Formulation and evaluation of ciprofloxacin suspension using natural suspending agent

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Abstract

The aim of the present study is to formulate and evaluate ciprofloxacin suspension using natural suspending agent. *Trigonella foenum graecum* Mucilage was used as natural suspending agent. Total 9 batches (C1-C9) were prepared by varying concentration of suspending agent from 0.5-2% and propylene glycol. Prepared suspension were evaluated by studying different parameters like pH, sedimentation volume, redispersibility, Flow rate (F), viscosity, degree of flocculation, effect of temperature etc. batches C6, C7 and C8 were found to be stable throughout the study. As the concentration of suspending agent increased viscosity also get increased which reduces the sedimentation and contributes to the stability of suspension. Increase in viscosity avoids the particle aggregation so particles remain in a flocculated state.

Keywords: Ciprofloxacin, Suspension, Sedimentation volume, redispersibility, flocculation

1. Introduction

In recent era, oral drug delivery is most prominent route amongst all other routes of drug administration [1]. Since long years, an oral pharmaceutical suspension has been one of the most preferable dosage forms for pediatric patients or patients incapable to tolerate solid dosage forms [2, 3]. A Pharmaceutical suspension is a coarse dispersion in which internal phase is dispersed uniformly throughout the external phase [4]. Suspension is thermodynamically unstable, so it is necessary to add suspending agent which reduces the rate of settling and permits easy redispersion of any settled particulate matter both by protective colloidal action and by increasing the consistency of the suspending medium [5, 6].

Natural suspending agents are mainly used as they are biodegradable and biocompatible [7]. Use of natural suspending agents likes okra gums [8], *Abelmoschus Esculentus* Mucilage [5], *Cassia tora* Mucilage[9] and *Trigonella foenum graecum* Mucilage [10] to formulate suspension of Active pharmaceutical agents, has been previously reported. *Trigonella foenum graecum* is an annual plant with leaves consisting of three small obovate to oblong leaflets [11]. Cuboid-shaped, yellow-to-amber colored fenugreek seeds are a common ingredient in the dishes from the Indian Subcontinent, used both whole and powdered in the preparation of pickles, vegetable dishes, daals, and spice mixes such as panch phoron and sambar powder [12, 13].

Ciprofloxacin (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl) 3 quinoline carboxylic acid) is a second-generation fluoroquinolone antibiotic . It is a faintly yellowish to light yellow crystalline substance and its chemical structure is as in figure 1 [14, 15],

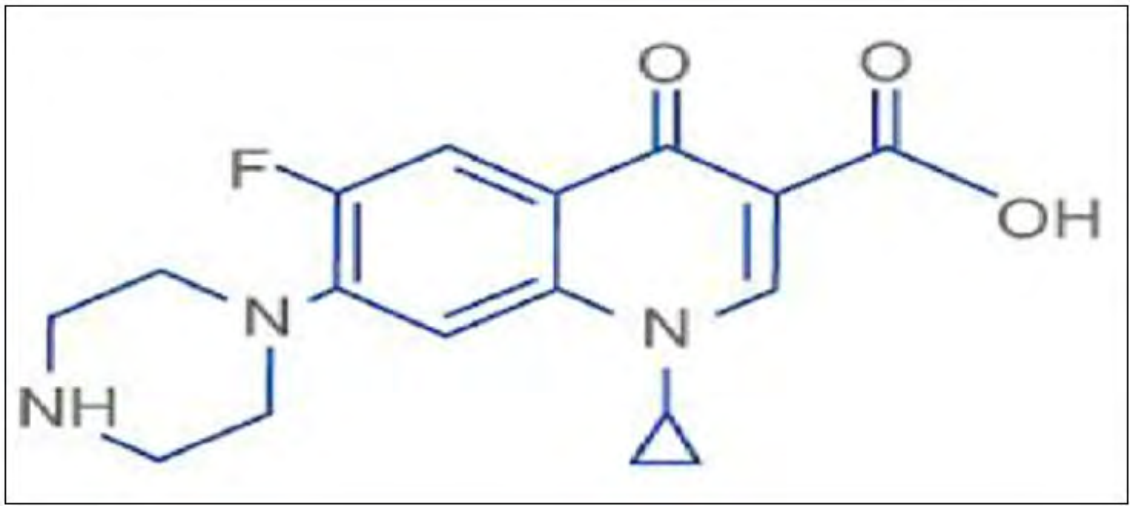

Figure 1. Structure of Ciprofloxacin hydrochloride

Its spectrum of activity includes most strains of bacterial pathogens mainly responsible for respiratory, urinary tract, gastrointestinal, and abdominal infections. It is helpful against some Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Moraxella catarrhalis*, *Proteus mirabilis*, *Haemophilus influenzae*, and *Pseudomonas aeruginosa*), and Gram-positive (methicillin-sensitive but not methicillin-resistant *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, and *Streptococcus pyogenes*) bacterial pathogens [16].

2. Materials and Methods

2.1. Materials

Ciprofloxacin were obtained as gift sample. Fenugreek seeds, Pine-apple flavour and tartrazine were purchased from local market. All other solvents used were of analytical grade.

2.2. Methods

2.2.1 Extraction of suspending agent from Trigonella foenum graecum (Seed):

Initially seeds of *Trigonella foenum graecum* were crushed and reduced in size using ball mill. The crushed seeds were soaked in distilled water for 12 hrs and boiled in water bath to prepare slurry. Further slurry was cooled and allows settling down unwanted material. Upper portion was collected and concentrated in water bath and after cooling acetone was added in it with continuous stirring. The precipitate was collected and dried at room temperature for 24 hrs. The air dried material further subjected to size reduction and passed through sieve no. 60 and stored in desiccators for further evaluation [17, 18].

3. Evaluation of mucilage

3.1. Determination of Swelling Index:

500 mg of isolated mucilage was taken in a Petri dish and then 10 ml of distilled water was added and the mixture was shaken and allowed to stand for 1 hour. After 1 hour the remaining water in Petri dish was discarded and the weight increase of the isolated mucilage was determined [19].

Swelling Index % (SI) = (W2 – W1/W1) x 100 ---------- (1)

W1= Weight of compact at time ‘0’

W2= Weight of compact t at time ‘t’

3.2. Phytochemical screening of mucilage

Preliminary tests were performed to confirm the nature of mucilage obtained. The chemical tests that were conducted are: Molisch’s test, Ninhydrin test, Wagner’s test, Ruthenium red test, Iodine test, Shinoda test, Keller-Killaini test and Ferric chloride test [20].

Table 1: composition of ciprofloxacin suspension

Ingredients	Batch Code							
	C1	C2	C3	C4	C5	C6	C7	C8
Ciprofloxacin (g)	2	2	2	2	2	2	2	2
Mucilage (g)	0.5	0.5	1	1	1.5	1.5	2	2
Glycerin (ml)	5	10	5	10	5	10	5	10
Propyl paraben (mg)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Methyl paraben (mg)	5	5	5	5	5	5	5	5
Aspartame (mg)	100	100	100	100	100	100	100	100
Pineapple flavour (ml)	1	1	1	1	1	1	1	1
Tartrazine (mg)	1	1	1	1	1	1	1	1
Purified water (q.s)	100	100	100	100	100	100	100	100

3.3. Micromeritic properties of mucilage

3.3.1. Bulk density & tap density

Accurately weighed mucilage was poured in 100 ml graduated cylinder. The volume occupied by mucilage, before (Vb) and after tapping (Vt) were determined in triplicate using bulk density apparatus (Lab Hosp, Mumbai, Maharashtra, India). The bulk density and tap density was calculated using the formulas [21, 22],

$$\rho b = \frac{M}{Vb} \dots\dots\dots\dots\dots (2)$$

$$\rho t = \frac{M}{Vt} \dots\dots\dots\dots\dots (3)$$

3.3.2. Angle of Repose, Carr's Compressibility Index (CCI) and Hausner's Ratio (HR)

Angle of Repose, (CCI) and (HR) were determined using following equations [21, 22]

$$\theta = Tan^{-1}\, \mathrm{H/R} \dots\dots\dots\dots\dots (4)$$

Where, 'θ' is angle of repose; 'H' is height between lower tip of the funnel and the base of heap of powder; and 'R' is radius of the base of heap formed

$$CCI = \frac{TD - BD}{TD}\, X\, 100 \dots\dots\dots\dots\dots (5)$$

$$HR = \frac{TD}{BD} \dots\dots\dots\dots\dots (6)$$

Where, TD and BD are tapped density and bulk density respectively.

4. Formulation of suspension

Suspension was prepared as per formula given in table 2. Fenugreek husk powder was taken in mortar to which methyl and propyl paraben were added and triturated for some time along with water to make paste. In beaker ciprofloxacin was mixed well with glycerin. This mixture was further added to the above paste and triturated for 20 min. Then colouring agent i.e. tartrazine and flavouring agent (Pine-apple flavour) were added mixed well in suspension. Volume made up with water upto 100 ml and further homogenized.

5. Evaluation of suspension

5.1 pH determination

The pH of all developed formulations was measured using digital pH meter.

5.2 Sedimentation volume

Sedimentation volume is determined by following equation[22],

$$F = \frac{Hu}{Ho} \ldots\ldots\ldots\ldots (7)$$

Where, Hu is ultimate or final height of sediment as suspension settles, Ho is original height of suspension.

5.3. Redispersibility

Fixed volume of each suspension (50 ml) was kept in calibrated tubes which were stored at room temperature for various time intervals (1, 5, 10, 15, 20, 30, 45 days). At regular interval one tube was removed and shaken vigorously to redistribute the sediment and the presence of deposit if any was recorded[23].

5.4. Flow rate (F)

The time taken for 10ml sample of suspension to flow through a 10ml pipette was determined and the flow rate calculated using the following equation:

$$F = Volume\ of\ pipette\ (ml)/Flow\ time\ (sec) \ldots\ldots\ldots\ldots (8)$$

5.5. Determination of viscosity

The viscosity of suspension samples was determined using the Brookfield viscometer at 100 rpm. All determinations were carried out in at least triplicates and results obtained were expressed as the mean values.

5.6. Degree of flocculation

Degree of flocculation (β) was determined using following equation[22],

$$\beta = \frac{(Vu)floc}{(Vu)defloc} \ldots\ldots\ldots\ldots (9)$$

Where, *(Vu)floc* is ultimate sedimentation volume in flocculated suspension and *(Vu)defloc* is ultimate sedimentation volume in deflocculated suspension.

5.7. Effect of temperature

Further, the effect of the temperature (30^o to 60^o) was investigated on the viscosity of the suspension of all formulations.

5.8. Drug content

10 ml of suspension (20mg/ml) was accurately measured and transferred into 100 ml volumetric flask. And volume made up with 0.1 N HCl. Further from above suspension 1 ml was withdrawn and added to 10 ml flask, volume made with 0.1 N HCl. Absorbance was measured using UV-Visible double beam spectrophotometer (Jasco V-530) at λ max 280 nm. Drug content was calculated by comparing the absorbance with standard curve.

5.9. Particle size measurement

Particle size determination is carried out by optical microscopy method using motic microscope. Suspension was spread on slide & observed under microscope. Diameters of 20 particles were measured.

5.10. In vitro dissolution studies

Dissolution study of formulated suspensions (n=3) was carried out in USP type II dissolution test apparatus (TDT 08 L, Electrolab, Mumbai, India) in 500 ml of water for 30 min (37 ± 0.5^0C and 25rpm). USP type II dissolution test apparatus although mainly designed for tablets and capsules, this apparatus has also been used by several investigators to study the dissolution behaviour of suspensions.[24, 25] 10 ml suspension was introduced carefully into the bottom of the apparatus. 5 ml aliquots were withdrawn at interval of 5 min for analysis and replenished by equivalent amount of blank. The aliquots were filtered through Whatman filter paper and further analyzed at respective wavelength by double beam UV visible spectrophotometer (Jasco V-530 UV). The data obtained were put in PCP Disso V 3.0 (Pune, India) software to type the drug release kinetics.

6. Results and Discussion

6.1 Evaluation of mucilage

6.1.1. Determination of Swelling Index

Swelling index of *Trigonella foenum graecum* was found to be 38% at end of 1 hr. Result shows that the swelling index was found to be increased with time. Swelling index was increased, because weight gain by mucilage was proportional to rate of hydration. The direct relationship was observed between swelling index and mucilage concentration, as mucilage concentration increase swelling index increased.

6.1.2. Phytochemical screening of mucilage

Phytochemical tests carried out on *Trigonella foenum graecum* mucilage confirmed the absence of alkaloids, glycosides starch and tannins. Treatment of mucilage with ruthenium red showed red coloration confirms the obtained product as mucilage. A violet ring was formed at the junction of two liquids on reaction with Molisch's reagent indicating the presence of carbohydrates. The results of phytochemical screening of mucilage are summarized in table 2.

Table 2: Phytochemical screening of Trigonella foenum graecum mucilage

Sr.No	Identification test	Name of test	Observation
1	Test for Carbohydrates	Molisch's test	Positive
2	Test for proteins	Ninhydrin test	Negative
3	Test for alkaloids	Wagner's test	Negative
4	Test for mucilage	Ruthenium red test	Positive
5	Test for starch	Iodine test	Negative
6	Test for flavonoids	Shinoda test	Negative
7	Test for glycosides	Keller-Killaini test	Negative
8	Test for Tannins	Ferric chloride test	Negative

6.1.3. Micromeritic properties of mucilage

Values of Hausner's ratio, CCI Angle of Repose showed that mucilage powder has excellent flow properties. Micromeritic properties of mucilage have been summarized in table 3.

Table 3: Micromeritic properties of Trigonella foenum graecum mucilage

Parameter	Value
Bulk density	0.779 g/ml
Tap density	0.865 g/ml
Hausner's ratio	1.11
CCI	9.942%
Angle of Repose	22.3°

6.2. Evaluation of suspension

Batch C1and C2 was found to be disturbed at initial phase so not consider for further evaluation. This could attribute to low viscosity of the suspension.

6.2.1 pH determination

pH of all formulation was found to be in the range of 7.01 – 7.31.Comparative profile of pH all batches is given in (Fig. 2).

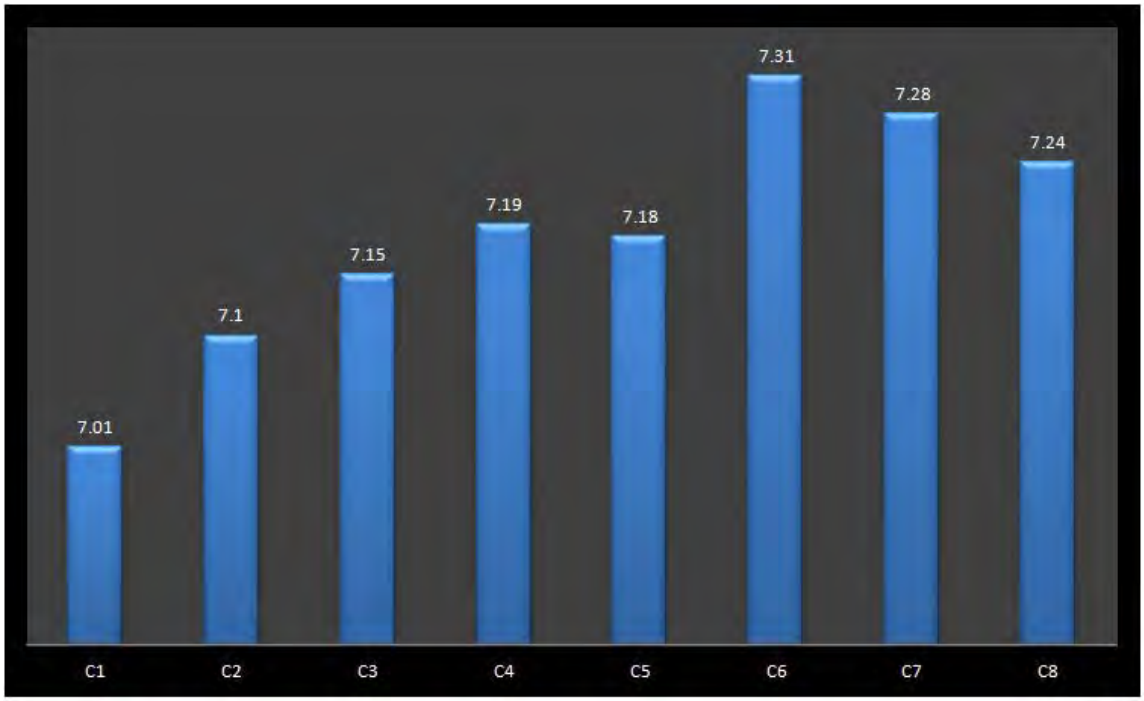

Figure 2. Comparative pH profile for all batches of suspensions

6.2.2. Sedimentation Volume

Sedimentation volume was found to be decreased at the end of 7 days. Result of values of Sedimentation Volume is reported in graph 1. Batch C6, C7 and C8 was found to be stable and dispersed at the end of 45 days. The dispersed particle were sediment at faster rate in suspension containing lower concentration of suspending agent compared to containing higher amount (Fig. 3).

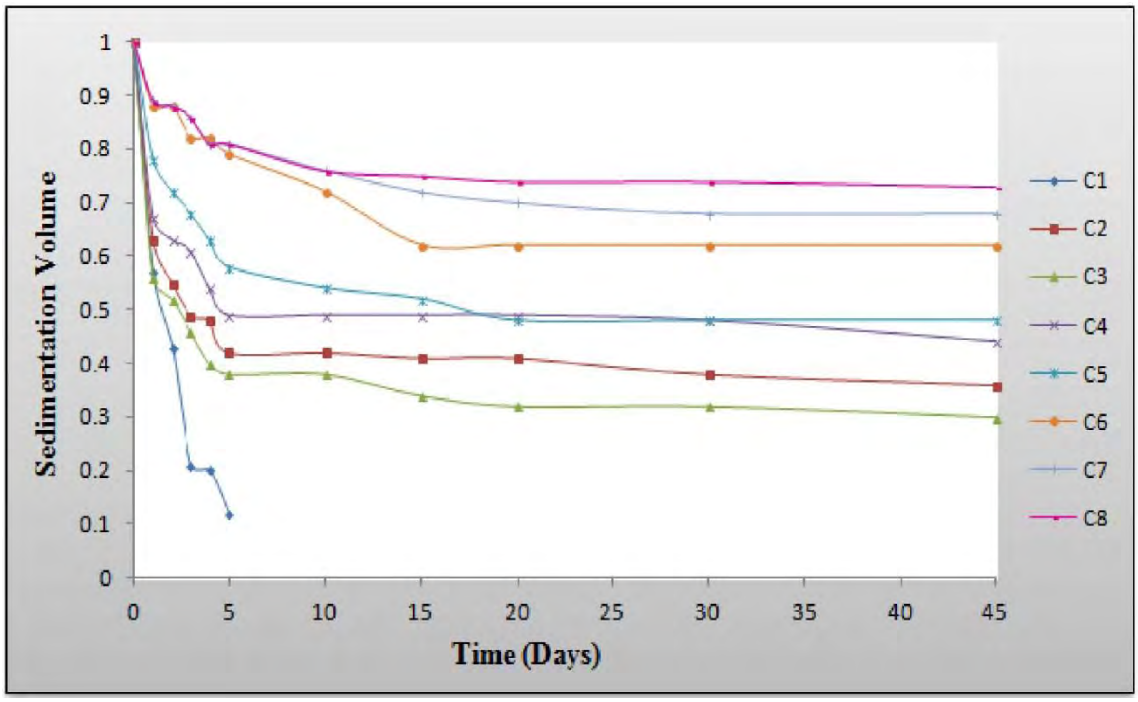

Figure 3. Comparative sedimentation volume profile of suspensions

6.2.3. Redispersibility

Since the suspension sediment on storage, it must be radially dispersible so as to ensure a more uniform dosage administration of medicament after shaking. Suspension is called as caked if sediment remains after vigorous shaking. All the suspension was found to be easily redispersible after maximum 13 shaking after 45 days (Table 4). Redispersibility was found to be faster for suspension with lower amount of suspending agent comparing to higher concentration. This may attribute to higher viscosity of these suspensions with higher concentration.

6.2.4. Flow rate (F)

Flow rate was found to be decreased as concentration of suspending agent and viscosity of suspension increased. It is found in the range of 0.1 -0.05 (Table 4).

Table 4: Evalution of suspension

Parameter	**Batch Code**						
	C2	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**
Particle size	21.98± 8.56	23.12 ± 14.36	19.89 ± 11.23	26.29 ± 6.34	21.06 ± 9.63	24.09 ± 10.12	22.65 ± 7.29
Degree of flocculation	2.57 ± 0.12	2.99 ± 0.18	3.10 ± 0.26	3.31 ± 0.32	3.55 ± 0.19	3.80 ± 0.38	4.01 ± 0.18
Flow rate	0.12	0.098	0.085	0.076	0.072	0.068	0.056
No. of shaking for complete dispersion	05	06	08	09	10	11	13
Drug content	96.09 ± 0.08	97.8 ± 0.09	95.96 ± 0.15	98.36 ± 0.19	97.97 ± 0.28	96.54 ± 0.18	99.12 ± 0.11
Viscosity (Poise)	1.18	1.93	3.28	4.48	5.94	6.90	7.10

6.2.5. Determination of viscosity

Viscosity of all formulation was found to be decreased with increasing rpm indicated shear thinning nature of suspension. Values of viscosity for all batches have been reported in table 4.

6.2.6. Degree of flocculation

Degree of flocculation was determined for all formulated suspension using different concentration of *Trigonella foenum graecum* mucilage. The values of degree of flocculation for all formulated suspension have been mentioned in table 4 and it found to be increased at higher concentration of suspending agent. This is due to higher viscosity of suspension at higher concentration, which ultimately reduces the sedimentation of suspension.

6.2.7. Effect of temperature

Increase in temperature reduces the viscosity for all formulation.

6.2.8. Drug content

Drug content for all batches was found to be in the range of 95- 99%.

6.2.9. Particle size measurement

Particle sizes of 20 particles of all formulated suspensions were determined and values are reported in Table 4.

6.2.10. In vitro dissolution studies

Result showed that all formulation releases almost 95% drug at the end of 30 min. For most of the batches, the release kinetics of' ciprofloxacin from the suspensions appeared to follow first order release kinetics. Some batches also follow Korsmeyer peppas kinetic model (n= 0.47) (Fig. 4).

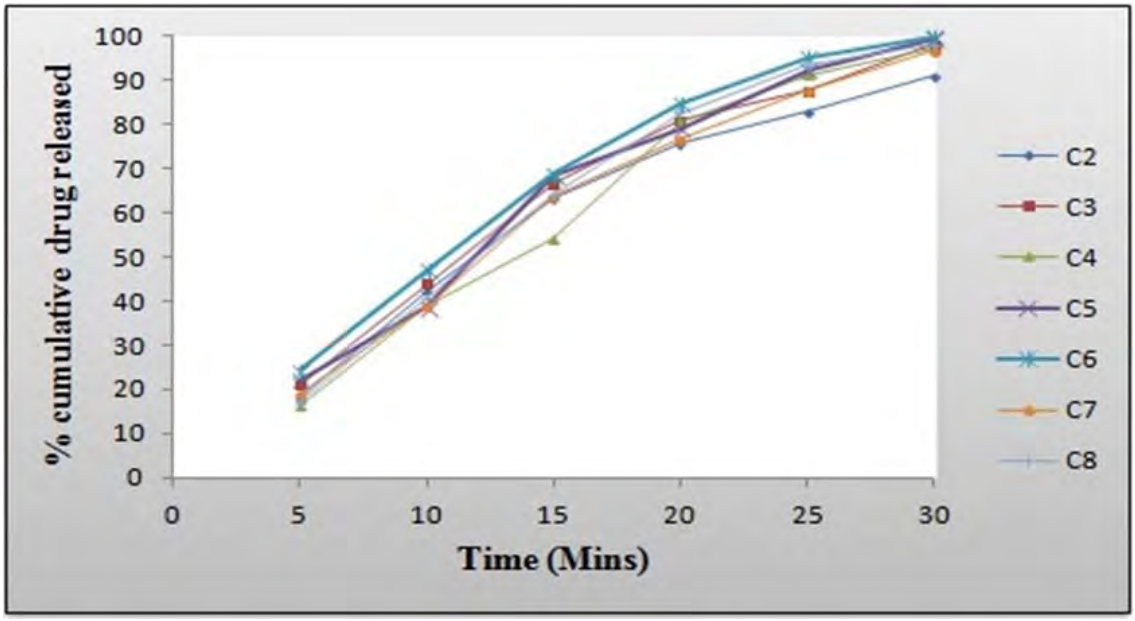

Figure 4. Plot of % cumulative drug release Vs time (min) of different batches of suspensions.

7. Conclusion

Formulated Ciprofloxacin suspension with natural suspending agent i. e. *Trigonella foenum graecum* showed superior stability over period of time. Increase in concentration of suspending agent increases the viscosity of suspension which ultimately reduces sedimentation and contributes to the stability of suspension.

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