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FORMULATION AND *IN VITRO* EVALUATION OF DICLOFENAC SODIUM *IN SITU* GELLING SYSTEM BY *FENUGREEK* SEED MUCILAGE

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Abstract

Diclofenac sodium has short half-life, frequent oral administration is necessary to maintain its therapeutic concentration, but this can increase chances of missing dose. This makes diclofenac sodium a good candidate for oral sustained release formulation. The aim of this study is to formulate and *in vitro* evaluate floating *in situ* gel of diclofenac sodium. Floating *in situ* gelling system is a liquid dosage form that can sustain the release of diclofenac sodium. It exhibits solution to gel transition phase when triggered by factors such as temperature, pH and presence of ions for cross linking. Method of pH activated ion cross linking was used in this study. Eight diclofenac sodium *in situ* gel formulations F1- F8 were formulated using different combinations of sodium alginate with Fenugreek seed mucilage and HPMC K4M together with other excipients. Eight formulations F1- F8 were evaluated for floating properties and in vitro drug release. Formulation F4 with diclofenac sodium, sodium alginate and Fenugreek seed mucilage in a ratio of 1:1.25:1.2 showed floating lag time of eight seconds and released 51% of drug within eight hours. F4 was selected as ideal formulation among eight formulations. It follows zero order kinetic and fit to Korsmeyer-Peppas model with release exponent of 0.6434, revealed non-fickian diffusion mechanism.

Keywords: Diclofenac sodium, *Fenugreek* seed mucilage, *in situ* gel, cross linking

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Introduction

Among five non-communicable diseases (high blood pressure, diabetes, asthma, coronary heart disease and arthritis), arthritis is the most common one. Statistics revealed that arthritis is more likely to occur in older women than men. It can result from injury, abnormal metabolism, inheritance, infections and immune system dysfunction[1]. According to bio pharmaceutics classification system (BCS), diclofenac sodium is classified under BCS class II active pharmaceutical ingredients. Various studies proposed that diclofenac sodium is a non-steroidal anti-inflammatory drug which has been widely employed in clinical management of inflammatory diseases, including rheumatoid arthritis and osteoarthritis[2]. It works by inhibiting cyclooxygenase-2 enzyme at higher potency compared to cyclooxygenase-1[3]. However, reports revealed that the use diclofenac sodium can lead to side effects like heart burn, diarrhoea, constipation and more[4]. Upon oral administration, it rapidly absorbed from gastrointestinal tract, achieving peak plasma levels within 2 to 3 hours. It has short half-life and high therapeutic index[5]. It eliminated from blood rapidly with half-life of less than three hours[6]. Frequent oral administration is required in order to maintain its drug plasma concentration within therapeutically effective rang e; this would have led to worsening of adverse events. To overcome various complications associated with conventional dosage form, sustained release formulation of diclofenac sodium is developed to reduce dosing frequency and improve patient compliance [3].

Fenugreek is *commonly* used as spice in India[7]. Various investigations discovered that *Fenugreek* can be used as natural treatment for migraine as well as reducing blood glucose levels[8]. Recently, it has been proposed that mucilage from plant seeds have polymerase rheological behaviour of gummy substances, widely used as stabilizers, thickener, emulsifier and foaming agent. One of them is *Fenugreek* seed mucilage, a neutral polysaccharide with pH around 7 [9]. Among various approaches of sustained drug delivery system, *in situ* gel has been the focus of research studies in recent years. *In situ* gel drug delivery system is in solution form. Upon administration, the system transforms from liquid state to gel in stomach[10]. Generally, floating agent will be incorporated into the formulation, causing the resulted gel lighter than gastric fluid, enable it to float on surface of stomach contents to lengthen gastric residence time. This contributes to prolongation of drug release in stomach. Transition from liquid to gel can occur due to various physiological conditions, can be either one or combination of different mechanism such as pH change, ion cross linking or temperature modulation[11]. pH activated ion cross linking method was employed in present study. Diclofenac sodium *in situ* gelling system was formulated to sustain the release of diclofenac sodium and liquid state of the formulation can ease the administration in patients with difficulty in swallowing. Resulting formulations were evaluated with several parameters.

Materials and Methods

The major drug diclofenac sodium salt was purchased from Sigma Aldrich chemicals Ltd. Hydroxy propyl methylcellulose K4M, sodium alginate and all other chemicals were purchased from Sigma Aldrich chemicals Ltd. All the solvents and chemicals used in this study were of analytical-reagent grade. Deionized double distilled water was used throughout the studies.

Isolation of *Fenugreek* seed mucilage

200 g of *Fenugreek* seeds were soaked in 1.5 L of distilled water at room temperature for a period of 24 hours. Then, it was boiled in water bath until slurry was formed. The slurry was cooled at room temperature. Upper clear solution was decanted and concentrated at 60 °C by water bath to one-third of its original volume. Solution was left to be cooled at room temperature, thrice the volume of acetone was added with continuous stirring. Resulting precipitate was rinsed repeatedly with acetone and was left to be dried at room temperature for 24 hours. Dried mucilage was grinded into powder form. Mucilage powder was sieved through sieve number 80 and stored in desiccators [13].

Evaluation of *Fenugreek* seed mucilage

Physicochemical properties of *Fenugreek* seed mucilage

The isolated *Fenugreek* seed mucilage was assessed for organoleptic properties such as colour, odour, texture and pH [14].

Percentage yield

Yield of *fenugreek* seed mucilage was calculated by the formula [15].

Percentage of yield = Practical yield x 100 /Theoretical yield

Swelling index

1 g (W1) of *Fenugreek* seed mucilage was placed in crucible dish. 10 ml of distilled water was introduced. The content was shaken vigorously and left to stand for 1 hour. Excess water in crucible dish was discarded[15]. Weight of content (W2) was measured and recorded. Percentage of swelling index was calculated by:

Swelling index % = (final weight – initial weight / initial weight) x 100

Were, W1: initial weight and W2: final weight

Solubility

Fenugreek seed mucilage was introduced into different types of solvent like water, methanol and chloroform with continuous stirring. Solubility of mucilage in each solvent was recorded [15].

Loss on drying

1 g of *Fenugreek* seed mucilage was heated in hot air oven at 105 °C until constant weight was obtained. Percentage of loss on drying of *Fenugreek* seed mucilage was calculated with the formula of weight of water in mucilage divided by weight of dried mucilage and times with hundred [16].

Bulk density

50 g of *Fenugreek* seed mucilage was weighed and filled into a graduated cylinder. Ratio of mucilage weight to mucilage volume was calculated [15].

Tapped density

Fenugreek seed mucilage was filled into cylinder up to 50 ml line. The cylinder was attached to tap density apparatus and was run for 150 taps. Ratio of weight of mucilage to final volume of mucilage was calculated [15].

Preparation of diclofenac sodium *in situ* gel

Fixed concentration of sodium alginate (1.25% w/v) were prepared in distilled water containing 0.2% w/v calcium chloride and 0.5% w/v sodium citrate, the solution was heated to 60°C with stirring and cooled to below 40°C. In another beaker, *Fenugreek* seed mucilage was added to distilled water with continuous stirring. *Fenugreek* seed mucilage solution was then introduced to sodium alginate solution. 1% w/v diclofenac sodium, 1% w/v sodium bicarbonate, 0.02% w/v propyl paraben, 0.18% w/v methyl paraben, 0.06% w/v saccharin sodium and tartrazine were added. Final volume was made up to 100 ml with distilled water. Procedure was repeated by using different concentration of *Fenugreek* seed mucilage. For formulations contained hydroxypropyl methylcellulose (HPMC K4M), steps were repeated by replacing *Fenugreek* seed mucilage with HPMC K4M [17]. The formulations of diclofenac sodium in situ gel F1- F8 were tabulated in Table 1.

Evaluation of diclofenac sodium *in situ* gel

The general appearance, colour and odour of formulation were physically visualised and recorded and the pH was determined by using digital pH meter [18].

***In vitro* floating test**

10 ml of *in situ* gel solution was introduced into 500 ml of 0.1N hydrochloric acid. Time taken for the formulation to float on the surface after addition of formulation (floating lag time) and total floating time were measured and recorded [19].

Drug content estimation

10 ml of formulation that composed of 100 mg of diclofenac sodium was introduced to 50 ml of 0.1N hydrochloric acid in conical flask and was stirred at high speed for a period of 30 minutes. 0.1N hydrochloric acid was used to make up the volume to 100 ml. Content was filtered by Whatman filter paper. Then, 10 ml of sample was withdrawn from filtrate and diluted with 0.1 N hydrochloric acid. Drug concentration was determined by using UV spectrophotometer against blank solution at wavelength of 276 nm [20].

***In vitro* drug release test**

In vitro drug released test was performed by using USP dissolution apparatus with paddle at speed of 50 rpm. 900 ml of a 0.1N hydrochloric acid was used as dissolution at 37°C. 10 ml of formulation sample was withdrawn by using a disposable syringe. The sample was introduced gently into a petri dish and it was immersed into the dissolution medium without much turbulence. At 1 hour intervals, 10 ml sample of the dissolution medium was accurately measured and withdrawn. Same amount of 37°C fresh medium was then replaced. Absorbance of the sample was measured at 276 nm by using a UV spectrophotometer [21].

Gelling capacity

500 ml of 0.1N hydrochloric acid was poured into a beaker. Then, 10 ml of formulation was accurately measured and added to the hydrochloric acid with mild agitation. The gel formed in beaker was observed visually and recorded in terms of strokes depending on the pattern of gel formed [17].

Drug release kinetic profile

Various kinetic models were performed to analyse kinetic profile of dissolution. Values of formulations that influence rate controlling mechanism which was determined [22].

Zero order kinetic model described drug release rate which is independent of its concentration of dissolved substances.

$Q_t = Q_o + K_o t$

Where, Q_t = cumulative drug release at time "t", Q_o = initial drug amount, K_o = zero order release constant, t = time (hours)

First order kinetic described absorption and/or clearance of drug. Release rate of drug depends on concentration.

$LogC_t = LogCo - kt / 2.303$

It has been observed that diclofenac sodium *in situ* gel contained *Fenugreek* seed mucilage (F1- F4) were light brown in colour and were odourless whereas diclofenac sodium *in situ* gel that contained HPMC K4M (F5- F8) were yellow in colour. Resulted formulations showed good pourability. Various other parameters such as pH,

Where, C_o = initial concentration of drugs, also indicates first order reaction constant.

Higuchi's model described kinetic profile of different geometric and porous drug delivery system which obeys Fick's law. Drug release that calculated in time per unit area was plotted against square of time.

$Q = K_H t^{1/2}$

Where, K_H = Higuchi dissolution constant to identify diffusion controlled process.

Korsmeyer-Peppas model determined drug release mechanism of particular dosage form either by fickian or non-fickian [22].

$Log (M_t / M\infty) = Log k + n Log t$

Where, $M_t / M\infty$ = release of drug at time t, n = release exponent that indicate release mechanism manipulated by formulation's polymer, K= kinetic constant.

Results and Discussion

Fourier transform infrared spectroscopy for diclofenac sodium, *Fenugreek* seed mucilage, the combination of diclofenac sodium, sodium alginate and *Fenugreek* seed mucilage, the combination of diclofenac sodium, sodium alginate and HPMC K4M were presented in Table 2 and Figure 1 to 4 respectively. Interpreted results of FTIR were summarised in Table 2. Peak frequency of substances in unit of cm^{-1} revealed specific vibration and bonds of compounds. Several peaks of each substance were chosen in order to investigate compatibility. Results revealed that there was no overlapping of peak value between combinations of ingredients, indicated that there is absence of interaction.

Evaluated parameters including organoleptic properties, yield, swelling index, solubility, loss on drying, bulk density, tapped density and pH of *Fenugreek* seed mucilage were summarised in Table 3.
floating time and gelling capacity were tabulated in Table 4.

As presented in Table 4, pH of developed formulations was found to be in a range of 7.2 to 7.8. This indicated that all formulation was neutral and slightly alkaline in nature. Sodium alginate as the main polymer in present study is anionic in nature composed of alpha L guluronate (G) residues, known as G blocks. These G blocks function as a crucial group for gelation process in the presence of divalent cations such as calcium ions. When *in situ* gel expose to acidic condition, free calcium ions will be released upon breakage of complexes between calcium chloride and sodium citrate, the free calcium ion will be entrapped in polymeric chain of sodium alginate, lead to intermolecular cross linking and formation of double helical junction zone. As a result, three dimensional matrices of gel will be formed. Hence, pH plays a crucial role to maintain fluidity of formulation. *In situ* gel should not be acidic before administration. Optimum concentration of calcium chloride can ensure better stiffness of gel[14]. It has been proposed that calcium chloride should be in the range of 0.1% w/v to 0.2% w/v. Hence, 0.2% w/v of calcium chloride had been incorporated into present study. Observations revealed that all formulation exhibited good gelling capacity; gels formed immediately in simulated gastric acid and remained for extended period. Resulted gels floated for more than 24 hours, ideal to provide sustained release of drug over 24 hours.

Floating lag time varies for each formulation. Optimum concentration of sodium bicarbonate for floating properties of formulation should be 1% w/v in order to give floating lag time of less than 30 seconds[14]. The same amount of sodium bicarbonate was incorporated in every formulation. All formulations exhibited good floating lag time which was less than 24 seconds. Result showed a decline of floating lag time from F1 to F4. As concentration of *Fenugreek* seed mucilage increased, formulation took shorter time to float. The reason for this may be attributed to better formation of gel matrix when *Fenugreek* seed mucilage increased, caused formulation to be more buoyant when contacted with hydrochloric acid. Apparently, floating lag time should be prolonged when amount of HPMC K4M in formulations was increased[22]. However, F5 – F8 had floating lag time of fifteen seconds, twelve seconds, ten seconds and nine seconds respectively, present study revealed that floating lag time was inversely proportional to concentration of HPMC K4M. HPMC K4M played an important role in the formation of three-dimensional network matrices of gel. According to Madan, J.R. et al (2015), optimum concentration of sodium bicarbonate for floating properties of formulation should be 1% w/v to give floating lag time of 24 seconds. Thus, increased HPMC K4M may have led to formation of gel in shorter time and gave shorter floating lag time. Among the eight formulations, F4 exhibited best floating properties which shown in Figure 5.

Drug content estimation of diclofenac sodium *in situ* gel was within the range of 89 to 96%.

Eight hours of *in vitro* dissolution test of diclofenac sodium *in situ* gel was performed to determine drug release pattern. Various studies proposed that increased amount of *Fenugreek* seed mucilage will prolong release of drug from formulation[23]. From F1 to F4, percentage of *Fenugreek* seed mucilage included in formulation was increased from 0.6% w/v to 0.8% w/v, 1.0% w/v and 1.2% w/v respectively. Present investigation showed that the percentage cumulative drug release of F1- F4 at eighth hour was in the range of 51 to 90% respectively. Drug release was better controlled when percentage of *Fenugreek* seed mucilage increase.

Viscosity enhancing polymer plays an important role in forming cross linking chain of gel, higher concentration of viscosity enhancing agent allowed more polymeric chains for cross linking in three-dimensional matrix of gel and hence reduce cumulative drug release, provide more sustained release of drug. In present study, concentration of HPMC K4M increased from F5- F8, led to significant reduction in drug release. It was observed that the percentage cumulative drug released at eighth hours for the four formulations were 90, 76, 63% and 59% respectively. F8 with highest amount of HPMC K4M exhibited better sustained drug release among the four formulations.

Formulation with 0.6% w/v of *Fenugreek* seed mucilage (F1) and 0.6% w/v of HPMC K4M (F5) released 89% and 90% of drug respectively at eighth hour. F1 showed slightly better sustained release of drug compared to F5. F2 and F6 contained 0.8% w/v of *Fenugreek* seed mucilage and HPMC K4M respectively. F2 released 71% of drug whereas F6 released 76% of drug at the end of dissolution test. Formulation with *Fenugreek* seed mucilage (F2) exhibited better sustained drug release. However, F3 that contained 1.0% w/v of *Fenugreek* seed mucilage released more drug at eighth hour compared to F7 that contained 1.0% w/v of HPMC K4M, F7 proposed a better sustained drug release property. F4 with increased concentration of *Fenugreek* seed mucilage exhibited drug release of 51% whereas F8 that contained increased amount of HPMC K4M released 58% of drug within eight hours. Summarisations of *in vitro* dissolution of F1 to F8 were presented in Table 5 and Figure 6.

Kinetic profiles of F4 have been presented in Table 6 and Figure 7 – 10. Regression correlation (R2) and release constant (k0) in zero order kinetic profile showed a value of 0.9758 and 0.1098 respectively. First order kinetic

described release rate of drug is dependent of concentration. Table 4.20 revealed regression correlation (R2) of first order kinetic was 0.7194 and first order release constant (k1) shown to be 0.0028. Regression correlation from zero order kinetic was closer to 1.0000 compared to first order kinetic indicated that F4 was more likely to follow zero order kinetic. Higuchi's model determined drug release of different geometric and porous drug system by obeying Fick's law. In this study, Higuchi's model of F4 showed regression correlation of 0.9383 with Higuchi constant of 2.5410. However, F4 had greater regression correlation for zero order kinetic when compared to Higuchi's model, so, F4 was more likely to follow zero order drug release kinetic. For Korsmeyer-Peppas, drug release mechanism was described by value of release exponent (n). When release exponent is equal to 0.5, the release of drug will be obeying Fickian diffusion. Korsmeyer-Peppas model of F4 showed release exponent of 0.6434 which was in the range of 0.4500 to 0.8900, revealed that F4 followed non-Fickian diffusion, drug released through polymeric matrix erosion. Overall, study revealed that formulations contained *Fenugreek* seed mucilage provided more sustained release of drug compared to formulation contained HPMC K4M. Present investigation showed that F4 is preferred over seven other formulations, F4 had ideal properties of sustained drug release formulation to provide once a day dosing to achieve objective of this study.

Conclusion

Ratio of polymer and thickening agent played essential part in manipulating drug release rate of formulations. Formulation F4 with diclofenac sodium, sodium alginate and *Fenugreek* seed mucilage in a ratio of 1: 1.25: 1.2 was chosen as optimum formulation by virtue of its too good floating properties more than 24 hours and better sustained release of drug compared to seven other formulations. . It follows zero order kinetic and fit to Korsmeyer-Peppas model with release exponent of 0.6434, revealed non-fickian diffusion mechanism. The principle of *in situ* gel preparation offers practical approach to achieve sustained release of drug.

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Table 1. Formulation of diclofenac sodium *in situ* gelling system

Materials (g)	F1	F2	F3	F4	F5	F6	F7	F8
Diclofenac sodium	1	1	1	1	1	1	1	1
Sodium alginate	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Fenugreek seed mucilage	0.6	0.8	1.0	1.2	-	-	-	-
HPMC K4M	-	-	-	-	0.6	0.8	1.0	1.2
Sodium bicarbonate	1	1	1	1	1	1	1	1
Sodium citrate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Calcium chloride	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Saccharin sodium	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Methyl paraben	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Propyl paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Distilled water (q.s.) ml	100	100	100	100	100	100	100	100

Table 2. Results of FTIR

Diclofenac sodium	
483.33 to 769.55 cm^{-1}	C-H bending
1452.30 to 1603.84 cm^{-1}	Aromatic C=C stretching
***Fenugreek* seed mucilage**	
1015.97 cm^{-1}	Carbocylic C-O stretching
3286.14 cm^{-1}	Alcoholic O-H stretching
Diclofenac sodium + sodium alginate + *Fenugreek* seed mucilage	
563.12 cm^{-1}	C-H bending
1029.78 cm^{-1}	C-O stretching
1414.93 cm^{-1}	C-H stretching
1631.83 cm^{-1}	Alkene C=C stretching
3307.75 cm^{-1}	Alcoholic O-H stretching
Diclofenac sodium + sodium alginate + HPMCK4M	
594.81 cm^{-1}	C-H bending
1365.9 cm^{-1}	C-H stretching
1634.68 cm^{-1}	C=C stretching
3306.72 cm^{-1}	Alcoholic O-H stretching

Table 3. Summarisation of evaluation for *Fenugreek* seed mucilage

Parmeters	Results
Organoleptic properties	Light brown, odourless, rough texture
Yield	3%
Swelling index	Method one: 30 ml, Method two: 735%
Solubility	Swell in distilled water, insoluble in chloroform and methanol
Loss on drying	8.70%
Bulk density	0.6 g/ ml
Tapped density	0.7 g/ ml
pH	6.25

Table 4. General properties of diclofenac sodium *in situ* gel

Formulation	pH	Floating lag time (seconds)	Floating time	Gelling capacity
F1	7.2	18	More than 24 hours	+++
F2	7.4	14	More than 24 hours	+++
F3	7.3	10	More than 24 hours	+++
F4	7.6	8	More than 24 hours	+++
F5	7.8	15	More than 24 hours	+++
F6	7.2	12	More than 24 hours	+++
F7	7.7	10	More than 24 hours	+++
F8	7.2	9	More than 24 hours	+++

(+): Gel formed after few minutes, dispersed rapidly.
(++): Gel formed immediately and remained for few hours.
(+++): Gel formed immediately and remained for extended period.

Table 5. Summarisation of *in vitro* dissolution of F1 to F8

Time	Formulations							
	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
60	25.1	15.5	20.3	10.5	27.3	21.3	16.6	12.0
120	36.6	32.4	27.1	14.1	34.3	33.3	25.5	16.9
180	51.6	42.7	34.7	21.2	47.1	40.6	34.8	21.8
240	62.1	49.0	42.1	31.8	56.2	51.7	38.7	29.1
300	71.6	53.8	45.7	40.2	66.8	55.3	44.5	38.8
360	76.6	60.1	50.4	44.1	74.7	62.7	49.3	46.3
420	83.8	65.5	58.4	47.6	84.0	70.1	56.5	53.7
480	89.7	71.0	63.4	51.2	90.2	76.4	62.6	58.8

Table 6. Kinetic profiles of F4

Zero order		First order		Higuchi model		Korsmeyer-Peppas model		Mechanism of drug release
R^2	K_0	R^2	k_1	R^2	K ($min^{-1/2}$)	R^2	n	
0.9758	0.1098	0.7194	0.0028	0.9383	2.5410	0.9828	0.6434	Non-fickian

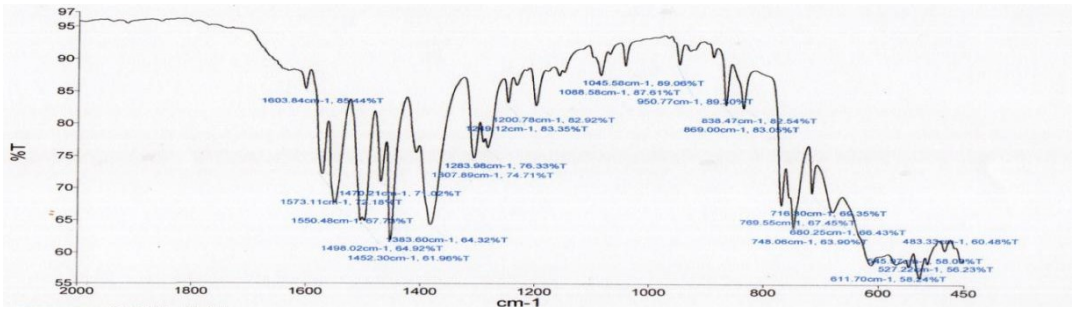

Figure 1. Fourier Transform Infrared Spectroscopy result of diclofenac sodium

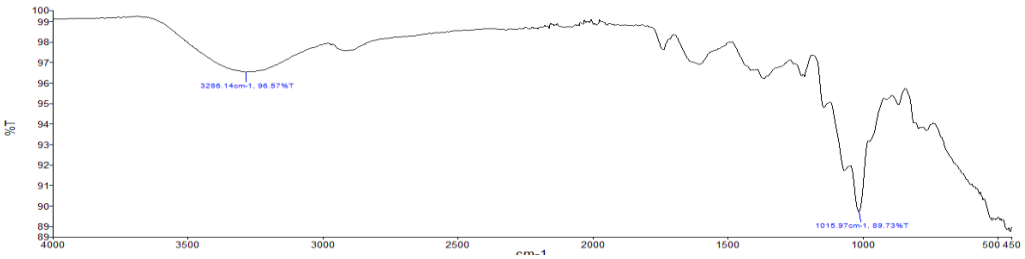

Figure 2. Fourier Transform Infrared Spectroscopy result of *Fenugreek* seed mucilage

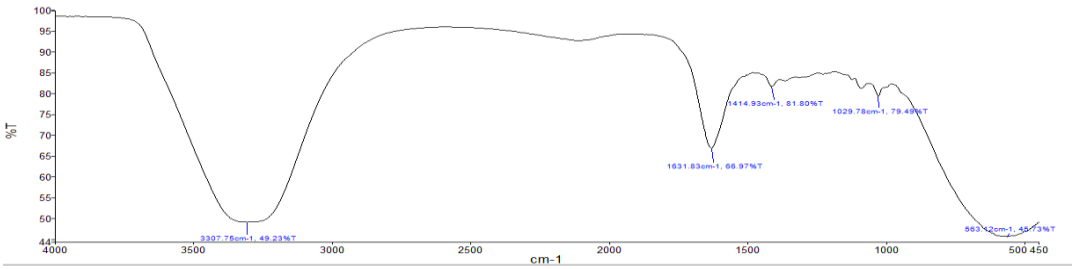

Figure 3. Fourier Transform Infrared Spectroscopy result for combination of diclofenac sodium, sodium alginate and *Fenugreek* seed mucilage

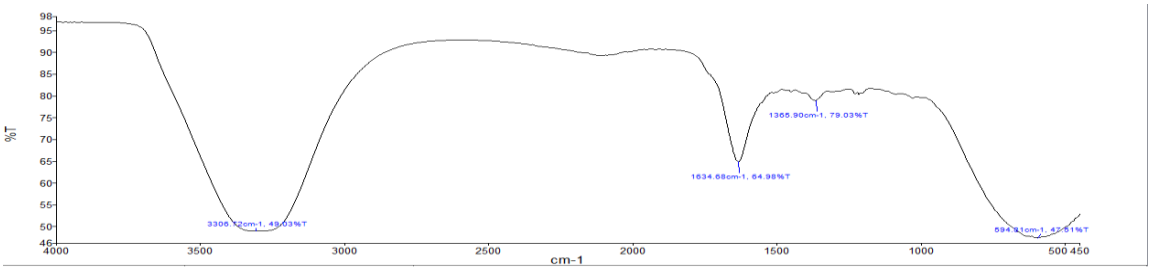

Figure 4. Fourier transform infrared spectroscopy result for combination of diclofenac sodium, sodium alginate and HPMC K4M

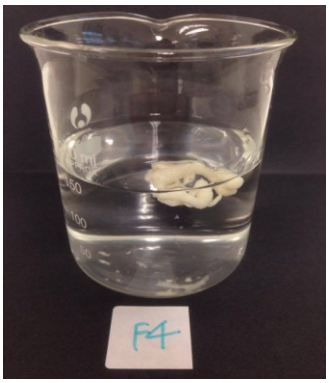

Figure 5. Diclofenac sodium *in situ* gel formulation F4 after 24 hours

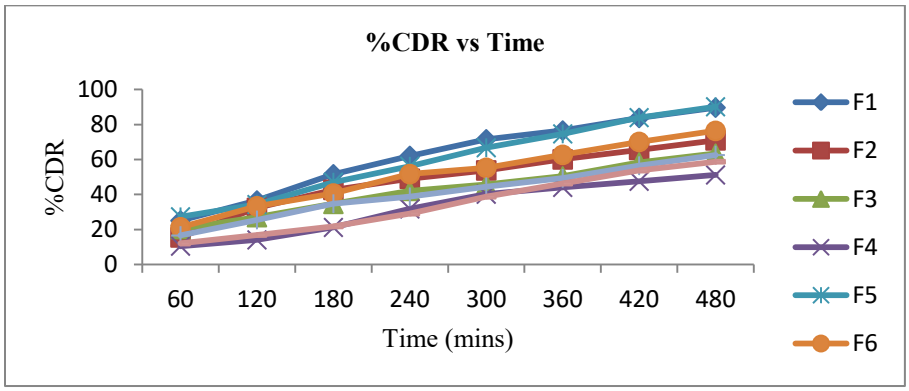

Figure 6. Summarisation of *in vitro* dissolution of F1 to F8

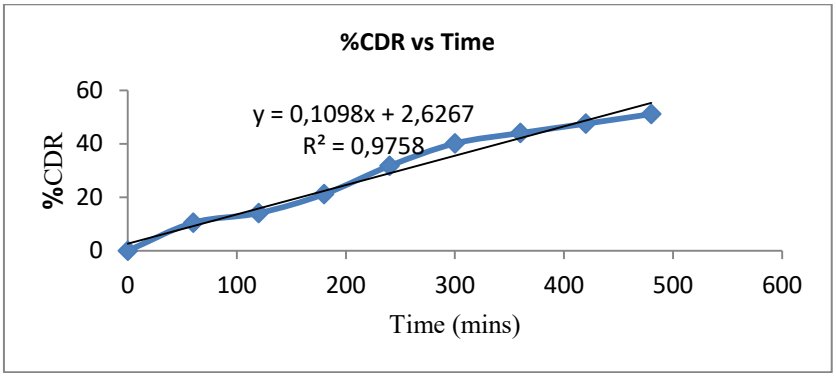

Figure 7. Zero order release of F4

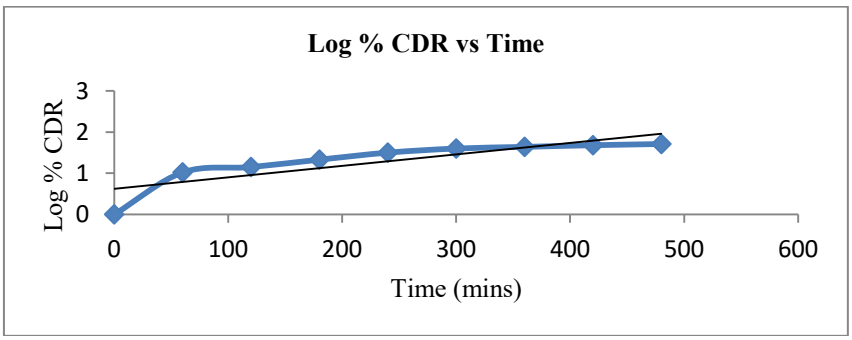

Figure 8. First order release of F4

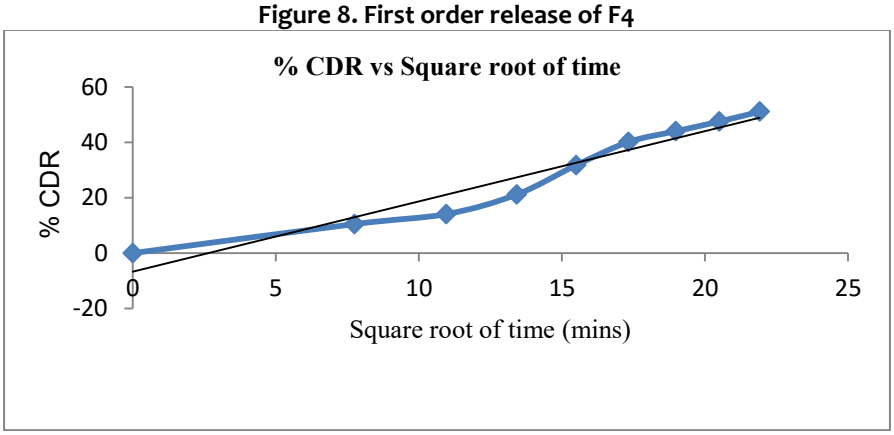

Figure 9. Higuchi model of F4

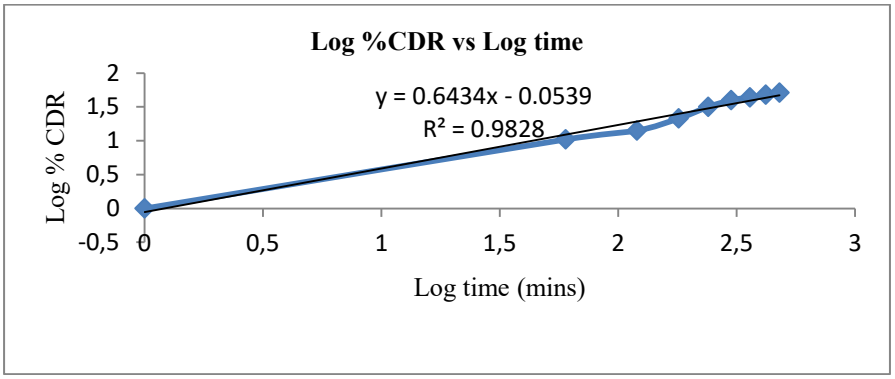

Figure 10. Korsmeyer-Peppas model of F4