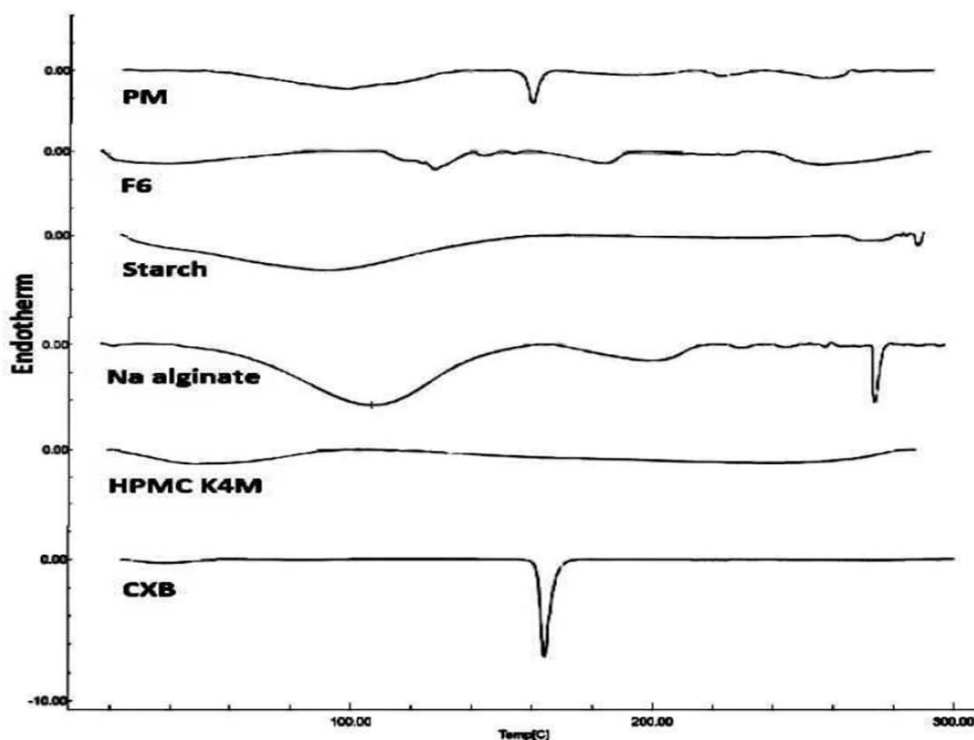


Fig. 6 DSC thermograms of pure celecoxib (CXB), HPMC K4M, Sodium (Na) alginate, Starch, optimized oil-entrapped beads containing celecoxib (F6), and Physical mixture (PM) of celecoxib and all polymers.

3.9 Swelling index of oil entrapped CXB beads :

Figure 7 indicates the swelling behavior of the optimized dried CXB oil entrapped beads (F6) at two different pH levels (1.2 and 6.8). The beads showed a high swelling rate at pH 6.8, while a lower swelling rate was observed at pH 1.2.

The replacement of cross-linking calcium ions with non-gelling Na or K ions (buffer constituents) caused bead swelling at pH 6.8 [50]. This eventually produces a rather flexible structure, and the beads absorb too much water till rupturing occurs and the beads begin to disintegrate.[51]

When exposed to an acidic environment with pH 1.2, the same beads tend to shrink. It was discovered that, at low pH media (< 4), the carboxylate groups of alginates are protonated and shrinkage occurs, causing the internal water to be rejected outside the bead and its weight to decrease .[52]

It was noticed that beads kept their intact form in an acidic pH 1.2 for 4 hours; no change in the beads' shape and no erosion occurred, while in phosphate buffer, pH 6.8, the bead swelled rapidly and remained intact only for 1.5 hours.

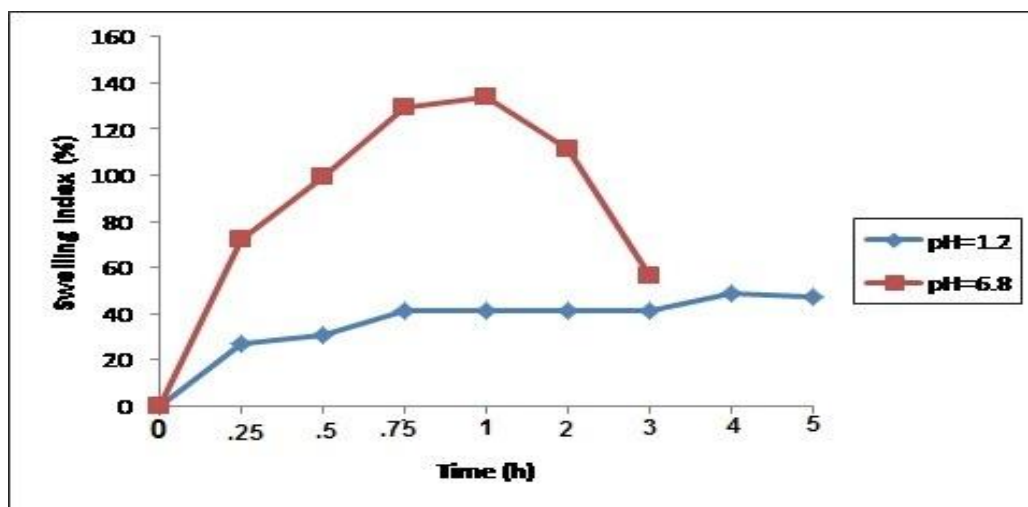


Fig. 7 swelling index of optimized oil entrapped CXB beads (F6) in 0.1N HCL, pH =1.2 and phosphate buffer, pH =6.8.

3.10 In vitro floating properties

All formulations containing celecoxib oil entrapped beads floated well throughout simulated gastric fluid (pH 1.2) with no floating lag time and 100% floatation after 6 hours. The liquid paraffin (embedded low-density oil) in these beads was concerned for capsule floatation by reducing the density of the multi-unit polymeric systems. Furthermore, the buoyancy of these prepared capsules may be explained by the greater gel strength of the polymeric matrices due to hydration and enlargement. Rapid hydration and swelling of the polymeric matrices, resulting in a floating mass in the gastric pH 1.2, also contribute to the buoyancy of these capsules [39]

3.11 In vivo X-ray radio imaging study

The optimized capsules of celecoxib oil embedded floating beads with 50 mg of barium sulfate for each capsule were formulated and tested in vivo for stomach-specific efficiency in rabbits using X-ray imaging. Figure 8 illustrates X-ray photographs. These X-ray images clearly show the existence of the administered floating capsule in the upper GIT. An in vivo X-ray imaging analysis demonstrated that the formulated optimized capsules of celecoxib oil embedded floating beads maintained in the rabbit stomach for an extended period of time, indicating good stomach-specific gastro retention efficiency.

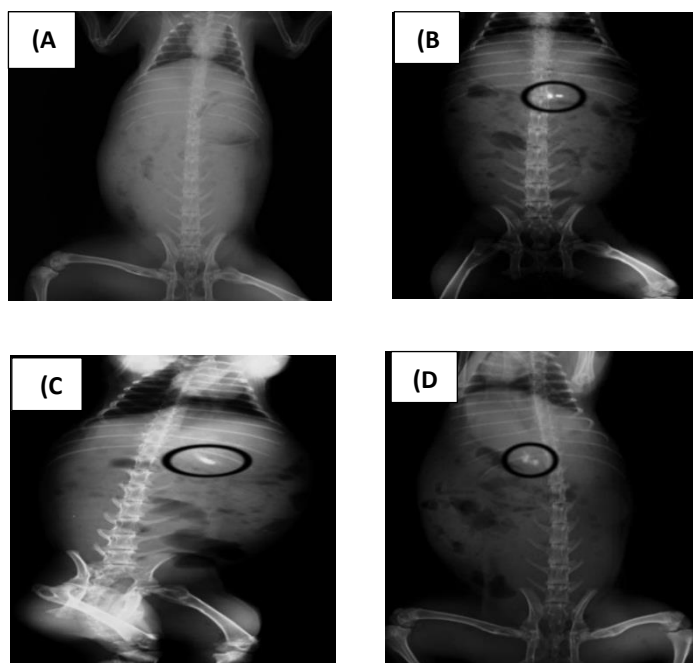


Fig. 8 In vivo X-ray images of optimized capsules of celecoxib oil embedded floating beads in rabbits' gastric area: (A) prior to administration, (B) after 1 hour, (C) after 2 hours, and (D) after 6 hours.

Time (hr)	% Inhibition Group 1 (control)	% Inhibition* Group 2 (Indomethacin®)	% Inhibition* Group 3 (Celeborg®)	% Inhibition* Group 4 (F6)
0.5	—	28.13±1.05	21.14± 0.88	23.86± 2.53
1	—	47.98± 2.31	27.49± 7.50	33.21± 4.71
2	—	87.51± 3.45	49.53± 5.42	69.71± 8.53
3	—	88.54± 7.63	54.54± 4.97	81.77± 9.43
4	—	90.17± 3.75	54.79± 8.51	77.25± 2.51
5	—	89.11± 2.41	31.18± 3.25	84.04± 3.10
6	—	53.81± 0.95	31.14±6.85	92.77± 1.13

Table 5: In vivo anti-inflammatory efficacy of CXB floating beads.

3.12 In-vivo anti-inflammatory efficacy of CXB floating beads

Table 5 illustrates the anti-inflammatory activity between the four groups on the rats' hind paws. Group 4 receives CXB oil entrapped floating beads (F6), which has a significant effect ($P < 0.05$) as an anti-inflammatory formulation. Over the time course studied (1–6 hours), the inhibition percentage was approximately (23.86–92.77%). During the first two hours of observation, CXB oil entrapped floating beads (F6) showed 69.71% inhibition, and Indomethacin® showed 87.51%, while Celeborg® showed 49.53%. After 6 hours, the inhibitory activity of CXB oil entrapped floating beads (F6), Indomethacin®, and Celeborg® formulations were 92.77%, 53.81% and 31.14%, respectively. These results show that CXB oil entrapped floating beads (F6) are more effective in reducing the edema than commercial capsules Indomethacin®, Celeborg®, also the anti-inflammatory effect of CXB oil entrapped floating beads (F6) was sustained for longer time (up till 6h).

Conclusion

In vitro and in vivo studies were studied on stomach-specific capsules containing celecoxib oil entrapped multi-unit floating beads for gastro retentive delivery. The celecoxib oil embedded floating beads were produced using an ionotropical emulsion-gelation technique and polymers such as HPMC K4M, sodium alginate, and maize starch. These low-density beads were encapsulated in vacant hard gelatin capsules (size 1). The influences of HPMC K4M, sodium alginate, and maize starch weights on the EE of celecoxib oil embedded multi-unit floating beads and R6h from stomach-specific capsules of formulated oil embedded floating beads were studied using a 2³ factorial design. These capsules floated for more than 6 hours without a floating lag time. All of these capsules containing celecoxib oil entrapped floating beads demonstrated prolonged sustained drug release in

gastric pH over 6 hours (in simulated gastric fluid, pH 1.2 for the first 2 hours, then shifting pH to 6.8 for the next 4 hours). The in vitro drug release of floating capsules from (F1) to (F4) followed diffusion-controlled release (Fickian diffusion) but from (F5) to (F6) obeyed the anomalous transport (non-fickian diffusion) mechanism controlled by both diffusion and polymeric chain enlargement or relaxation mechanisms due to presence of starch according to Korsmeyer–Peppas model. An in vivo X-ray imaging study in rabbits revealed that the optimized capsules containing floating beads were maintained in the rabbit stomach for an extended period of time, showing good stomach-specific gastro-retention efficiency. An in vivo anti-inflammatory efficacy of optimized CXB floating beads showed sustained drug release and better inhibition of rats' hind paw edema. These formed stomach-specific floating systems may be preferable over traditional oral dosage forms for sustained gastro-retentive drug delivery with enhanced bioavailability and clinical effects.

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