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Formulation and Evaluation of Herbal Gel Containing *Punica Ganatum*

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ABSTRACT:

Research in the field of anti-inflammatory is very recent. The concept of anti-inflammatory is changing day to day. The medicinal plants Punica granatum are richest source for management of the anti-inflammatory. Family Lythraceaeis used as hemostatic and anti-inflammatory. The aim of present study is to formulate and evaluate the herbal gel containing pomegranate fruit extract. The pomegranate fruit extract is shows the anticancer activity, analgesic and anti-inflammatory activity, Antiepileptic activity, Antidiabetic, hypolipidaemic and antioxidant activity, Prevention of skin damage, Cardio protective, Musculoskeletal. The gel formulation was designed by using Carbopol 934, pomegranate fruit extract propylene glycol, methyl paraben, Propyl paraben and required amount of distilled water. The skin pH was maintained by drop wise addition of Tri-ethanolamine. The prepared gel was characterized for their physicochemical parameters, preliminary phytochemicals analysis, appearance, quantitative analysis, Spreadability, pH, viscosity and extrudability, In-vitro study & stability study.

Keywords: Pomegranate fruit, Carbopol 934, anti-inflammatory, gel formulation etc.

INTRODUCTION:

The pomegranate belongs to the family Lythraceae (previously Punicae) with only two different species namely *Punicagranatum*and *Punicaprotopunica*. These are planted either for its edible Fruit or as an ornamental tree. Commonly found in India is used for its patent anti-inflammatory activity. Focusing on the treatment and prevention of various diseases. The constituents of pomegranate have been reported to have antioxident,anti-carcinogenic and anti-inflammatory properties, focusing on the treatment and prevention of various diseases. The pericarp of Punicagranatum has been commonly employed as a crude drug in Indian traditional medicine for treatment of diarrhea as well as for use as an astringent, anti-helmenthis, laxative, diuretic, stomachic, cardio tonic and refrigerant. The anti-inflammatory components of Pop, i.e., punicalagin, punicalin, strictinin A and granatin B significantly reduce production of nitric oxide and PGE2 by inhibiting the expression of pro- inflammatory proteins Currently there is a greater global interests in non synthetic natural drug derived from plant herbal sources due to better tolerance and decreased adverse drug reaction. However, there is a lack of supporting studies regarding the formulation and evaluation aspect. A document on quality control for medicinal plant material by the WHO And the note for guidance on specifications, by the committee for proprietary medicinal product (CPMP) are positive measured in this direction thus the present study was carried out to formulate gel of punicagranatum fruit extract using different gelling agent in varying proportion and to evaluate its physical parameters and to set up specifications for the finished medicinal product. The oral bioavailability with an elimination of half-life of1.87 to 4.58 h.the topical delivery is difficult due to its high lipophilicity[1].

Inflammation (from Latin: inflammation) is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants, and is a protective response involving immune cells, blood vessels, and molecular mediators.

The function of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and initiate tissue repair.

The five classical signs of inflammation are heat, pain, redness, swelling, and loss of function (Latin calor, dolor, rubor, tumor, and functiolaesa).

Inflammation is a generic response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen. Therefore, the uses of anti-inflammatory herbal agents are helpful in the therapeutic treatment of these pathologies medicinal plants are widely used in folk medicine of many countries to treat different inflammatory conditions and, in particular, skin inflammations. However, for many of the plants in use the real efficacy and/or the relevant active principles are unknown. Consequently, experimental studies aimed to demonstrate the pharmacological properties of these plants and to identify the relevant active principles are needed.

Punica granatum Linn.Has been investigated extensively for its anti-inflammatory activities.
The plant is used as haemostatic and anti-inflammatory agent from ethno pharmacological point of view .*P. granatum* peels showed intermediate anti- inflammatory effect than allopathic anti-inflammatory drug.

The anti-inflammatory components of PoP, i.e., punicalagin, punicalin, strictinin A and granatin B significantly reduce production of nitric oxide and PGE2 by in Topical anti-inflammatory and analgesic activities of a standardized pomegranate rind extract of which ellagic acid (EA) was assessed and finding reported that rind extract and the equivalent ellagic acid dose-dependently reduced the ear edemainhibiting the expression of pro- inflammatory proteins[2].

Classical signs of inflammation:

English	Latin
Redness	Rubor
Swelling	Tumor
Heat	Calor
Pain	Dolor
Loss of function	Funtiolaesa

Table no.1 Classical signs of inflammation

1. Acute Inflammation
2. Chronic Inflammation

Inflammation can be classified as follows:

	Acute	Chorinc
Causative agent	Bacterial pathogens, injured tissues	Persistent acute inflammation due to non-degradable pathogens, viral infection, persistent foreign bodies, or autoimmune reactions
Major cells Involved	neutrophils (primarily), basophiles (inflammatory response), and eosinophils (response to helmenthis worms and parasites), mononuclear cells (monocytes, macrophages)	Mononuclear cells (monocytes, macrophages, lymphocytes, plasma cells), fibroblasts
Primary Mediators	Vasoactive amines, eicosanoids	IFN-γ and other cytokines, growth factors, reactive oxygen species, hydrolytic enzymes
Onset	Immediate	Delayed
Duration	Few days	Up to many months, or years
Outcomes	Resolution, abscess formation, chronic inflammation	Tissue destruction, fibrosis, necrosis

Table No.2 classification of inflammation

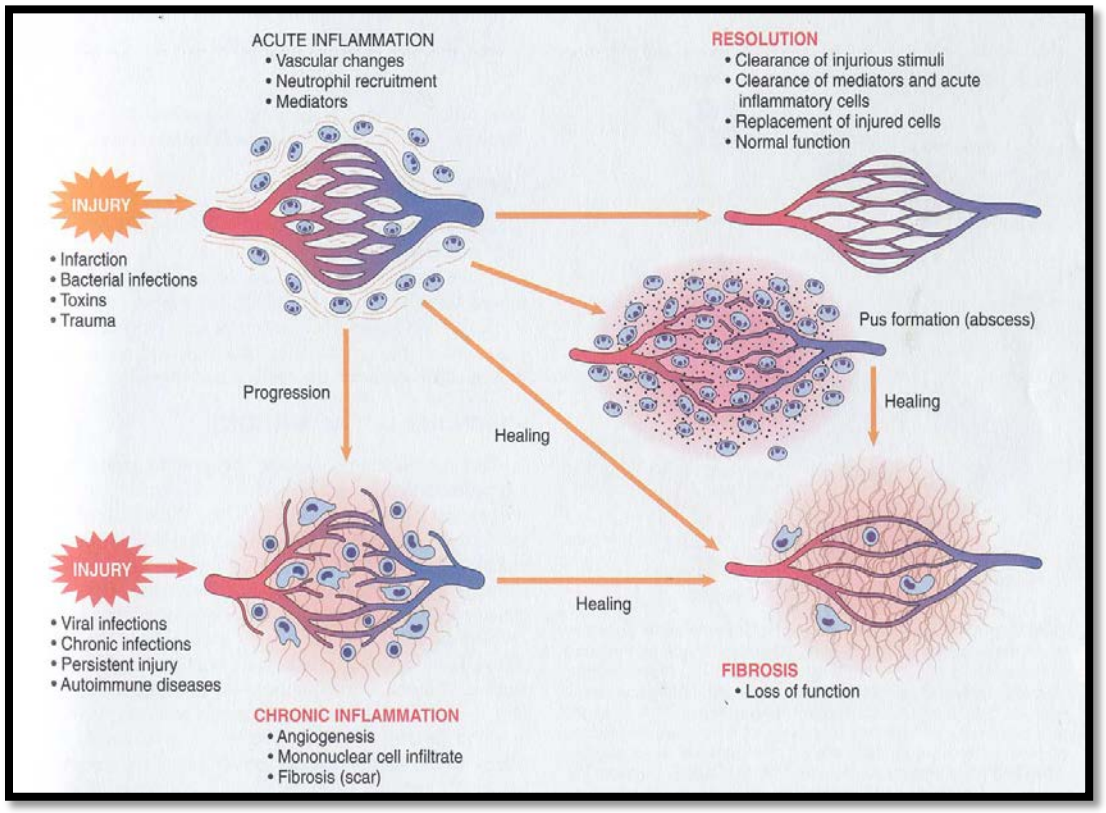

Fig No.1 chronic and acute inflammation

Mechanism of action of anti-inflammatory agent:

- NSAIDs inhibit cyclooxygenase (COX) enzymes responsible for the production of prostaglandins (PGs) which promote inflammation necessary for healing, pain and fever.
- As a consequence, ongoing inflammation, pain and fever are reduced by NSAIDs. When these phospholipids are acted upon by phospholipase A2, arachidonic acid is formed [3].

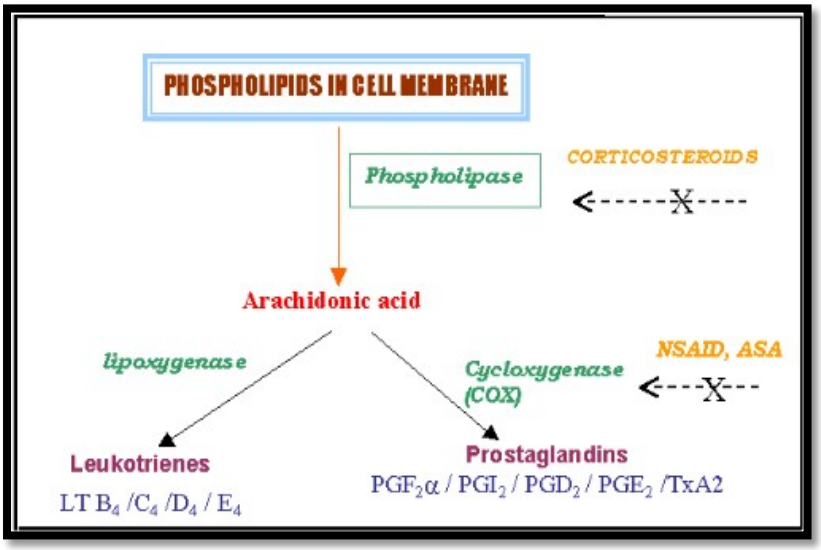

Fig No.2.**Mechanism of action of anti-inflammatory**

Mechanism of action pomegranate fruit as anti-inflammatory agent:

Cold pressed pomegranate seed oil has been shown to inhibit both cyclooxygenase and Lipoxygenase enzymes in vitro. Cyclooxygenase, a key enzyme in the conversion of arachidonic acid to prostaglandins (important inflammatory mediators), was inhibited by 37 percent by a CPSO extract. Lipoxygenase, which catalyzes the conversion of arachidonic acid to leukotrienes, also key mediators of inflammation, was inhibited by 75 percent by a CPSO extract. By comparison, an FPJ extract resulted in a 23.8-percent inhibition of lipoxygenaseinvitro [4].

2. MATERIALS AND METHODS

Sr. No.	Ingredient	Category
1.	Pomegranate fruit extract	API
2.	Carbopol934	Gelling agent
3.	Sodium CMC	Gelling agent
4.	Propylene glycol	Penetration enhancer
5	Methanol	Extracting solvent
6.	Glycerine	Solvent
8.	Methyl paraben	Preservative
9.	Triethanolamine	Maintain the pH

Table No.3.MATERIALS

Punicagranatum (pomegranate fruit) [5]

Figure No.3: ***Pomegranate fruit***

Biological Name: *Punica granatum*

Common Name: Hindi : Anar, Sanskrit : Dadimah, English : Pomegranate, Marathi : Dalimba, Gujarati : Dalimba, Bengali : Dadim, Tamil : Madalai, Telgu : Danimma, Malayalam : Talimatatalum, Pharsi : Anartursa, Arabi : Roman Hamiz, German : Granatapfels.

Family: Lythraceae / Pomeceae

- **BOTANICAL DESCRIPTION:**
- **Leaves:**

Colour: Dark green.

Size: 3-7 cm long and 2 cm broad.

Shape: Glossy and have a leathery leaves that are narrow and lance-shaped.

- **Blossoms:**

  Produced in summer where rainfall is minimal during late summer.

- **Flower:**

  Colour: Bright red with 5 to 8 crumpled a petal which persists on the fruit.

  Size: 3 cm in diameter.

- **Fruit:**

  Size: Typicall**y** ranges from 2 to 5 inch wide.

  Colour: leathery skin or rind is typically yellow overlaid with light or deep pink or rich red.

2. Formulation of topical gel preparation:

The PFE gel was prepared using Carbopol 934, sodium CMC as a gelling agent in a 1% w/w concentration with deionized water using mechanical stirrer. The methyl paraben heat on water bath and place to cool after that the glycerin and propylene glycol added into it. This solution added into the gel. The pH of the gel was adjusted to neutral by addition of sufficient quantities of Triethanolamine and DMSO with continuous stirring. The given quantity of methanolic extract of pomegranate fruit added into the gel and stirred for sufficient time for homogeneous mixing of extract in gel base. Prepared gel was filled in collapsible tubes and stored at a cool and dry place. Physical parameters such as colour, appearance, and feeling on application were recorded. pH of the gel was recorded using a pH meter[6].

2.4 Formulation table

Ingredient(mg)/ Formulation	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Punicagranatum fruit extract	0.5gm	0.5gm	0.5gm	0.5gm	0.5gm	0.5gm	0.5gm	0.5gm	0.5gm
Carbopol 934(gm)	0.5	0.75	1.0	-	-	-	0.5	0.75	1.0
Sodium CMC(gm)	-	-	-	1	2	3	0.5	0.75	1.0
Propylene glycol	2.5ml	2.5ml	2.5ml	2.5ml	2.5ml	2.5ml	2.5ml	2.5ml	2.5ml
Methanol	20ml	20ml	20ml	20ml	20ml	20ml	20ml	20ml	20ml
Methyl paraben	0.2gm	0.2gm	0.2gm	0.2gm	0.2gm	0.2gm	0.2gm	0.2gm	0.2gm
Glycerine	3ml	3ml	3ml	3ml	3ml	3ml	3ml	3ml	3ml
Triethanolamine	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Distilled H_2O	Up to 100	Up to 100	Up to 100	Up to 100	Up to 100	Up to 100	Up to 100	Up to 100	Up to 100

Table No.4 Formulation table

3. Evaluation of Gel[7-10]:

1. **Physical Evaluation:** The colour& appearance of the prepared herbal gel formulations were observed physically.
2. **pH measurement**: The pH of the gel was determined using digital pH meter. Gel formulation was dissolved in water and pH was determined by dipping the glass electrode completely into gel solution. Then instrument reading was recorded as a pH of solution.
3. **Homogeneity** All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container for their appearance and presence of any aggregates.
4. **Spreadability**

It was determined by wooden block. Spreadability was measured on the basis of slip and drag characteristics of gels. An excess of gel was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide. The upper slide was then pulled apart horizontally with a string, then weight was applied. The time required separating the two slides was measured as spreadability using following formulae

$S = M \times L / T$

Where,

S = Spreadability

M = Weight in the pan (tied to the upper slide)

L = Length moved by the glass slide

T = Time (in sec.) taken to separate the slide completely each other.

.

5. **In-vitro Drug Release Study :**

The dissolution studies were performed USP rotating basket method in 6.8 phosphate buffer solution. The compartment containing 900 ml of 6.8 phosphate buffer solution. The sample of 5 ml each was withdrawn at predetermined time interval and were replenished immediately with the same volume of phosphate buffer maintaining sink condition throughout the experiment. The aliquots following suitable dilution with phosphate buffer were analyzed spectrophotometrically at λmax 285 nm. The concentrations of test samples were calculated using regression equation.

6. Viscosity:

Viscosity of herbal gel was determined by using Brookfield rotational viscometer at 6rpm. Each reading was taken after equilibrium of the sample at the end of two minutes. The viscosity determination of samples was repeated three times

7. Stability Study:

The stability study conducts by ICH guideline. It showed No significance change in properties of the optimized formulation & the drug release. Short term stability studies were performed in a Stability chamber over a period of 3 month on the promising *pomegranate fruit extract* gel. Sufficient quantity of gel formulation were packed in stability container and kept in a Stability chamber at Temperature 45^0c &RH 75%. Samples were taken for the P^H and viscosity, were performed to determine the stability profile.

4. RESULT AND DISCUSSION

1. Estimation by UV spectroscopy:

1.1 Calibration of *Pomegranate fruit* extract:

Table No.5: Calibration of *Pomegranate fruit* extract

Sr. no.	Concentration (µg/ml)	Absorbance (λ_{max} at 285nm)
1.	2	0.205
2.	4	0.425
3.	6	0.665
4.	8	0.890
5.	10	1.254
6.	12	1.521

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Calibration curve:

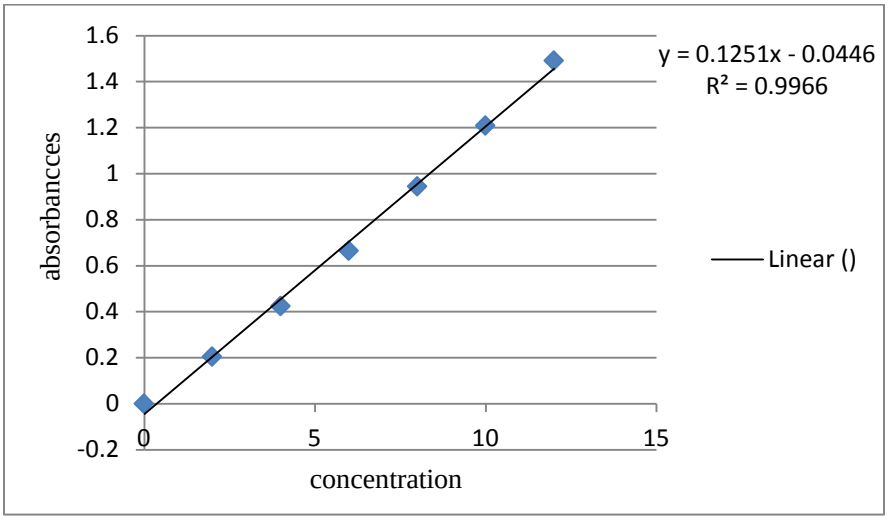

Figure No.4: Calibration curve

2. **Interpretation of FTIR Spectra**

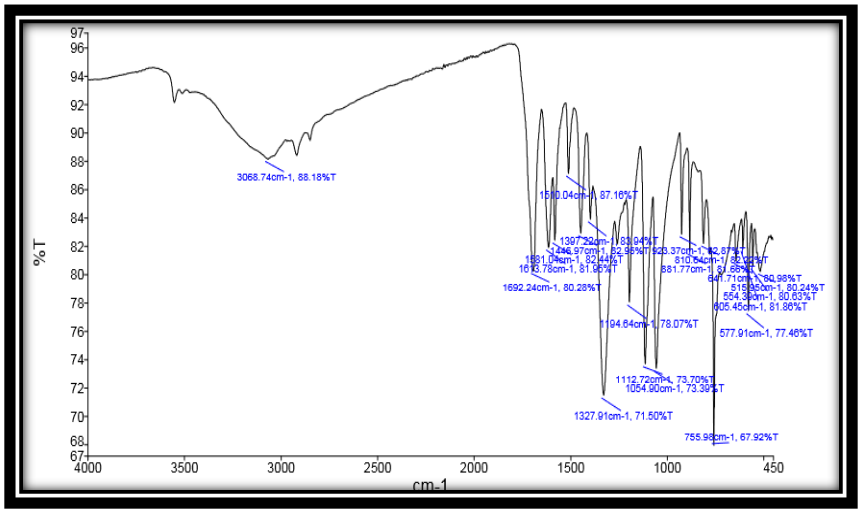

Figure No.-5: Interpretation of FTIR Spectra of *pomegranate fruit* extract

Table No. 6: Interpretation of FTIR of Pure *Pomegranate fruit* extract

Functional Group	Standard Frequency	Observed Peak
C-H stretching(alkenes)	3040-3010	3068
C=C Stretching(alkene)	1680-1620	1692
C=N Stretching	1630-1690	1613
N=N stretching	1575-1630	1581
N=H bending	1500-1650	1510
C-C Stretching	1450-1600	1446

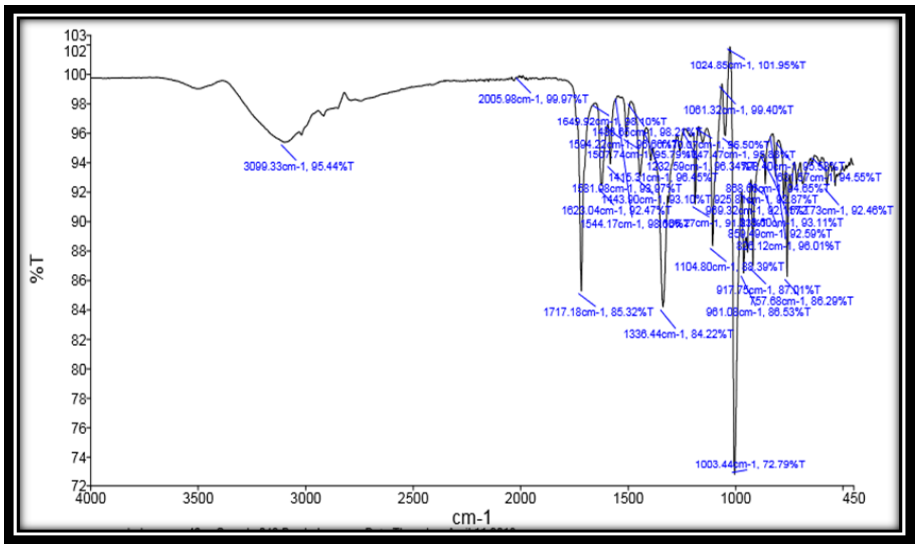

Figure No.-6: FTIR Spectra of formulations of Herbal Gel

Evaluation Studies

Table No.-7: ***Evaluation of HERBAL GEL CONTAINING PUNICA GANATUM***

Sr.no	Batch	Appearance	Homogeneity	P^H	Spreadability(gm.\sec)	Viscosity
1	F1	Light Brown and translucent	Homogeneous	6.5	20.83	14830
2	F2	Light Brown and translucent	Homogeneous	6.4	20.27	21600
3	F3	Brown and translucent	Homogeneous	6.8	19.16	22800
4	F4	Dark Brown And translucent	Homogeneous	6.6	17.22	58000
5	F5	Brown and translucent	Homogeneous	6.7	18.19	66800
6	F6	Light BrownAnd translucent	Homogeneous	6.5	19.15	33000
7	F7	Brown and	Homogeneous	6.3	16.16	32666.6

		translucent				
8	F8	Dark Brown and translucent	Homogeneous	6.8	18.15	70500
9.	F9	Dark Brown and translucent	Homogeneous	6.7	20.86	23170

In-vitro drug release study:

The in-vitro diffusion studies were carried out using Franz diffusion cell apparatus and semi-permeable cellophane membrane. Cellophane membrane, previously soaked overnight in phosphate buffer 5.5 was mounted by tied and sandwiching between the donor and receiver compartment. Franz diffusion cell with a diameter 3.7 cm was used in in-vitro release studies. A glass tube with both end open, 10 cm height and 3.7 cm outer diameter was used as a permeation cell. A one gram sample was accurately weighed and placed on a semi permeable cellophane membrane to occupy a circle of 3.7 cm diameter. The loaded membrane was stretched over the lower open end of a glass tube of 3.7 cm diameter and made water tight by rubber band. The tube (donor compartment) was immersed in a beaker containing 100 ml of phosphate buffer pH 7.4(receptor compartment). The cell was immersed to a depth of 1 cm below the surface of buffer. The system temperature was maintained at 37°±1° and speed was maintained at 30 rpm throughout the experiment by magnetic stirrer Samples 5 ml were withdrawn at intervals of 1, 2, 3, 4, 5, 6, 7 and 8 hour, the volume of each sample was replaced by the same volume of fresh buffer to maintain constant volume. The samples were filtered through Whatman filter paper, diluted up to 10 ml and absorbance was taken by UV spectrophotometer at respective λ_{max} 285nm. The experiment was carried out triplicate and average value is reported[11].

Cumulative drug release

Table No. 8 Cumulative drug release

Time (hr.)	% Cumulative drug release								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	8.40	07.06	8.39	7.43	6.54	9.19	11.55	9.65	8.45
2	14.34	15.4	12.03	18.15	19.18	17.18	17.12	17.80	20.19
3	22.85	23.20	21.17	28.42	29.25	26.25	25.85	30.10	32.46
4	29.15	30.25	28.02	39.90	40.23	38.19	39.40	41.55	45.35
5	36.92	37.12	35.89	51.53	52.42	53.20	46.10	53.09	58.30
6	43.22	44.81	42.02	63.55	62.60	61.55	60.15	63.13	70.15
7	50.15	52.17	49.75	74.78	73.10	74.27	83.42	74.19	82.10
8	59.08	60.32	62.45	82.15	87.12	91.07	89.15	90.10	92.85

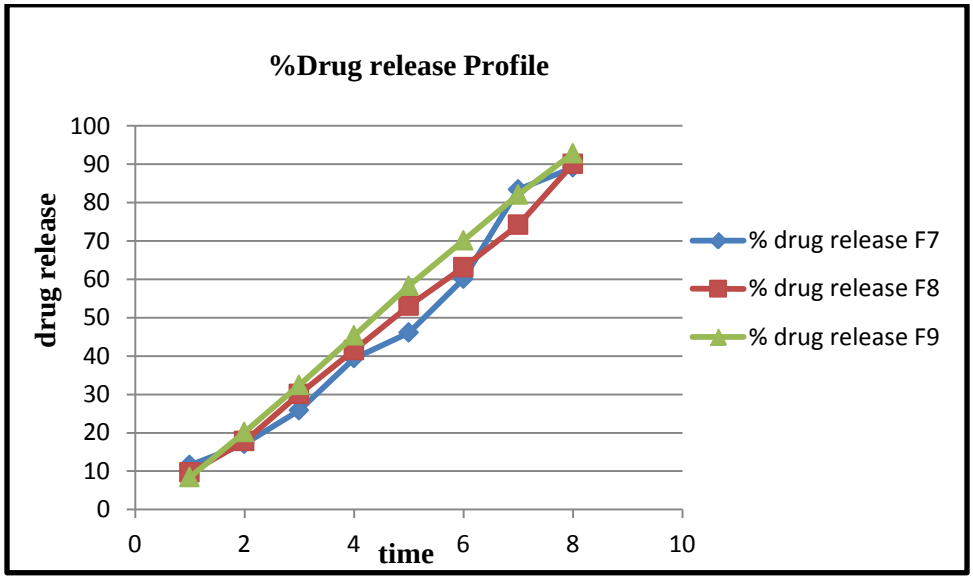

Figure No.7: Cumulative drug release

12. Stability Study:

Sufficient quantity of Optimized gel formulation were packed in container and kept in a Stability chamber at Temperature 40^0c & RH 75%. For 3 month stability will be checked its physical

appearance, pH, viscosity, Homogeneity, Consistency, Drug Content (%) and % In Vitro Drug release Study were been checked and a result was tabulated in table 8.

Table No.-8: Stability Study

Sr.no.	Parameters	Initial	Stability after 1St month	Stability after 2nd month	Stability after 3rd month
1.	Physical Appearance	Brown and Translucent	Brown and Translucent	Brown and Translucent	Brown and Translucent
2.	pH	6.7	6.6	6.5	6.4
3	Consistency	Smooth	Smooth	Smooth	Smooth
4	Viscosity	23170	22550	22010	21510
5	Drug Content (%)	98.75%	98.55%	97.55%	96.85%
6	In vitro drug release	92.85	91.12	90.85	88.85
7	Homogeneity	Homogeneous	No change	No change	No change

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