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FORMULATION, OPTIMIZATION AND EVALUATION OF GLYCEROGELATIN INSITU FILM CONTAINING ETHANOLIC LEAF EXTRACT OF CALOTROPIS GIGANTEA

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ABSTRACT

The aim of the study was to design, optimize and evaluate transdermal glycerogelatin insitu film containing *Calotropis gigantea* leaves extract for the treatment of Arthritis. Skin is considered as an important route of administration for both local and systemic effects. Topical film forming systems are developing drug delivery system ment for topical application to the skin, which adhere to the body, forming a thin transparent elastic film which provide delivery of active ingredient to the body tissue. The formulation was optimized by mixture design (design expert software, version 11.03) with glycerin, gelatin, water as the factors and spreadability, elasticity, drying time, tensile strength as the responses. *Calotropis gigantea* having significant anti -inflammatory potential and its ethanolic extract was incorporated into the optimized formula of glycerogelatin insitu film. The optimized formula contained 1.5% of drug exract and showed a drug release of 79 % at 8th hour, and 96% in 24 hour time period.

KEYWORDS: Arthritis, insitu film, *Calotropis gigantea*, Optimization, Mixture design.

INTRODUCTION

Arthritis is a chronic inflammatory disease that affects people of age ≥60. It is reported to affect 14-47% of Indian population.[1-2] Hormonal, genetic, aging, metabolic and mechanical factors regulate the biology of the articular cartilage by complex molecular mechanisms.[3] Rheumatoid arthritis is both an extravascular immune complex disease and a disorder of cell-mediated immunity leads to chronic inflammation, granuloma formation and joint destruction. *Calotropis gigantea R.Br* (Asclepiadaceae) known as Arka and Jayanti in

Ayurveda, have been widely documented in the ayurvedic and traditional medical literature for various therapeutics applications. Traditionally extracts and preparations from roots and leaves are used against rheumatism, wounds, piles, tuberculosis and cancer.

Film forming systems (FFS) are novel approach which can be used as an alternative to the conventional topical and transdermal formulations. The polymeric solution applied to the skin as a liquid turn to an film in situ by solvent evaporation with in few minutes.[4-6] Transdermal drug delivery system (TDDS) can provide some desirable performances, such as avoiding gut and hepatic first-pass metabolism, improving drug bioavailability, reducing dose frequency and stabilizing drug delivery profiles, easy application, avoid fluctuation of drug level, spreads easily. Glycerogelatins are melted before application, cooled to slightly above body temperature and applied to the affected area.[7-9] Following application, the glycerogelatin hardens, is usually covered with a bandage. The formulation was optimized by design expert (11.03) with gelatin, glycerin, water is factors spreadability, elasticity, drying time, and tensile strength is responses. 14 formulations were prepared and evaluated for tensile strength, percentage moisture content, percentage moisture uptake, spreadability, elasticity, drying time. One of the formulae suggested by the software having desirability = 1 as optimal and was prepared and evaluated to conform the responses. To the optimized formula 1.5% drug extract was incorporated and further evaluated for drug content and in vitro drug release study.

MATERIALS AND METHODS

Materials

The Tween 80 was obtained from chemdynes corporation, Rajkot, Gujarat. The other chemicals and reagents used were analytical grade.

Collection of medicinal plant

The Indian medicinal plant *Calotropis gigantea* was collected from the medicinal garden of DPS CPAS Puthuppally, Kottayam, Kerala. India. The plant was authenticated at the Department of botany, CMS College Kottayam, Kerala.

Preparation of Plant extract

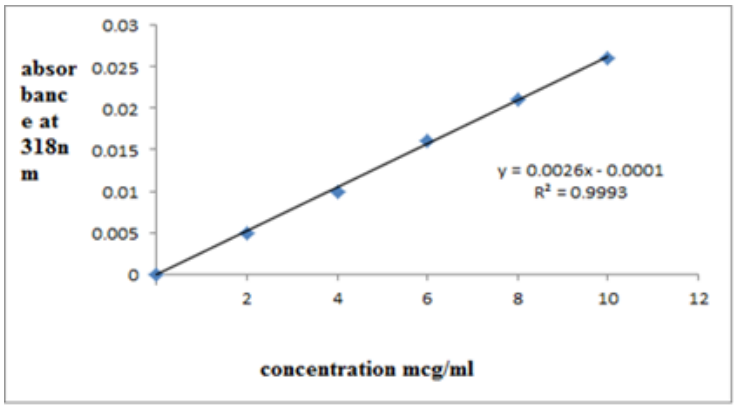
The ethanolic extract of dried leaves of *Calotropis gigantea* was used in the study. The leaves were separated, freed from adhering moisture, dried in sunshade and powdered. The powdered material (32gm) was packed in soxhlet apparatus and extraction was done using

450 ml of ethanol (100%) at 60 to 70 `c for 56 hours. The extracts was filtered using whatman filter paper (No.1) while hot, concentrated in vacuum under reduced pressure using rotary flask evaporator, and dried under vaccum. The ethanolic extract yielded was a dark greenish semi solid residue. The extract was then kept in sterile bottle, under refrigerated conditions at 2 to 4°C until further use.[10,11]

Preparation of standard curve

Accurately measured *Calotropis gigantea* equivalent to100mg and was dissolved in 1ml of ethanol and made-up to 100ml with phosphate buffer PH 7.4 from that 10ml was taken and made up to 100 ml using phosphate buffer from that 0.2ml, 0.4ml, 0.6ml, 0.8ml, 1.0ml were pippeted out and made up to 10ml using phosphate buffer to contain 2, 4, 6, 8, 10 mcg extract/milliliter of solutions.[12]

Calibration curve of calotropis gigantea



UV scanning revealed a prominent peak at 318 nm along with other peak. Components phytol, octadecatrienoic acid (GCMS) has the UV absorbance at 318 nm. Further two components have high anti -inflammatory activity. Therefore 318 nm was kept as the λmax for further studies.

Design of experiments (mixture design)

Mixture design was the technique used to determine the combination of constituent that deliver the desired response with minimum number of runs. The key attribute of mixture design is that proportions of ingredients are used and sum of which is always one. 14 runs with11 different combinations of gelatin, glycerin and water, 3 out of 11 run were duplicated. Observed responses spreadability, elasticity, drying time, tensile strength was then applied to the model to get optimum levels of combination. The design was generated by design expert

11.03 software, the design region for mixture proportion is a simplex i.e., with 3 factor, simplex is a triangle.[13]

Table number 1

Run	Component 1 A:Gelatin grams	Component 2 B:Glycerin grams	Component 3 C:Water grams	Response 1 Spreadability cm/second	Response 2 Elasticity percentage	Response 3 % drying in 15 minutes	Response 4 Tensile strength kg/cm2
1	22.5	12.5	65	148	389	3.276	4695
2	25	15	60	192	375	3.619	4557
3	20	10	70	250	375	3.328	2628
4	27.5	10	62.5	150	333	4.052	3010
5	20	25	55	296	500	2.105	2883
6	22.5	20	57.5	300	457	2.962	2822
7	35	10	55	192	190	3.5	2355
8	35	10	55	200	184	3.2	3015
9	27.5	17.5	55	196	316	3.1	2146
10	20	10	70	288	366	3.088	2110
11	20	25	55	288	311	2.12	2208
12	30	12.5	57.5	118.4	323	4.041	2983
13	20	17.5	62.5	100	388	3.2	1927
14	27.5	17.5	55	176	191	3.619	1651

Preparation of glycerogelatin insitu film

The required quantities of ingredients were measured accurately as per the table. The gelatin was hydrated in warm water at 50° C to get a clear solution to this glycerin was added. The solution was evaluated for various properties are given below.[14,15]

Evaluation of insitu film

Spreadability

A grounded glass plate of 15×30 cm was fixed on the work table and excess of the prepared gel (2 gms) was placed on the grounded slide and was then sandwiched with smaller grounded glass slide (3×10 cm). 1 kg weight was placed on the top of the slide for 3 minutes to expel the air and to get a uniform film of gel between the slides. The excess of gel was craped from the edges. The top slide was hooked horizontally and pulled by 80 grams weight over a pulley. The time taken in seconds by the top slide to cover a distance of 7.5cm was noted. Spreadibility was calculated using the following formula. Shorter time indicates better spreadability.

Spreadibility was calculated using the following formula:

Spreadibility = M x L / T

Where, S = Spreadibility, M = Weight in the pan to pull the slide, L = Length moved by the glass slide and T = Time (in sec.) taken to cover the distance of 7.5 cm.[16,17]

METHODOLOGY

Preparation of film

The molten formula was poured into different molds to get films of uniform size and thickness. Circular molds of thickness 2.5mm was used for the preparation.

Drying time

A definite volume of film forming formula (7.5ml) was poured into the mold fixed on a small glass plate. The initial weight was determined and the loss of weight was further determined at regular intervals for 3 hr. on an electronic balance.[18]

Elasticity/ percentage elongation

Elasticity was determined using custom designed elongation testing apparatus. The dried film of 1cm width was cut out. The film was held strongly between two clips and the upper clip was fixed permanently on a vertical wooden board. The weight was added to the weight pan which was fixed to the lower clip and the weight was gradually increased to effect the elongation of the film. The film length at any time could be read from the scale attach on the wooden board. The initial length between the clips was noted the weight was gradually added until the film was broken. The elongated length was read at from the scale just before breaking the film.

The percentage elongation was determined using formula

$$\% \text{ elongation} = [L_2\text{-}L_1] / L_1 \text{ X } 100$$

Where, L_1 is the initial length and L_2 is the final length of the film before breaking.

Tensile Strength

Tensile strength was determined similar way as elasticity. It is determined using the formula

$$\text{Tensile strength} = (\text{break force} / a \times b) \times (1+L/I) \quad (2)$$

Where “a” is width, “b” is thickness, “L” is length, and “I” is elongation of the films.[19,20]

OPTIMIZATION

ANOVA for Special Quartic model

Response 1: Spreadability

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	58155.49	8	7269.44	36.55	0.0005	significant
(1)Linear Mixture	13292.30	2	6646.15	33.41	0.0013	
AB	4476.82	1	4476.82	22.51	0.0051	
AC	5453.15	1	5453.15	27.42	0.0034	
BC	26210.13	1	26210.13	131.77	< 0.0001	
A^2BC	2503.41	1	2503.41	12.59	0.0164	
AB^2C	17352.35	1	17352.35	87.24	0.0002	
ABC^2	164.61	1	164.61	0.8276	0.4047	
Residual	994.55	5	198.91			
Lack of Fit	8.55	1	8.55	0.0347	0.8613	not significant
Pure Error	986.00	4	246.50			
Cor Total	59150.03	13				

ANOVA for Linear model

Response 2: Elasticity

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	71270.29	2	35635.14	8.45	0.0060	significant
(1)Linear Mixture	71270.29	2	35635.14	8.45	0.0060	
Residual	46367.14	11	4215.19			
Lack of Fit	20635.64	7	2947.95	0.4583	0.8270	not significant
Pure Error	25731.50	4	6432.88			
Cor Total	1.176E+05	13				

ANOVA for Quadratic model

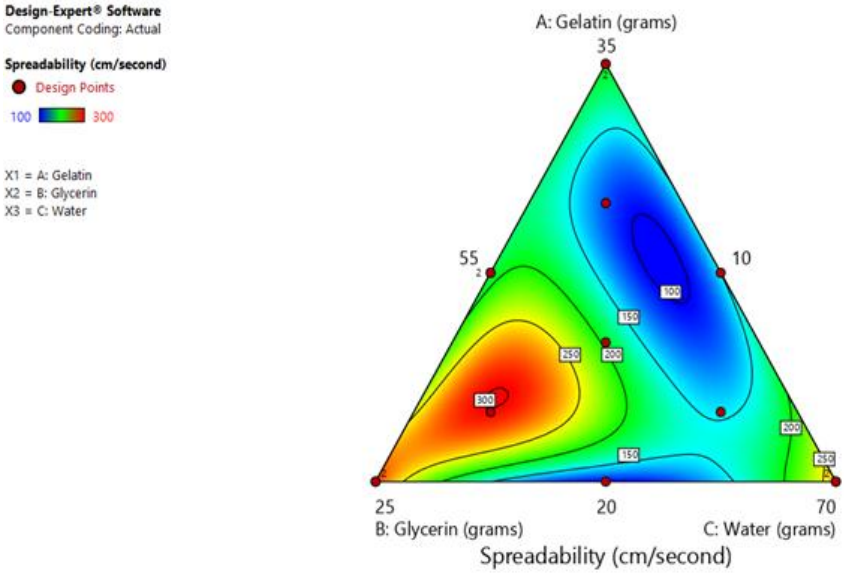
**Response 3: % drying in 15 **

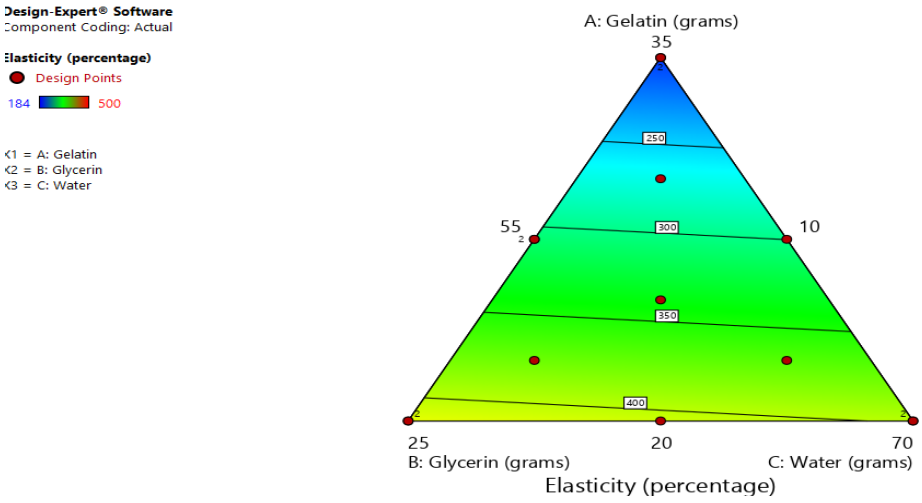
Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3.91	5	0.7817	14.86	0.0007	significant
(1)Linear Mixture	2.57	2	1.29	24.48	0.0004	
AB	0.5655	1	0.5655	10.75	0.0112	
AC	0.5277	1	0.5277	10.03	0.0132	
BC	0.1649	1	0.1649	3.14	0.1146	
Residual	0.4208	8	0.0526			
Lack of Fit	0.2122	4	0.0530	1.02	0.4936	not significant
Pure Error	0.2086	4	0.0521			
Cor Total	4.33	13				

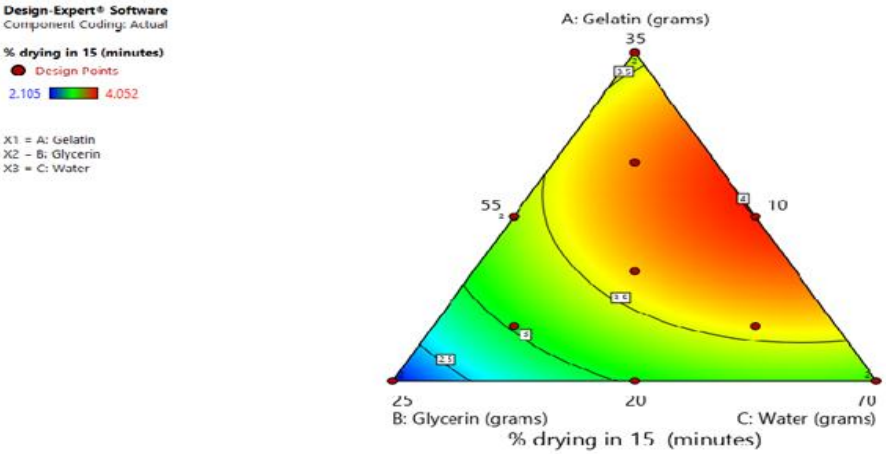
ANOVA for Special Quartic model

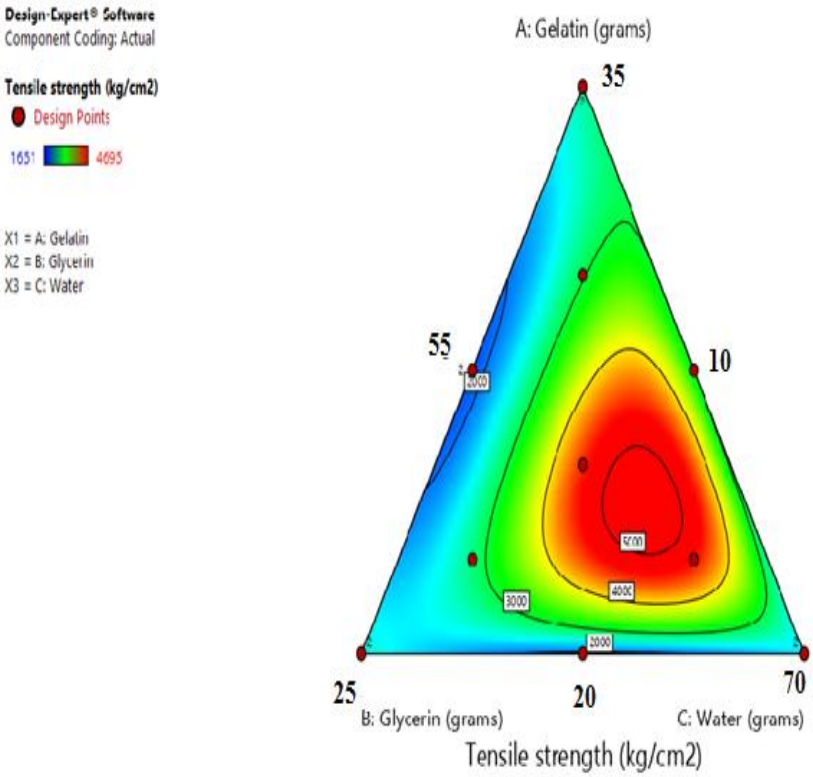
Response 4: Tensile strength

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	9.665E+06	8	1.208E+06	8.57	0.0150	significant
$^{(1)}$Linear Mixture	4.842E+05	2	2.421E+05	1.72	0.2706	
AB	6.827E+05	1	6.827E+05	4.84	0.0790	
AC	1.925E+05	1	1.925E+05	1.37	0.2952	
BC	2.224E+05	1	2.224E+05	1.58	0.2646	
A^2BC	9114.20	1	9114.20	0.0647	0.8094	
AB^2C	30277.76	1	30277.76	0.2148	0.6625	
ABC^2	3.434E+06	1	3.434E+06	24.36	0.0043	
Residual	7.048E+05	5	1.410E+05			
Lack of Fit	2560.46	1	2560.46	0.0146	0.9097	not significant
Pure Error	7.023E+05	4	1.756E+05			
Cor Total	1.037E+07	13				

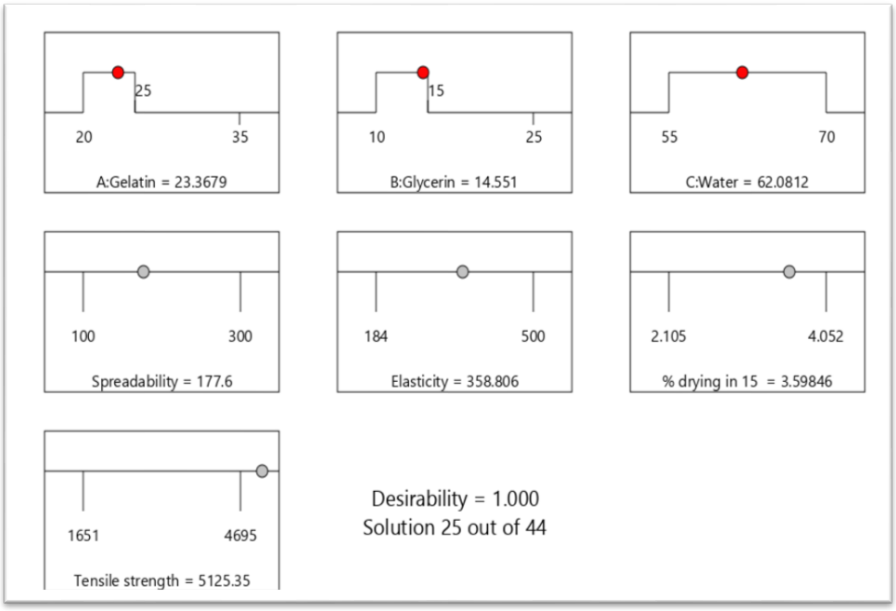








The formula suggested by design expert software 11.03 with expected responses.



Evaluation results of optimized formula.

EVALUATION	PREDICTED	OBSERVED
Spreadability	177.6gm/sec	175gm/sec
Elasticity	358.806%	375%
Drying time	3.59846%	4.80%
Tensile strength	5125.35gm/cm^2	4552gm/cm^2

1. Film thickness

A definite volume of film (7.5ml) was poured into the mold fix on a small glass plate. Film was left overnight for drying and then the film was peeled off and the thickness was determined from three different points on the film. Film thickness was measured by screw gauge.

2. Weight Uniformity

The prepared patches were dried at room temperature for 4hrs before testing. A specified area of patch were cut in different parts of the patch and weighed on digital balance. The average weight and standard deviation values were calculated from the individual weights.

3. Folding endurance

The folding endurance was determined manually by taking a strip of patch (4X2cm) and repeatedly folding it at the same place till it breaks. Folding endurance is considered as the number of times the film is folded at the same place without breaking/cracking.

4. Drug content

Film of specific area (1cm^2) was cut and placed in a 50 ml volumetric flask. To this 25ml of PH 7.4 was added; gently heated to 45°C for 30 minutes and kept for 24 hours; with occcional shaking the volume was made up to 50 ml with phosphate buffer PH 7.4. The solution was filtered and suitable dilutions were made against blank solution which was prepared by following same procedure containing film without drug.[21,22]

5. In vitro drug permeation study

An in vitro drug diffusion study was performed using modified Franz diffusion cell. It consists of a donor and receptor compartment. The receptor compartment filled with 25ml of phosphate buffer PH7.4 as diffusion medium. The cellophane membrane was mounted between the donor and receptor compartment of the diffusion cell. The prepared film 1cm^2 was placed in the donor compartment. The whole assembly was fixed on a hot plate magnetic stirrer and the solution in the receptor compartment was continuously stirred at 100rpm using magnetic beads and the temperature was maintained at 37± 2°C. 1ml of sample of the receptor fluid was withdrawn at predetermined time intervals through the sampling port and replaced immediately with same volume of phosphate buffer. Similar i*nvitro* drug diffusion study was also carried out for a similar composition but with added with 0.5% tween 80.The samples were analyzed for drug content at 318 nm using UV spectrophotometer. The suitable dilution with phosphate buffer PH7.4 and cumulative amount of drug permeated was calculated and plotted against time.[23]

RESULT AND DISCUSSION

1. Folding endurance

Folding endurance of film was (265 ±2) and lowest (234 ± 40).

2. Film Thickness

All the film have uniform thickness throughout. The thickness was found in range of 0.18 mm to 0.29 mm.

3. Percentage Drying

The percentage drying at 15 minute is 0.86 and 3 hrs ranged from 9.7 to 53.

4. Tensile strength

The tensile strength of the film varied with the polymer concentration. It increased proportionately and ranged between 2146 to 4695 g/cm^2.

5. Elasticity

The elasticity of film ranged from 184 to 388.89%.

6. Drug content and percentage entrapment.

The drug content was 5.95mg/ cm^2 and the percentage entrapment was 97.86%.

7. *In vitro* drug permeation study

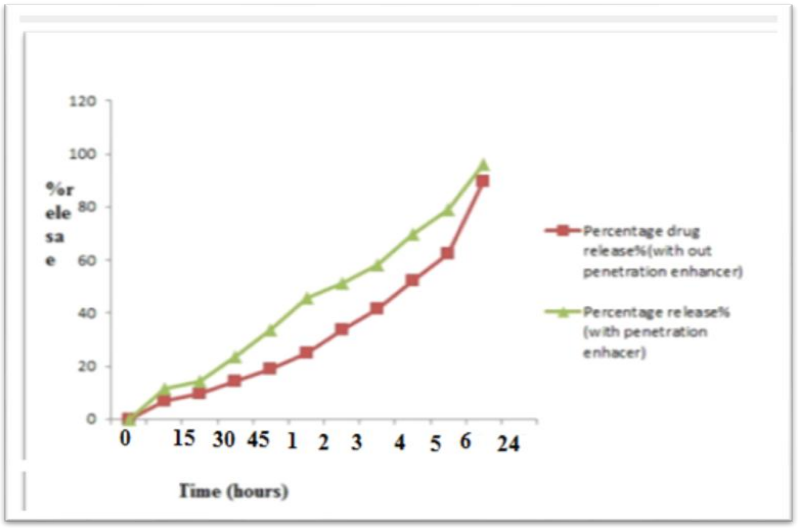


Figure - *in vitro* drug release profile for two formulation.

CONCLUSION

Calotropis gigantea using glycerogelatin insitu films were successfully developed and evaluated. Insitu film were prepares by solvent evaporation method using gelatin, glycerin, water, tween 80, It was evaluated for the spreadability, elasticity, drying time, tensile strength. The results were fed to the software and it suggested n-number of compositions and corresponding responses. One among the different compositions with desirability one was selected as optimum. That was then prepared and the drug extract was incorporated into it. The prepared insitu film was evaluated and the responses were very close to the predicted. Further the permeation was also evaluated with and without permeation enhancers. The optimized insitu film showed good permeation.

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