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Formulation and Evaluation of *Withania somnifera* Dunal Emulgel for Treatment of Rheumatoid Arthritis

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Abstract

Rheumatoid arthritis is an inflammatory condition associated with painful joint destruction. The lack of possible cure and associated disadvantages in allopathic medicine has led to extensive research in natural products with anti-rheumatoid activity. *Withania somnifera* (Ashwagandha) was found to be efficacious and cost effective anti-inflammatory drug with least side effects as compared to the synthetic drugs used in the treatment of rheumatoid arthritis. Therefore, this drug selected for the present study. Considering above justification an attempt was made to design and develop herbal anti-rheumatic transdermal drug delivery system wherein biphasic drug delivery system of herbal drug in the form of emulgel was formulated and their effect was compared to gel formulation. The factorial and optimized batches evaluated for the various parameters like Drug content, % Drug release and Stability study. From this studies F3 formulation showed maximum % drug release and optimum parameters like pH,Viscosity, Consistency, Spreadability (Spreading Coefficient),Drug Content, Swelling Index and Extrudability Study.

Keywords: *Withania somnifera*; Dunal Emulgel; Rheumatoid Arthritis; Ashwagandha Emulgel; Biphasic Drug Delivery; Herbal Emulgel

Introduction

Rheumatoid arthritis (RA) is a chronic and usually progressive inflammatory disorder of unknown aetiology characterized by polyarticular symmetric joint involvement and systemic manifestations. Rheumatoid arthritis is a systemic disease and it involve rheumatoid nodules, vasculitis, eye inflammation, cardio pulmonary disease are manifestation of the disease. Rheumatoid arthritis is not an inherited disease [1,2]. Antigenic exposure(e.g. infectious agent, environmental factors) in genetic all predisposed individual(HLA-DR) causes activation of CD4+ T cell which elaborate TNF-α,interferons, IL-1,IL-6 followed by activationofβ – lymphocytes, Macrophages and activation of IgM antibody against IgG (i.e. Anti-IgG) [3]. This is termed as Rheumatoid factor. IgG and IgM immune complexes formed trigger inflammatory damage to synovium, small blood vessels and collagen. The current the rapies for Rheumatoid Arthritis are as follows:

- Non-steroidal anti-inflammatory drugs (NSAIDs)
- Glucocorticoids
- Non-biologic disease-modifying anti-rheumatic drugs (DMARDs) and
- Biologic DMARDs [4]

There is increasing evidence that many current drug therapies attempt to suppress symptoms than addressing the underlying disease processes. The allopathic system of

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medicine includes conventional line of the treatment for rheumatoid arthritis, which come along with certain side effects. Hence, turning to safe, effective and time tested Ayurvedic herbal drug formulation would be a preferable option. So, there is need to investigate such drugs and their effective formulation for the better patient acceptance. There are many synthetic drugs that are being used as standard treatment for rheumatoid arthritis but they have adverse effect that can compromise the therapeutic treatment so these adverse effects increase the chances for the use of herbal plants for the rheumatoid arthritis treatment [5,6].

Various parts of plant like roots, stems, flowers, seeds, leave and barkcontains variety of chemical constituents which are given in various herbal traditional formulations likeoralroute (e.g.Pills,Tablets, Capsules, Arishtas, Aasavas, Ghritaetc.), External application(e.g.Taila, Lepa, Malametc.).

Novel Drug Delivery System: Emulgel

Emulgel is an emulsion, either of water-in-oil type or oil-in-water type emulsion system, which are gelled by mixing it with an appropriate gelling agent. Emulgel have emerged as one of the most important topical delivery system as it has dual control release system i.e. Gel and emulsion [7,8].

The Emulgel can be easily formulated from the gels, the material used are easily available and cheaper so the preparation cost of the Emulgel is very low. Its formulation also involves the simpler steps. Emulgel have one important advantage that it can be used to in formulating the controlled release drug delivery system to prolong the effect of the drugs having shorter half-life (t1/2). Emulgel have lesser spreading coefficient and therefore need to apply with rubbing action [9,10].

Materials and Method

Materials

Alcoholic extract of Ashwagandha (*Withania somnifera* Dunal.) dried root powder purchased from Chakrapani Clinic and Research Centre, Jaipur along with the Certificate of Analysis. All excipients Carbopols, parabens, tween 80, span 20, ethanol, triethanolamine, propylene glycol, camphor, menthol and linseed oil were purchased from Research Fine Laboratory, Mumbai. Thymol was purchased from Dawa-saj, Aurangabad.

Method

Preparation of Carbopols gel: Carbopols gel was prepared by dispersing Carbopols powder in sufficient quantity of deionized water with the aid of magnetic stirrer (1500rpm) and then the pH was adjusted to pH 6 to 6.5.

Preparation of Emulsion: The oil phase was prepared by dissolving certain amount of Span 20 in 6gm of Linseed oil, while the aqueous phase was prepared by dissolving the required amount of Tween 80 in deionized water. 1 gm of herbal extract was dissolved in sufficient Water: Ethanol mixture, while 0.18 gm of methyl parabens and 0.02gm of propyl parabens was dissolved in 7gm propylene glycol and both are mixed with aqueous phase. Both oily and aqueous phases were separately heated to 700 – 800C. During heating when temperature reaches at 400 – 450 C, camphor, menthol and thymol were added in little quantity of ethanol which was mixed in aqueous phase. Then, the oil phase was added to the aqueous phase with continuous gentle stirring (50rpm) until cooled to room temperature to form emulsion.

Preparation of Emulgel: All experimental batches prepared by dispersing the obtained emulsions with the gel in 1:1 ratio with gentle stirring until homogenous emulgel is obtained.

Experimental Batches and Optimization Using 32 Full Factorial Designs

A 32 randomized full factorial design was used in this study. Two factors were evaluated each at three levels and experimental trials were performed on all nine possible combinations. The amount of Carbopol 940 (X1) and the amount of emulsifying Agent (X2) were selected as independent variables as shown in Tables 1 & 2. The percent drug release was selected as dependent variable.

Sr. No.	Ingredients (in g)	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	Drug	1	1	1	1	1	1	1	1	1
2	Ethanol	7	7	7	7	7	7	7	7	7
3	Tween 80	1.1	1.65	2.2	1.1	1.65	2.2	1.1	1.65	2.2
4	Linseed oil	6	6	6	6	6	6	6	6	6
5	Span 20	0.9	1.35	1.8	0.9	1.35	1.8	0.9	1.35	1.8
6	Carbopol 940	1	1	1	1.5	1.5	1.5	2	2	2
7	Methyl paraben	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
8	Propyl paraben	0.02	0.02	0,02	0.02	0.02	0.02	0.02	0.02	0.02

9	Propylene glycol	7	7	7	7	7	7	7	7	7
10	Camphor	1	1	1	1	1	1	1	1	1
11	Menthol	1	1	1	1	1	1	1	1	1
12	Thymol	1	1	1	1	1	1	1	1	1
13	Triethanolamine	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
14	Water (q.s)	100	100	100	100	100	100	100	100	100

Table 1: Master formula of F1 to F9 batches (quantities in g).

Sr. no.	Ingredients	A1	A2	A3
1	Drug	1	1	1
2	Ethanol	7	7	7
3	Carbopol 940	1	1.5	2
4	Methyl paraben	0.18	0.18	0.18
5	Propyl paraben	0.02	0.02	0.02
6	Propylene glycol	7	7	7
7	Camphor	1	1	1
8	Menthol	1	1	1
9	Thymol	1	1	1
10	Triethanolamine	q.s	q.s	q.s
11	Water (q.s)	100	100	100

Table 2: Master formula of batches A1 – A3 (in g).

Evaluation Tests for Emulgel and Gel [11-15]

- **Physical Parameters:** The prepared emulgel and gel formulations were inspected visually for colour, homogeneity, consistency, grittiness and phase separation.
- **pH:** The pH values of 1% aqueous solutions of the prepared emulgel and gel were measured by a pH meter. pH of emulgel and gel formulation were taken in triplicate and average value was taken.
- **Viscosity:** The viscosity of the emulgel and gel formulations was determined using Brookfield viscometer with spindle no. 4 at 10 rpm.
- **Spreadability:** The Spreadability of the gel formulations was determined at 24 h after permeation, by measuring the spreading diameter of 1 g of emulgel and gel between two horizontal plates (20 cm × 20 cm) after one min.
- **Drug content:** About 1 g of emulgel and gel was accurately weighed and transferred to 100 ml volumetric flask to which about 70 ml of 50% ethanol was added. After mixing the volume was made up to 100 ml 50% ethanol followed by 2 h shaking by mechanical shaker. The content was filtered through a suitable filter paper. An aliquot of 1 ml was pipetted out from filtrate. Blank solution is prepared by in same way as sample by using blank emulgel and gel. The extract was estimated spectrophotometrically by using Shimadzu UV/VIS spectrophotometer-1700 at 278 nm. Absorbance taken in triplicate and average value was calculated.
- **Extrudability study:** After the emulgels and gels were set in the container, the extrudability test was carried out using hardness tester. A 15 g of gel was filled in aluminum tube. The plunger was adjusted to hold the tube properly. The presence of 1kg/cm2was applied for 30 sec. The quantity of gel extruded was weighed. The procedure was repeated at 3 equidistance places of the tube.
- **Swelling Index:** To determine the swelling index of prepared emulgels and gels, 1 gm of gel was taken on petri dish and then placed separately in a 50 ml beaker containing 10 ml deionized water. Then samples were removed from beakers at different time intervals and put it on dry place for some time after it reweighed. Swelling index was calculated as follows:

Swelling Index (SW) % = [(Wt – Wo) / Wo] × 100.
Where, (SW) % = Equilibrium percent swelling,
Wt = Weight of swollen gel after time t,
Wo = Original weight of gel at zero time.

- **In Vitro diffusion study:** Studies of the prepared gels were carried out in Franz diffusion cell for studying the dissolution release of gels through a Cellophane

membrane. Gel sample (1g) was taken in cellophane membrane and the diffusion studies carried out at 37±1º C using as pH 7.4 Phosphate buffer as dissolution medium. One ml of each sample was withdrawn periodically at 0,30 min,1,2,3,4,5,6,7,8,9,10,11 and 12 h and each sample was replaced with equal volume of dissolution medium. The samples were analysed in triplicate for the drug content by using pH 7.4 Phosphate Buffer as blank.

- **Stability study:** Batch No. F-3 was showing results meeting standard specification requirement for Emulgel hence to be considered as optimised batch. Batch no. F-3 was subjected to stability testing for 3 months as per ICH norms at temperature of 300 ±20 C / 65% ± 5% RH and 400 ± 20 C / 75% ± 5% RH for intermediate and accelerated stability. The formulations were analysed for change in colour, appearance, Spreadability, pH and drug content.

Result and Discussions

- **Physical parameters:** The physical parameters observed visually for all batches of emulgel F1 to F9 and gel A1 to A3 shown in Table 3.

Sr. No.	Formulation code	Colour	Phase separation	Grittiness	Homogeneity
1	F1	Light brown	None	No	Homogenous
2	F2	Light brown	None	No	Homogeneous
3	F3	Light brown	None	No	Homogeneous
4	A1	Light brown	None	No	Homogeneous
5	F4	Light brown	None	No	Homogeneous
6	F5	Light brown	None	No	Homogeneous
7	F6	Light brown	None	No	Homogeneous
8	A2	Light brown	None	Yes	Aggregated
9	F7	Light brown	None	Yes	Aggregated
10	F8	Light brown	None	Yes	Aggregated
11	F9	Light brown	None	Yes	Aggregated
12	A3	Light brown	None	Yes	Aggregated

Table 3: Physical parameters of Emulgel (F1 to F9) and Gel (A1 to A3) formulations.

- **PH:** The pH of the emulgel and formulations was in the range of 6.30 to 6.57 which lies in the normal pH range of the skin and would not produce any skin irritation. The pH values of F1 to F9 emulgel formulations and A1 to A3 gel formulations shown in Table 4.
- **Viscosity:** The viscosity of the emulgel and gel formulations generally reflects its consistency. Decrease in viscosity of emulgel formulations showed increase in drug release. Viscosity of F1 to F9 emulgel and A1 to A3 gel formulations shown in Table 4.
- **Spreadability:** The Spreadability of the gel formulations was determined at 24 h after permeation, by measuring the spreading diameter of 1 g of gel between two horizontal plates (20 cm × 10 cm) after one min. The Voltaren emulgel (Novartis Pharma) was considered as reference standard. From results it was observed that F1, F2 and F3 emulgel formulations showed maximum Spreadability than F4, F5, F6, F7, F8, F9 Spreadability of F1, F2 and F3 was 56.58 mm, 54.80mm and 52.25 mm respectively. Order of Spreadability of emulgel formulations were found to be F3>F2>F1>F6>F5>F4>F9>F8>F7.Out of 9 formulations, F3 emulgel formulations showed maximum Spreadability than other emulgel formulations. Values of Spreadability (mm) of Emulgel (F1 to F9) and Gel (A1 to A3) formulations shown in Table 4.
- **Drug content:** Drug content of F3 emulgel formulation was found to be 99.34 % followed by F2 (98.47 %), F1 (98.03 %), F6 (98.18 %), F5 (98.03%), F4 (98.12), F9 (98.15%), F8 (97.30%) and F7 (97.16%). Drug content of gel formulation was found to be A1 (97.67%), A2 (97.56%) and A3 (95.76%) as shown in Table 4.
- **Extrudability study:** Extrudability study of F1 to F9 emulgel and A1 to A2 gel formulation were carried out and F3 (0.98gm), F2 (0.87gm) and F1 (0.82gm) showed that extrudability decrease when increase in percent of Carbopol 940 as shown in Table 4.
- **Swelling index:** Swelling index of F9 emulgel formulation was found to be 140% followed by F8 (142%), F4 (142%), F5 (138%) and F3 (91.21%). Maximum swelling index indicates matrix formulation which is useful in control drug release from gel formulations. Swelling index of F1 to F9 and Gel (A1 to A3) formulations was showed in Table 4.

Sr. No.	Formulation Code	pH	Viscosity	Spreadability	Drug content	Extrudability	Swelling Index
			(in cps)	(in mm)	(in %)	(in gm)	(in %)
1	F1	6.5	1308	52.25	98.03	0.82	93.18
2	F2	6.45	1312	54.8	98.47	0.87	92.16
3	F3	6.3	1345	56.58	99.34	0.98	91.21
4	A1	5.62	1756	30.49	97.67	0.67	101.13
5	F4	6.58	1567	36.78	98.12	0.69	142
6	F5	6.57	1564	37.93	98.03	0.71	138
7	F6	6.44	1597	39.29	98.18	0.73	135
8	A2	5.51	1900	24.12	97.56	0.54	148
9	F7	6.46	2010	22.12	97.16	0.53	145
10	F8	6.53	2005	23.73	97.3	0.6	142
11	F9	6.51	2023	24.27	98.15	0.63	140
12	A3	5.08	2498	20.14	95.76	0.48	161

Table 4: Evaluation tests of Emulgel (F1 to F9) and Gel (A1 to A3) formulations.

- **In vitro diffusion study:** In vitro diffusion study for F1 to F9 emulgel and A1 to A3 gel formulations for 12 h shown in Table 5. In vitro diffusion study carried out in diffusion cell for 12 h F3 formulation shown maximum drug release (96.11 %) as compared to the other gel formulation A3. As F3, F2, F1 shown maximum release as compare to the F4, F5, F6, F7, F8, F9. It may conclude that the % of Carbopol 940 contribute to the drug release of emulgel and gel formulation as decrease in % of Carbopol 940 there is increase in percent drug release as shown in Table 5 and Figure 1.

Time	F1	F2	F3	A1	F4	F5	F6	A2	F7	F8	F9	A3
0	0	0	0	0	0	0	0	0	0	0	0	0
30	5.733	9.63	14	45.1	5.36	5.4	5.66	39.12	4.13	4.88	5.07	34.02
60	6.7	10.88	15.14	57.19	6.15	6.72	6.88	40.49	5.19	5.9	6.04	41.39
120	20.09	25.02	32.15	61.23	19.64	21.1	22.01	52.09	18.17	18.87	20.19	50.12
180	39.11	43.2	47.67	70.17	38.55	39.07	39.89	60.13	37.19	38.2	40.14	63.13
240	42.14	46.2	51.87	82.16	40.26	42.1	44.11	73.17	39.1	40.11	42.91	72.1
300	48.16	52.72	58.74	93.18	47.5	47.79	48.09	82.16	46.359	48.16	48.6	79.12
360	53.47	58.03	63.39	--	53.35	55.81	57.15	--	52.51	53.1	56.01	--
420	57.45	62.06	68.58	--	56.09	58.07	59.66	--	54.01	55.16	57.11	--
480	63.12	67.12	71.96	--	57.07	59.18	60.89	--	55.06	60.11	62.01	--
540	66.87	72.03	84.33	--	66.36	69.73	70.17	--	65.11	67.18	69.12	--
600	75.65	80.15	91.37	--	75.53	78.11	79.27	--	67.09	68.11	71.03	--
660	82.19	88.26	92.78	--	77.54	80.88	86.12	--	69.19	69.19	74.01	--
720	85.27	90.28	94.65	--	81.83	82.09	84.17	--	70.15	72.2	76.1	--

Table 5: Percent cumulative drug release of Emulgel (F1 to F9) and Gel (A1-A3) formulations.

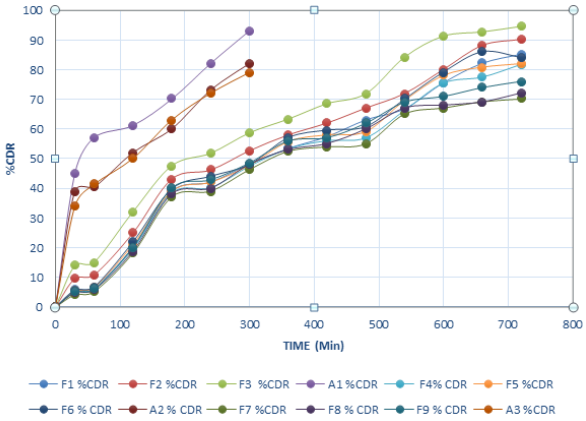

Figure 1: Drug Release Profile for all F1 – F9 Emulgel Formulations and A1- A3 Gel Formulations.

- **Stability studies:** Stability study for F3 formulation performed for 3 months in Intermediate and Accelerated level, reported in Tables 6, 7 respectively.

Intermediate stability					
30 ±2^{0}C / 65% ± 5% RH	**Colour**	**Appearance**	**Spreadability (mm)**	**pH**	**Drug content (%)**
Initial	Brown	Creamy	56.58	6.3	99.34
1 mo	No change	No change	56.23	6.23	99.21
2 mo	No change	No change	56.1	6.25	99.1
3 mo	No change	No change	55.78	6.2	98.96

Table 6: Stability studies of F3 Emugel formulation (Intermediate).

Accelerated stability					
40 ± 2^0 C / 75% ± 5% RH	**Colour**	**Appearance**	**Spreadability (mm)**	**pH**	**Drug content (%)**
Initial	Light brown	Creamy	56.58	6.3	99.34
1 mo	No change	No change	56.12	6.18	98.45
2 mo	No change	No change	56.4	6.22	98.12
3 mo	No change	No change	55.1	6.25	97.78

Table 7: Stability studies of F3 Emulgel formulation (Accelerated).

- **Factorial Designing:** Optimization was performed using State ease design of experiment software version 9.0.4.1 suite-480; predicted vs actual, contour plot and surface response plot were plotted. ANOVA study for Formulations was performed by Carbopol 940 (A) and emulsifying agent (B) formulation variables and for percent drug release at 12 h as response. ANOVA results were as summarized in Tables 8-10. Predicted vs actual, surface response plot and contour plot were given in Figures 2-4 respectively. Two equations were obtained as Equation 1 and Equation 2.

Source of Variation	Degrees of Freedom	Sum of Squares [Partial]	Mean Squares [Partial]	F Ratio	P Value
Model	3	500.36	166.79	59.24	0.0002
A-A	1	444.62	444.62	157.91	<0.0001
B-B	1	52.63	52.63	18.69	0.0075
AB	1	3.12	3.12	1.11	0.341
Residual	5	14.08	2.82	-	-
Cor Total	8	514.44	-	-	-

Table 8: ANOVA.

Term	Coefficient	Standard Error	Low Confidence	High Confidence	VIF
Intercept	81.85	0.56	80.41	83.29	-
A: Factor 1	-8.61	0.69	-10.37	-6.85	1
B: Factor 2	2.96	0.69	1.2	4.72	1
A • B	-0.88	0.84	-3.4	1.27	1

Table 9: Regression analysis.

Significant terms	
Name	**P Value**
A: Factor 1(Carbopol 940)	< 0.0001
B: Factor 2(Emulsifying Agent)	0.0075
A • B	0.341

Table 10: Significant terms.

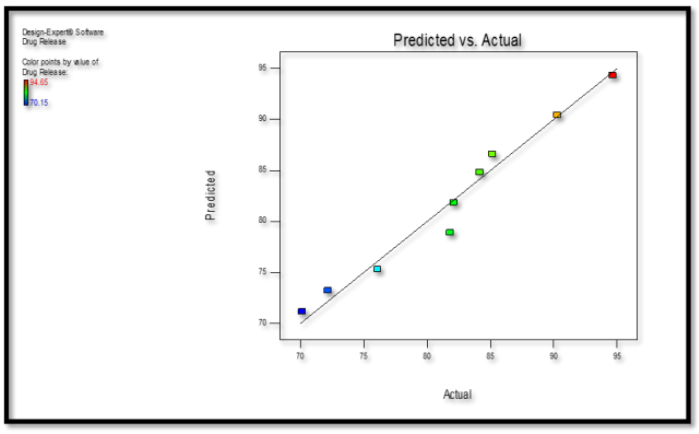

Figure 2: Predicted vs actual.

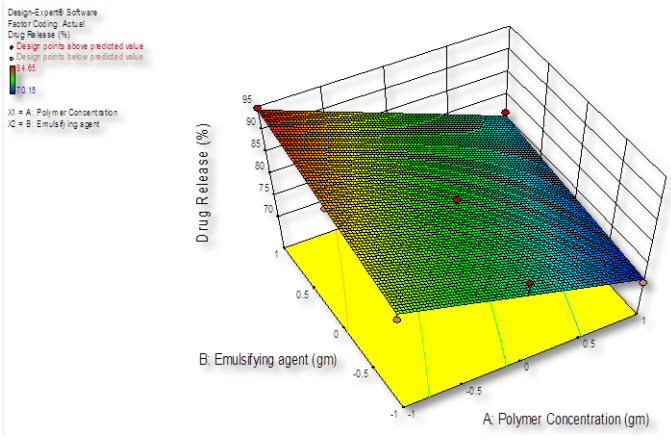

Figure 3: Surface response plot.

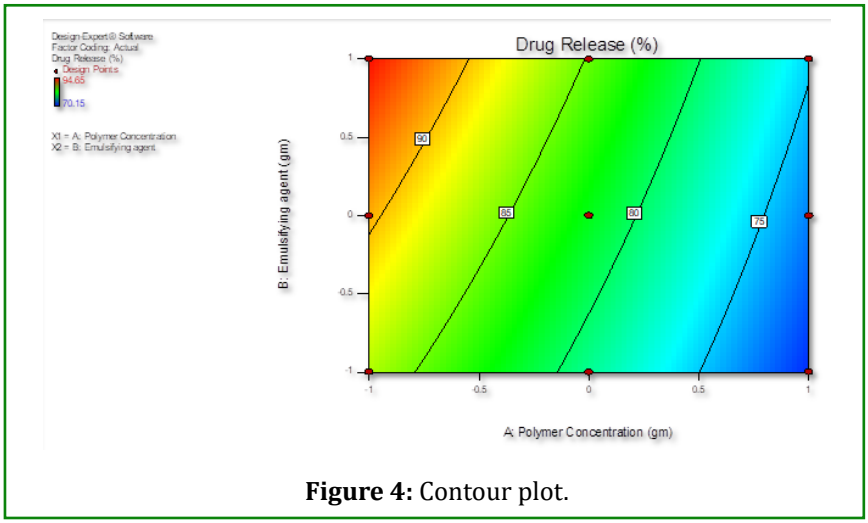

Figure 4: Contour plot.

Equation (Coded values):
Drug Release = +81.85 – 8.61 * A + 2.96 * B – 0.88 * A* B
Equation 1: Equation Coded values

Equation (Actual values):
Drug Release = +81.84889 – 8.60833 * Carbopol 940 + 2.96167 * Emulsifying agent – 0.88250 *Carbopol 940 * Emulsifying agent
Equation 2: Equation of Actual value

Conclusion

On the basis of results of preliminary batches, Carbopol grade, concentration of Carbopol and concentration of emulsifying agents were selected. Experimental batches were formulated by 32 Factorial designs by considering two independent variables and percent drug release was set as dependant variable. Comparative studies were performed between emulgel and gel. Emulgel formulations were light brown viscous creamy preparation with a smooth homogeneous structure and glossy appearance while gel was brown transparent preparation. Increase in the concentration of polymer formation of aggregates and lumps in formulations. There was no significant change in pH values as a function of time for all formulations.

The Rheological property was tested by Brookfield viscometer and it can be concluded that increase in concentration of polymer increases viscosity of formulation. Gel formulation showed greater viscosity than emulgel. Formulations containing lowest concentration of polymer were found to be good.

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