# Strategy for Improving Skin Permeation by using Topical Nanoparticulate Gel of Aloe Vera and In-Vivo Evaluation using Wistar Rats

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**Abstract**

The objective of the present study was to formulate nanoparticulate gel of *Aloe vera* for improved skin permeation and in-vivo investigation using Wistar rat as an experimental animal. The nanoparticles were prepared by internal gelation method and dispersed in carbopol 940 gel for improving the stability. Fifteen different formulations of *Aloe vera* with sodium alginate and chitosan in different concentrations were prepared. Chitosan-sodium alginate nanoparticles were investigated for differential scanning calorimetry (DSC), Particle size analysis and zeta potential, stability study, in-vitro skin permeation test and anti-inflammatory and skin blanching effect. Anti-inflammatory activity study was performed using carrageenan induced rat paw edema model in male Wistar rats and % inhibition of paw edema at different time intervals with different dose was investigated. Result revealed that 3% w/v sodium alginate concentration, 5% calcium carbonate, 100% w/v and stirring time of 75 minutes were chosen as the best optimized nanoparticles of *Aloe vera* kept in carbopol gel. It was concluded that our formulae could be very promising topical alternative for the treatment of skin fungal infections. However, further preclinical and clinical studies are required. From the *in-vitro* drug release kinetic study, we have concluded that the topical gel prepared from the natural polymer releases the drug from gel by following zero-order release kinetic model upholding the natural polymer as a key attributor to control the release of drug from the topical gel.

**Keywords:** Nanoparticulate gel; Skin-blanching; Anti-inflammatory; Optimization; Wistar rat.

## 1. INTRODUCTION

Nanoparticles are solid, submicron-sized carriers of drug and other substances with diameters ranging from 1 to 1000 nm that may or may not be biodegradable. Depending on the drug encapsulation, nanoparticles are of two types: nanospheres and nanocapsules. Nanospheres are matrix in nature where drugs may be absorbed and sometimes encapsulated within the particle to form a nano-carrier. Nanocapsules are similar to that of a vesicular systems where the drugs are encapsulated inside consisting of an inner liquid core which is surrounded by a polymeric membrane [1-2].

There are different materials like Polymers, lipids and inorganic materials are used to create nanoparticles with a diversified nature in delivery systems that vary with their physicochemical properties and their applications. Labile drugs can be stabilized by Nano-encapsulation. It also provides controlled drug release profile and drug bioavailability may be increased because of their nano size range which can effectively cross the permeability barriers.

Out of different routes, Skin is the promising routes for drug administration because of its large surface area and bypassing first-pass effect. It avoids gastrointestinal irritation and metabolic degradation which is associated with oral administration. The topical route of administrations are used to produce local effect for the effective treatment of skin disorders or sometimes to produce systemic drug effects [3].

Inflammation is the response of living tissues to injury. It associated with a complex arrangement of enzyme activation, release of mediator, fluid extravasations, migration of cell, tissue breakdown of tissue and repair. Conventional drugs for the treatment of inflammation are either too expensive or toxic and not commonly available for the peoples of the rural areas that constitute the major populace of the world. Therefore, this study aimed for observing *Aloe vera* for their anti-inflammatory activity effects in experimental animal models. *Aloe vera* (syn *Aloebarbadensis Miller*) belongs to the Liliaceae family. *Aloe vera* is a juicy plant which grows in dry and hot climate. Mucilaginous tissue present in the *Aloe vera* leaf is widely used to prepare cosmetic and some medicinal products. The peripheral bundle sheath cells of *Aloe vera* which commonly termed Aloe produce strongly bitter, yellow latex that has laxative effects. Total leaf extracts of *Aloe vera* also contain anthraquinones. Pharmacological effects of Aloe include anti-inflammatory, anti-arthritic activity, antibacterial and hypoglycemic effects also investigated *in-vitro* and *in-vivo* in animal models. *Aloe vera* contains 75 potentially active constituents: vitamins, lignin, saponins, salicylic acids, amino acids enzymes, minerals and sugars. The most significant constituents of Aloes are the two Aloins, namely Barbaloin and Isobarbaloin. Aloe leaf exudates also shows anti-diabetic and cardiac stimulatory activity. *Aloe vera* is the promising substances known to effectively decrease inflammation and promote wound healing. *Aloe vera* gel also helps in healing of burns, other cutaneous injuries and ulcer [4-6].

## 2. MATERIALS AND METHODS

### 2.1. Chemicals and Reagents

All the reagents used for experimental purpose were of analytical grade. *Aloe vera* Extract was gift sample from Palsons Derma Pvt. Ltd., Kolkata, West Bengal, India. Span 80, Sodium Alginate and Carbopol-940 was from Loba Chemic Pvt. Ltd. (Mumbai). Chitosan was from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Light Liquid Paraffin and Glacial Acetic Acid was from Merck Specialities Pvt. Ltd.

### 2.2. Preparation of nanoparticles by Internal Gelation Method

Sodium Alginate, Starch Soluble and Chitosan Aqueous solution was prepared by suspending them in distilled water. Solution was stirred on an orbital shaker for overnight. The solution was kept for one hour to allow deareation. Drug (Aloe vera Extract) was added to the above solution. An aqueous suspension of Calcium Carbonate was added to previous solution after sonicated for 30 minutes. Then the above dispersion was emulsified with liquid light paraffin and an emulsifier (Span 80, 1.5 % w/v). This w/o emulsion was prepared by using mixing impeller with a rmp of 1600. 15 minutes of emulsification and continued agitation was done and Light Liquid Paraffin was added to induce gelation with subsequent addition of different amounts of glacial acetic acid. After 60 minutes, the oil particle suspension was added with gentle mixing to an acetate buffer solution (pH 4.5, U.S.P XXVIII) with dehydrating solvents (100 ml) followed by centrifugation (10,000 rpm for 10 minutes). The nanoparticles were lyophilized fallowed by frozen in an alcohol bath at 50°C [4-7].

### 2.3. Preparation of Aloe vera Nano gel

Then Carbopol- 940 (2%) was dispersed in distilled water with glycerol (10%) and allowed to swell overnight. The prepared nanoparticles were dispersed in hydrogel and mixed using a high-speed stirrer at 1000 rpm for 5 min. pH was adjusted to the resultant mixture by drop wise addition of Triethanol amine to adjust the pH to 7. The topical Aloe vera gel was stored in a container maintaining 4°C [8-9].

**2.4. Formulation Design**

Design of Experiment (DOE) was used for preparation and optimization of *Aloe vera* topical Nano gel and effects of independent variables were investigated by using the response of dependent variables.

Sodium Alginate concentration in % w/v ($X_1$), Calcium Carbonate concentration, % w/v ($X_2$) and Stirring Time in Minutes were used as independent variables to determine the response at drug release at 8 hour ($Y_1$) and entrapment efficiency ($Y_2$) respectively [10].

**2.5. Statistical Optimization**

RSM was employed for Statistical Optimization using 45 days Trial Version of Design-Expert software (Version 8.0.6, Stat-Ease Inc., and Minneapolis, MN). Statistical validity of the model was established on the basis of Analysis of variance (ANOVA) and the 3D response graphs were plotted using Design-Expert software.

3D surface plots and 2D contour plots were obtained based on model polynomial functions using Design Expert Software. Ten optimum checkpoints were selected from the experimental data and polynomial equations. According to the ten checkpoints, formulations were prepared and evaluated for various response properties. The obtained experimental data were compared with the predicted value and were analyzed (Tables 1 and Table 2). $3^2$ full factorial designs by using 3 independent variables at 2 levels were used for obtaining response model. The general form of multiple linear regression analysis model is represented by following equation:

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + + \beta_{33}X_3^2$$

Where, Y is the determination value index. $\beta_0$ is the intercept representing the adequatic average of all the quantitative outcomes. $\beta_1$ to $\beta_{33}$ are all coefficients calculated from the observed experimental value of Y.

$X_1$, $X_2$, $X_3$ are all the coded level of factors [11-12].

**2.6. Characterization of prepared nanoparticulate gel formulation**

***2.6.1. Differential scanning calorimetry (DSC) study***

DSC study was performed by using differential scanning calorimeter (Shimadzu Corp, Model TA-60 WSI), which was previously calibrated with indium. Sample of pure *Aloe Vera*, Sodium Alginate, Carbopol and their mixture was subjected to Differential Scanning Calorimetry analysis. Analysis was performed on 5mg samples sealed in standard aluminium pans. Thermograms were obtained as a scanning rate of 20$^o$C/min. Each sample was subjected for the study between 0$^o$C - 300$^o$C. The temperature of maximal excess heat capacity was defined as the phase transition temperature [13].

***2.6.2. Analysis of particle size and Zeta potential***

The average particle size (Z-average size) and Polydispersity Index (PI) was measured by Photon Correlation Spectroscopy (PCS, Nano ZS90, Zetasizer, Malvern Instruments, Crop, U.K) at 25$^o$C under fixed angle of 90$^o$ in disposable polystyrene curettes. The measurement was obtained using a He-Ne laser of 633 nm. Multi-scattering phenomenon was not observed. Deionized water was used to dilute the nanoparticle formulation to a proper concentration. Surface charges was determined by Electrophoretic cell.

***2.6.3. Stability study***

Stability testing of prepared Aloe Vera Nano particulate gel was evaluated for three consecutive batches at different temperature conditions, that is, refrigeration temperature at 4$^o$C - 8$^o$C, room temperature at 25$^o$C ± 2$^o$C and at temperature 45$^o$C ± 2$^o$C for 30 days. During the study Aloe Vera nanoparticulate gel formulations were kept in stability chamber with aluminum foil packing in a glass vial. The samples were withdrawn at different time intervals during the period of days (1, 3, 7, 14, 30 days) and drug content was analyzed using UV-VIS Spectrophotometer (Shimadzu-1700, Japan).

**2.7. *In-vivo* study**

***2.7.1. Animal Model***

Male Wistar Rat of 170-200 g weight was selected for the *in-vivo* study of the *Aloe vera* Nano gel. Optimum and a standard environmental condition with standard diet was maintained for the animals before the *in-vivo* study. . The experimental protocol was approved by local animal ethical committee. The *in-vivo* study was performed with optimized formulation (by RSM) of *Aloe vera* nanoparticulate gel. The anti-inflammatory and skin blanching activity was performed under *in-vivo* investigation [14].

***2.7.2. In-Vitro Skin permeation test***

Skin permeation study of *Aloe vera* nanoparticles was performed using hairless abdominal skin (3.5 sq. cm.) of Wistar rat of weight ranges between 150 - 200 g, using modified Franz diffusion cells. After getting approval of the animal ethics committee animals were sacrificed. Hair on the dorsal side of the animal was removed. After removing the hair, the skin was rinsed with physiological saline and fat tissue was chopped carefully to get uniform thickness. The skin is clamped between the donor and the receptor chamber of vertical diffusion cell. The receptor chamber is filled with freshly mixture of saline and ethanol (7:3 v/v). Ethanol was used to solubilize *Aloe vera*. The diffusion cells were maintained at 37$^{o}$C using a re-circulating water bath. The fluid is continuously rotated at 300 rpm. The nanoparticulate gel was placed uniformly on the dorsal side of rat skin in the donor chamber.

At 1-4 hours, 0.5 ml of sample is periodically withdrawn and replaced with same amount of saline and 95% ethanol. The permeation rate of *Aloe vera* through rat skin is calculated from the slope of the linear portion of the cumulative amount permeated per unit area versus time plot [15-16].

***2.7.3. Anti-inflammatory activity study***

The prepared Aloe Vera nanoparticulate gel formulations were subjected for anti-inflammatory activity study using a carrageenan induced rat paw edema model. First the animals were anesthetized by using 100µl of a 1% w/v λ-carrageenan of Sigma-Aldrich in saline solution for the inflammation to induced in male Wistar rat. The solution was carefully injected into the planar surface of the left hind paw of the rats. The same volume of sterile saline was given to the contra-lateral paw. Four groups of animals were subjected for topical anti-inflammatory study using carrageenan induced paw edema of Wistar rat. A same group of rats was also used as control and remained untreated and they only received carrageenan solution [17- 19].

The formulation was applied in 100, 200, 300, 400 mg of drug/kg body weight of rat in the plantar surface of the hind paw of the male Wistar rat with gentle rubbing for 30 times. After one-hour inflammation was induced in individual rats. The % inhibition of paw volume was measured at 0, 1, 2, 3, 4, 5 hours immediately after induction of carrageenan by plethysmometer using mercury displacement method. Percentage inhibition of paw-edema was calculated for both control and test group by using following method:

% Inhibition = $(V_c - V_t) / V_c$

Where, $V_c$ is the inflammatory increase of paw volume for control group and $V_t$ is the inflammatory increase of paw volume for test group.

***2.7.4. Skin-blanching study***

Skin blanching (or vasoconstriction) response technique provides information on effectiveness and efficacy of the formulation in comparison with the topical availability of Aloe Vera Nano particulate formulations. The vasoconstriction assay has been used in many investigations for visual evaluation of the degree of blanching.

In this study, a Chromameter was utilized to measure and compare the vasoconstrictor effect of Aloe Vera Nano particulate gel. Chroma meters are compact portable instruments used for the measurement of surface color based on the tristimulus analysis of a reflected xenon light pulse. This is a noninvasive technique which involves only one investigator for visual scoring. Chroma meter records three-dimensional color reflectance including "*L*", "*a*" and "*b*". *L* represents luminance, which is related to the brightness value with the range of 0 to 100 meaning pure black to pure white. The balance between red (100)/green (−100) and yellow (100)/blue

(−100) is represented by "*a*" and "*b*", respectively. Blanching effect was formed after administration of formulation on male albino Wistar rat. The effect was measured with the Chroma meter and correlated with the results of ex-vivo permeation studies. The difference in color (Δ*a*) between the gel-treated site and the untreated site as the control were visual evaluated [20].

## 3. RESULTS AND DISCUSSION

### 3.1. Preparation and Statistical Optimization of Aloe verananoparticulate gel

*Aloe vera* nanoparticulate gel was formulated successfully by Internal Gelation Method using Sodium Alginate confirming the concept of producing controlled release nanoparticles. Use of Response Surface Methodology (RSM) has been proved to be useful tool for development and optimization. Mathematical relationship by polynomial equation for the response for measured response drug release at 8 hour and entrapment efficiency was taken as a dependable variables obtained from Stat-ease software. The polynomial equations relating the different responses and independent variablesare:

$$Y_1 = 69.79 + 1.55X_1 - 1.12X_2 + 0.42X_3 + 0.23X_1X_2 + 0.14X_1X_3 - 1.23X_2X_3 + 2.56X_1^2 + 0.67X_2^2 + 0.87X_3^2$$

$$Y_2 = 36.82 + 0.60X_1 + 0.83X_2 - 1.40X_3 - 4.39X_1X_2 + 1.35X_1X_3 + 6.25X_2X_3 - 1.89X_1^2 + 6.05X_2^2 + 2.55X_3^2$$

It was revealed from the above equation that the quantitative effect based on process variables and their interaction on the response for estimating the model significance, ANOVA was determined by Design Expert Software using 5% significance level with p-value less than 0.05 which is described in Tables 3 and Table 4. The Response Surface analysis of *Aloe vera* extract for drug release at 8 hour and entrapment efficiency represented by 3-dimensional analysis for the studied response properties. The response changes the variable in a linear and descending manner.

The 3-dimensional plot for the studied response properties of drug release at 8 hour and drug entrapment efficiency are depicted in (Fig. 1 and 2). The independent variables showed greater influence on drug release. The improvement in drug release in presence of Sodium Alginate of certain level is depicted in (Fig. 1) but above the level there is no significant improvement is observed. However, a very little effect of Calcium Carbonate concentration was observed on drug release at 8 hours. The effect of Calcium Carbonate and Sodium Alginate concentration on drug entrapment efficiency is described in (Fig. 2), where it is shown that Calcium Carbonate concentration has no effect on entrapment efficiency. The optimization of the independent variables, which was carried out taking, optimized release profile and entrapment efficiency. The system has generated 25 solutions in steepest analysis out of which 10 formulations were selected. For all 10 checkpoints of the formulations the result of nanoparticulate gel is found to be within limit where all checkpoints were compared with their predicted and experimental values on response variables and percentage error prognosis. The linear correlation plot demonstrated the values of $r^2$ indicating the excellent goodness of fit. Comprehensive evaluation formulation F8 was chosen as a best-optimized composition.

### 3.2. Differential scanning calorimetry (DSC) analysis

DSC thermogram of Aloe-Vera pure drug and drug polymer physical mixture was evaluated by DSC of Pyris Diamond TG (Model: Maker-Perkin Elmer, Singapore) with nitrogen atmosphere 150 ml/min. Platinum crucible was used with alpha alumina powder as reference.

The corresponding DSC thermograph of pure drug and its physical mixture is nearly superimposible to each other, indicates there is no interaction between drug and polymers. That is why there is no significant change in thermographic peak. DSC thermograms of pure drug and physical mixture are shown in (Fig. 3 and Fig. 4).

### 3.3. Particle size analysis and Zeta potential

The average particle size (Z-average size) and polydispersity index (P.I) were measured by photon correlation spectroscopy (PCS, Nano ZS90 Zetasizer, Malvern Instruments, Corp., U.K.) at 25$^o$C under a fixed angle of 90$^o$ in disposable polystyrene curettes.

The measurements obtained using a He-Ne laser of 633nm are shown in (Fig. 5). No multi scattering phenomenon was observed. The pH values of 1% aqueous solutions of the prepared gels were checked by using a calibrated digital pH meter (Sartorius, GD103) at constant temperature.

**3.4. Stability study**

Result of the stability study shows that nanoparticulate gel formulation of Aloe Vera was quite stable at different specified temperature, which is shown in (Fig. 6). Slight decrease in drug content was found for 45$^{o}$C on 30 days. It may be because of elevated temperature for consecutive days.

**3.5. In-vivo study**

***3.5.1. In-vitro skin permeation study***

*In vitro* skin permeation was subjected for each formulation of *Aloe Vera* nanoparticulate gel and cumulative percent of drug release was calculated for each formulation. The observations of *in vitro* drug release are shown (Fig. 7 Fig. 8 and Fig. 9). From the results, it was observed that the F8 formulation has the highest cumulative percent of drug release upto 8 hours as compared to other formulations. The formulation of F8 was chosen as the best topical Aloe-Vera nanoparticulate gel with 3% w/v of Sodium alginate concentration, 5% w/v of calcium carbonate concentration and 75 minutes of stirring time.

Furthermore, the cumulative amount of drug permeated was higher for formulations prepared with combination of intermediate amount of sodium alginate and calcium carbonate indicates that excess amount of both the substance can adversely affect the permeability of the formulation. The amount of drug permeated from F8 formulation at 8 hours was found to be 61.086±3.109, 83.012±2.301 at 10 hours and 92.286±2.229 at 12 hours, which was significantly better as compared to the other existing formulations. Stirring time plays an important role as better results observed with increased stirring time.

The result of the *in-vitro* dissolution study, drug release at 8 hour and drug entrapment efficiency was subjected for statistical optimization and best optimized formulation was subjected for *in vivo* test.

***3.5.2. Anti Inflammatory Effect***

Four groups of animals for test and four groups of animals for control were subjected for this test. Each groups of animals was given the dose of 100 mg/kg, 200 mg/kg, 300 mg/kg and 400 mg/kg of drug and result was measured at different time intervals mentioned in Table 5 and depicted in (Fig. 10).

**3.6 Skin Blanching Assay**

The effect of *Aloe-Vera* on depigmentation of skin, skin blanching study was performed with abdominal skin of anesthetized rat. The hair of the skin was removed by shaving and formulations were applied. The skin was kept untouched for one hour. The degree of balancing was observed at different time intervals before and after application of formulations by using Chroma meter. The instrument was previously calibrated and the baseline readings (zero time) were taken before the application of formulation [21-23].

The present study was performed to formulate and evaluate Topical *Aloe-Vera* nanoparticulate gel to increase the bioavailability, permeability and sustainability. *Aloe-Vera* nanoparticle was prepared using sodium alginate; calcium carbonate and light liquid paraffin was used as lipid phase. The Drug-Excipient compatibility studies conducted through examination of physical stability, FTIR and DSC thermograph. Vividly suggest that there is no interaction between the drug and excepients used in the formulation. *In Vitro* skin permeation study was carried in Franz Diffusion cell using hairless abdominal skin of Wister rat of weight ranges between 150-200 g. The results explicate that F8 formulation has highest percent of drug release having 92.286 percent of drug release at 12 hours. So, the formulation F8 is suited for better bioavailability of the *Aloe-Vera* in the treatment for topical diseases.

## 4.CONCLUSION

*Aloe-Vera* nanoparticles were successfully formulated by internal-gelation method using sodium alginate, confirming that the concept of producing controlled release nanoparticles. The results suggest that alginate is a potentially useful polymer for making controlled release nanoparticles by internal-gelation method. From among all the developed formulation the formula F8 shows drug release. Therefore, it was concluded that our formulation could be very promising topical alternative for the treatment of skin fungal infections. However, further preclinical and clinical studies are required. From the *in-vitro* drug release kinetic study, we have concluded that the topical gel prepared from the natural polymer releases the drug from gel by following zero-order release kinetic model means natural polymer plays important role to controls the release of drug from topical gel.

## CONFLICT OF INTREST

All the authors confirm that the contents of the proposed article have no conflict of interest.

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## Table 1. Independent Variables with Coded Value and Actual Value

| Serial No. | $X_1$ (Sodium Alginate Conc.) %w/v | X2 (Calcium Carbonate Conc.) %w/v | $X_3$ (Stirring Time) Minute |
|---|---|---|---|
| $F_1$ | 0 (3) | 0 (5) | 0 (60) |
| $F_2$ | 0 (3) | -1 (4) | 0 (60) |
| $F_3$ | 0 (3) | 1 (6) | 0 (60) |
| $F_4$ | 1 (4) | -1 (4) | 1 (75) |
| $F_5$ | -1 (2) | 1 (6) | -1 (45) |
| $F_6$ | -1 (2) | 0 (5) | 0 (60) |
| $F_7$ | -1 (2) | 1 (6) | 1 (75) |
| $F_8$ | 0 (3) | 0 (5) | 1 (75) |
| $F_9$ | 1 (4) | -1 (4) | -1 (45) |
| $F_{10}$ | 1 (4) | 0 (5) | 0 (60) |
| $F_{11}$ | 1 (4) | 1 (6) | -1 (45) |
| $F_{12}$ | -1 (2) | -1 (4) | 1 (75) |
| $F_{13}$ | -1 (2) | -1 (4) | -1 (45) |
| $F_{14}$ | 1 (4) | 1 (6) | 1 (75) |
| $F_{15}$ | 0 (3) | 0 (5) | -1 (45) |

**Table 2. Quantity of Raw Materials used in the Preparation of Formulation as per RSM**

| Sl No. | Drug (mg) | $X_1$ (% w/v) | Starch Soluble (% w/v) | $X_2$ (% w/v) | Chitosan (% w/v) | Stirring Speed (rpm) | $X_3$ (min) | Carbopol 940 (% w/v) |
|---|---|---|---|---|---|---|---|---|
| F1 | 100 | 3 | 500 | 5 | 75 | 500 | 60 | 2 |
| F2 | 100 | 3 | 750 | 4 | 75 | 750 | 60 | 2 |
| F3 | 100 | 3 | 1000 | 6 | 75 | 1000 | 60 | 2 |
| F4 | 100 | 4 | 500 | 4 | 100 | 500 | 75 | 2 |
| F5 | 100 | 2 | 750 | 6 | 50 | 750 | 45 | 2 |
| F6 | 100 | 2 | 1000 | 5 | 75 | 1000 | 60 | 2 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| F7 | 100 | 2 | 500 | 6 | 100 | 500 | 75 | 2 |
| F8 | 100 | 3 | 750 | 5 | 100 | 750 | 75 | 2 |
| F9 | 100 | 4 | 1000 | 4 | 50 | 1000 | 45 | 2 |
| F10 | 100 | 4 | 500 | 5 | 75 | 500 | 60 | 2 |
| F11 | 100 | 4 | 750 | 6 | 50 | 750 | 45 | 2 |
| F12 | 100 | 2 | 1000 | 4 | 100 | 1000 | 75 | 2 |
| F13 | 100 | 2 | 500 | 4 | 50 | 500 | 45 | 2 |
| F14 | 100 | 4 | 750 | 6 | 100 | 750 | 75 | 2 |
| F15 | 100 | 3 | 1000 | 5 | 50 | 1000 | 45 | 2 |

**Table 3. Response 1- Significant Data for Drug Release at 8 hr**

| Source | Sum of Squares | df | Mean Square | F Value | p- value Prob> F | |
|---|---|---|---|---|---|---|
| *Model* | 1217.9 | 7 | 173.99 | 5.77 | 0.0170 | Significant |
| *A- Sodium Alginate* | 1.13 | 1 | 1.13 | 0.037 | 0.8522 | |
| *B- Calcium Chloride* | 0.18 | 1 | 0.18 | 5.980E-003 | 0.9405 | |
| *C- Stirring Time* | 205.94 | 1 | 205.94 | 6.83 | 0.0347 | |
| *BC* | 93.30 | 1 | 93.30 | 3.10 | 0.1219 | |
| $A^2$ | 281.77 | 1 | 281.77 | 9.35 | 0.0184 | |
| $B^2$ | 820.85 | 1 | 820.85 | 27.23 | 0.0012 | |
| $C^2$ | 598.40 | 1 | 598.80 | 19.85 | 0.0030 | |
| *Residual* | 210.99 | 7 | 30.14 | | | |
| *Cor Total* | 1428.90 | 14 | | | | |

**Table 4. Response 2 - Significant Data for Entrapment Efficiency**

| Source | Sum of Squares | df | Mean Square | F Value | p- value Prob> F | |
|---|---|---|---|---|---|---|
| *Model* | 324.78 | 3 | 108.26 | 12.20 | 0.0008 | Significant |
| *D- Sodium Alginate* | 235.64 | 1 | 235.64 | 26.55 | 0.0003 | |
| *E- Calcium Chloride* | 87.61 | 1 | 87.61 | 9.87 | 0.0094 | |
| *F- Stirring Time* | 1.53 | 1 | 1.53 | 0.17 | 0.6864 | |
| *Residual* | 97.63 | 11 | 8.88 | | | |
| *Cor Total* | 422.40 | 14 | | | | |

**Table 5.** Paw Volume in ml (% Inhibition of Paw Edema) at Different Time Intervals with Different Doses

| **Time (hrs)** | **Control (Nil)** | **1st Group (100 mg/kg)** | **2nd Group (200 mg/kg)** | **3rd Group (300 mg/kg)** | **4th Group (400mg/kg)** |
|---|---|---|---|---|---|
| 0 | 0.46±0.012 | 0.44±0.025(4.34) | 0.45±0.031(2.17) | 0.43±0.027(6.52) | 0.43±0.038(6.52) |
| 1 | 0.43±0.022 | 0.4±0.02(6.97) | 0.41±0.031(4.65) | 0.4±0.021(6.97) | 0.38±0.014(11.62) |
| 2 | 0.4±0.016 | 0.37±0.039(7.5) | 0.35±0.051(12.5) | 0.34±0.032(15.0) | 0.31±0.022(22.5) |
| 3 | 0.36±0.019 | 0.32±0.021(11.11) | 0.3±0.037(16.66) | 0.28±0.033(22.22) | 0.27±0.021(25.0) |
| 4 | 0.34±0.018 | 0.28±0.029(17.64) | 0.25±0.025(26.47) | 0.24±0.012(29.41) | 0.21±0.015(38.23) |

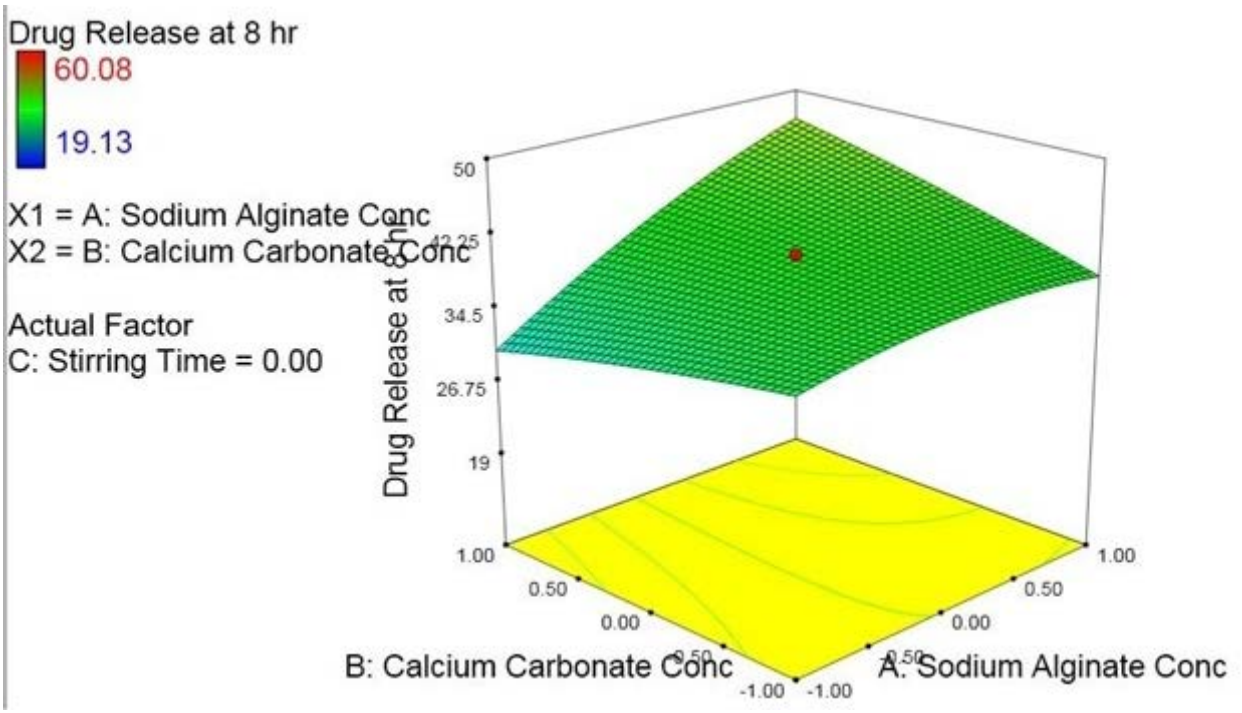



**Figure 1:** 3D response surface plot showing the effect of the Sodium Alginate concentration, Calcium Carbonate concentration, Stirring Time on drug release at 8 hour of the formulation

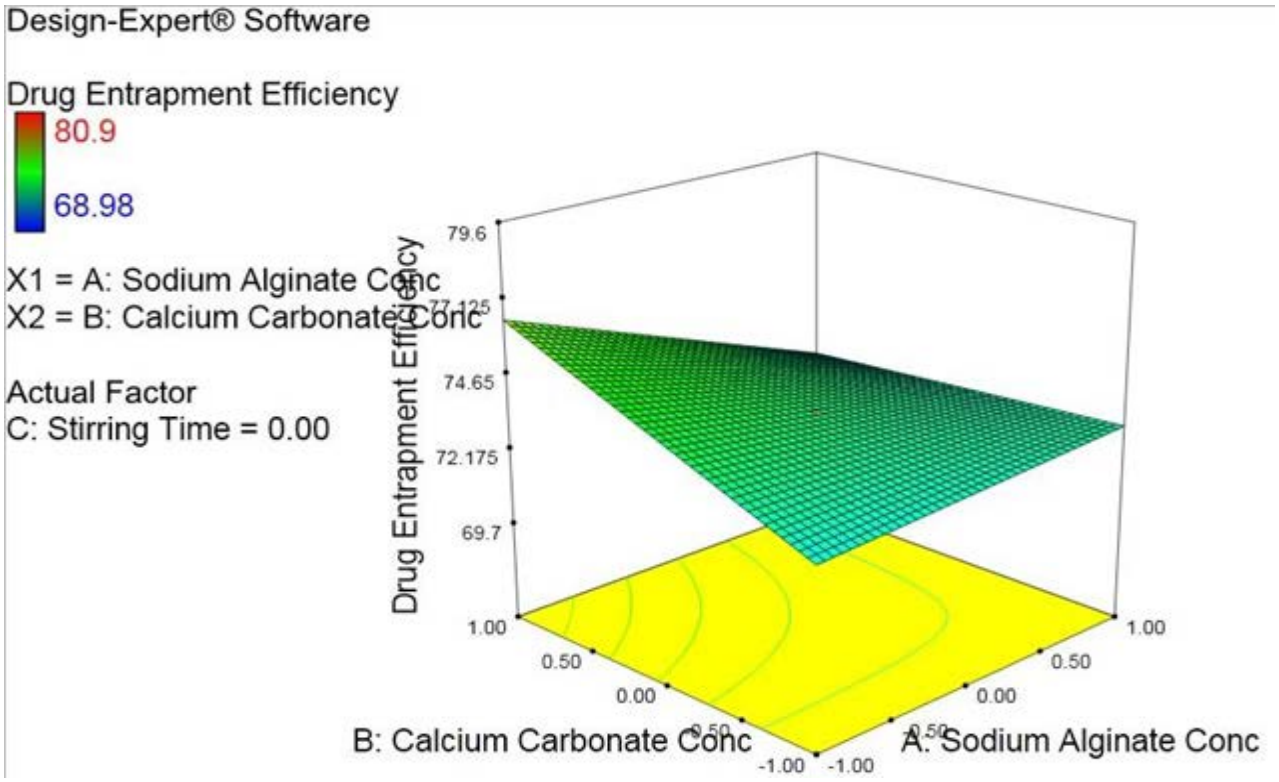



**Figure 2:**3D response surface plot showing the effect of the Sodium Alginate concentration, Calcium Carbonate concentration, tirring Time on entrapment efficiency of the formulation

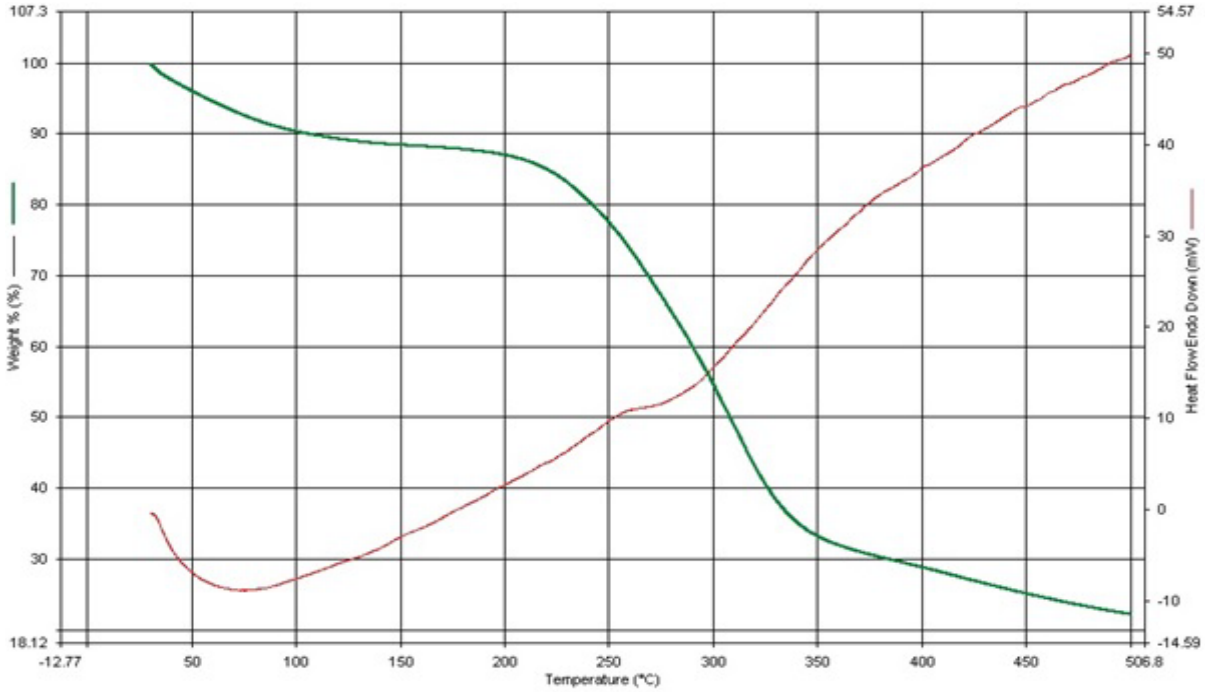



**Figure 3:** DSC thermogram of pure *Aloe Vera* drug

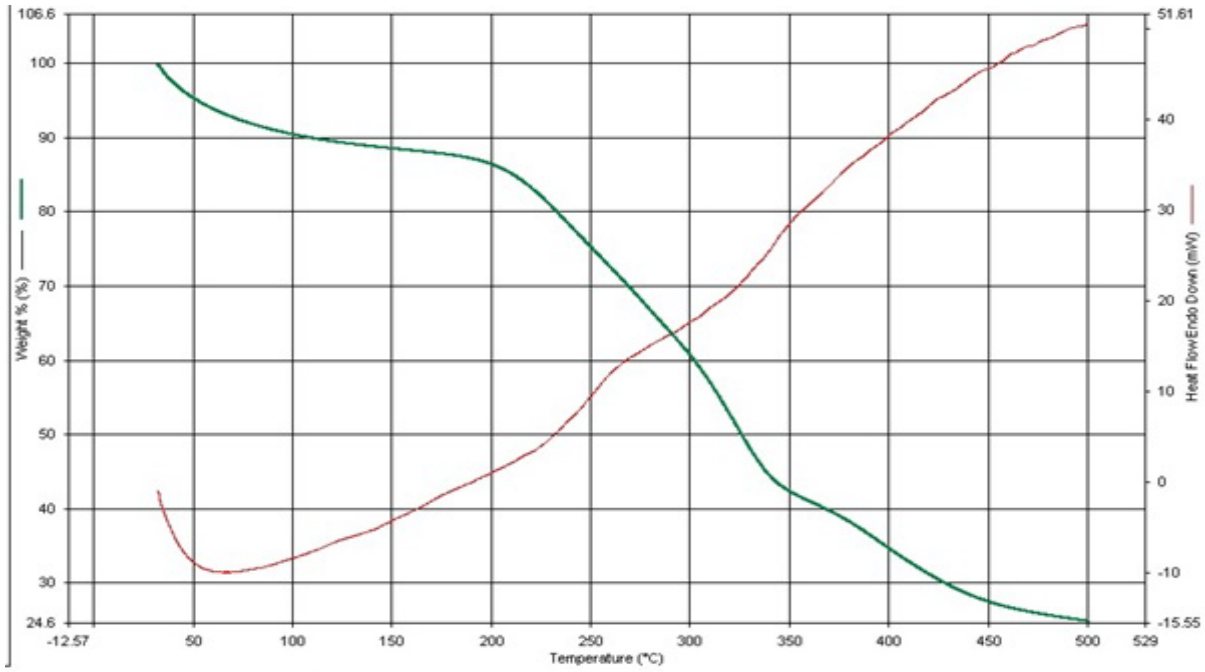



**Figure 4:** DSC thermogram of pure *Aloe Vera* drug and polymer mixture

| | | | |
|---|---|---|---|
| Temperature (°C): | 25.0 | Zeta Runs: | 12 |
| Count Rate (kcps): | 70.6 | Measurement Position (mm): | 2.00 |
| Cell Description: | Clear disposable zeta cell | Attenuator: | 7 |

| | | Mean (mV) | Area (%) | St Dev (mV) |
|---|---|---|---|---|
| Zeta Potential (mV): | -36.2 | Peak 1: -36.2 | 100.0 | 7.08 |
| Zeta Deviation (mV): | 7.08 | Peak 2: 0.00 | 0.0 | 0.00 |
| Conductivity (mS/cm): | 0.0353 | Peak 3: 0.00 | 0.0 | 0.00 |

Result quality : Good

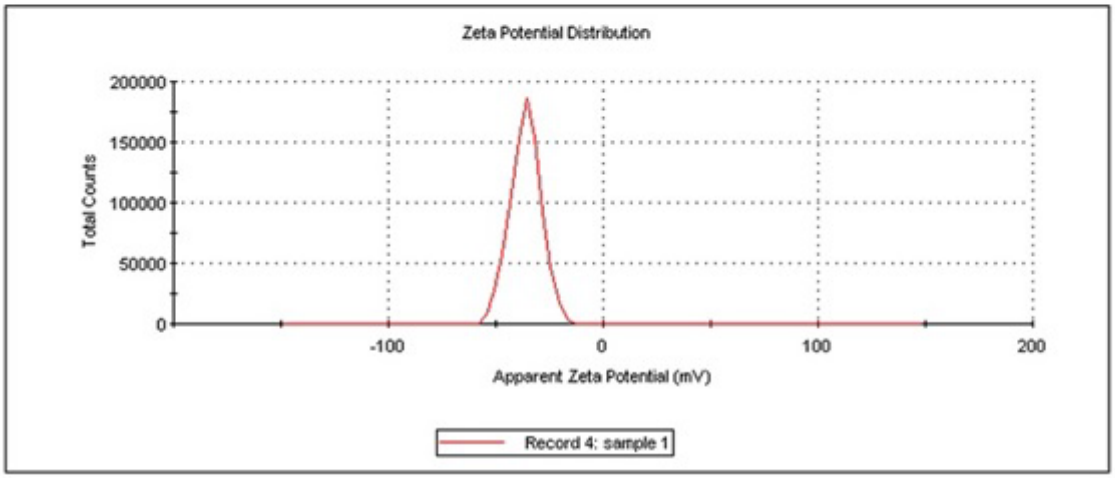
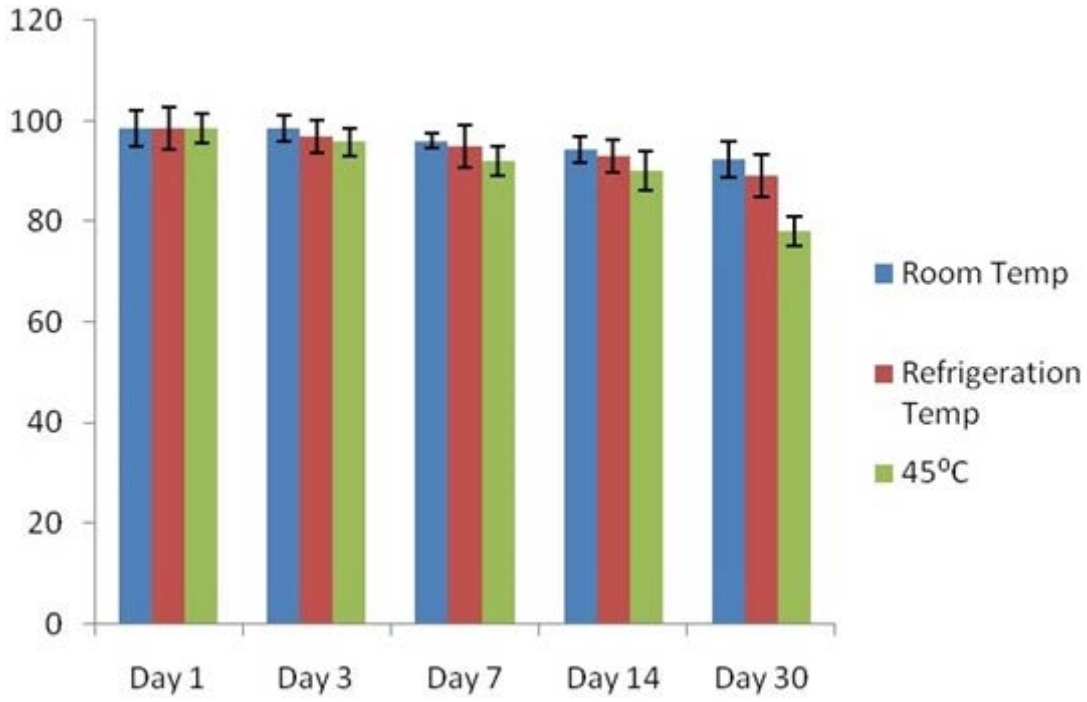



**Figure 5:** Zeta Potential distribution




**Figur 6:** Drug retained in nanoparticulate gel at various temperature conditions for 30 days

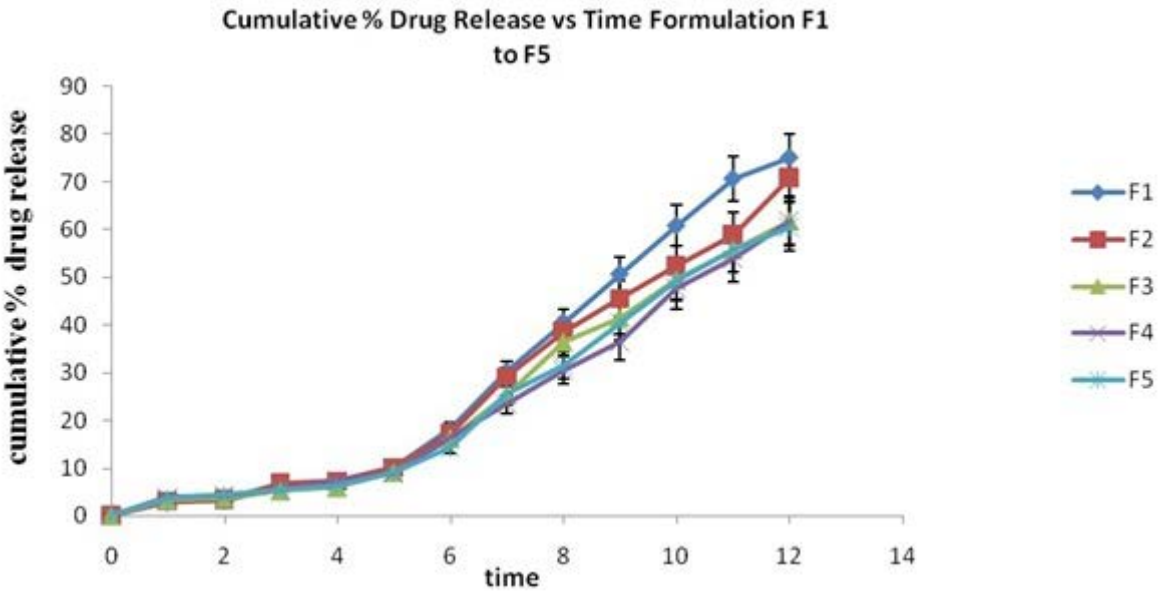



**Figure 7:** Zero order drug release kinetics of F1, F2, F3, F4, F5 batch

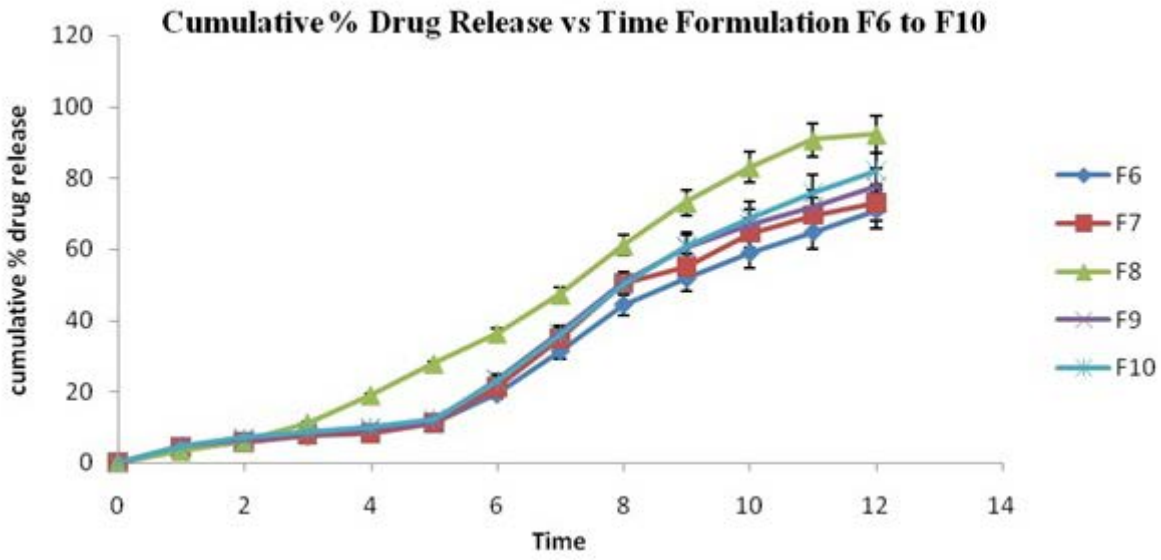



**Figure 8:** Zero order drug release kinetics of F6, F7, F8, F9, F10 batch

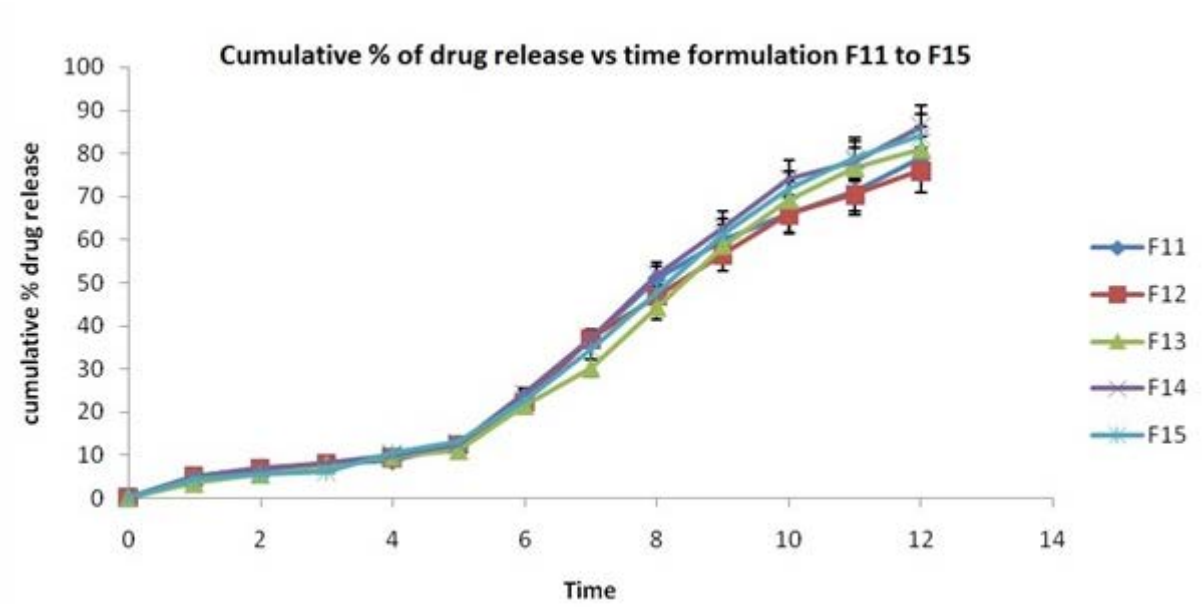



**Figure 9:** Zero order drug release kinetics of F11, F12, F13, F14, F15 batch

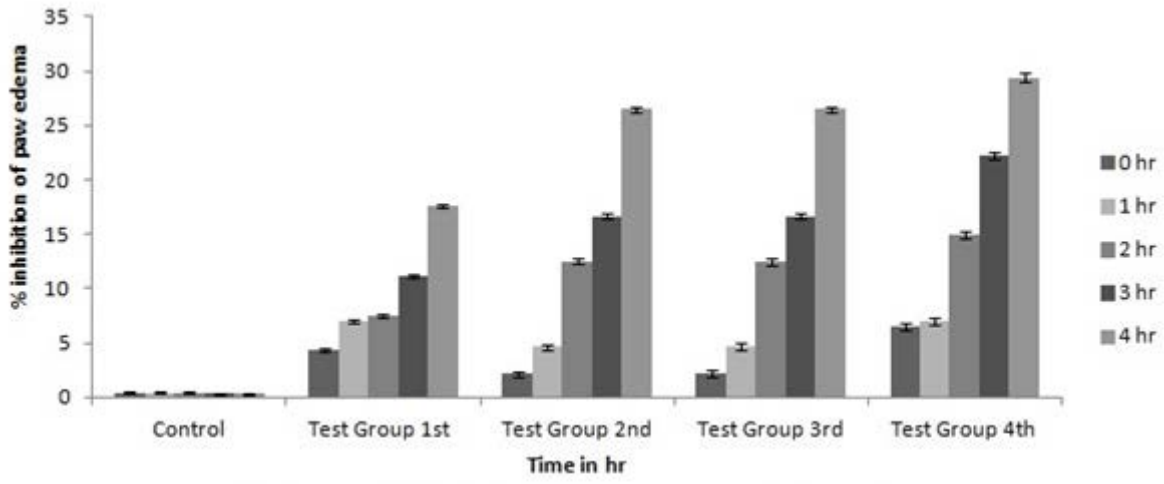



**Figure 10:** Plot of % inhibition of paw edema at different time periods

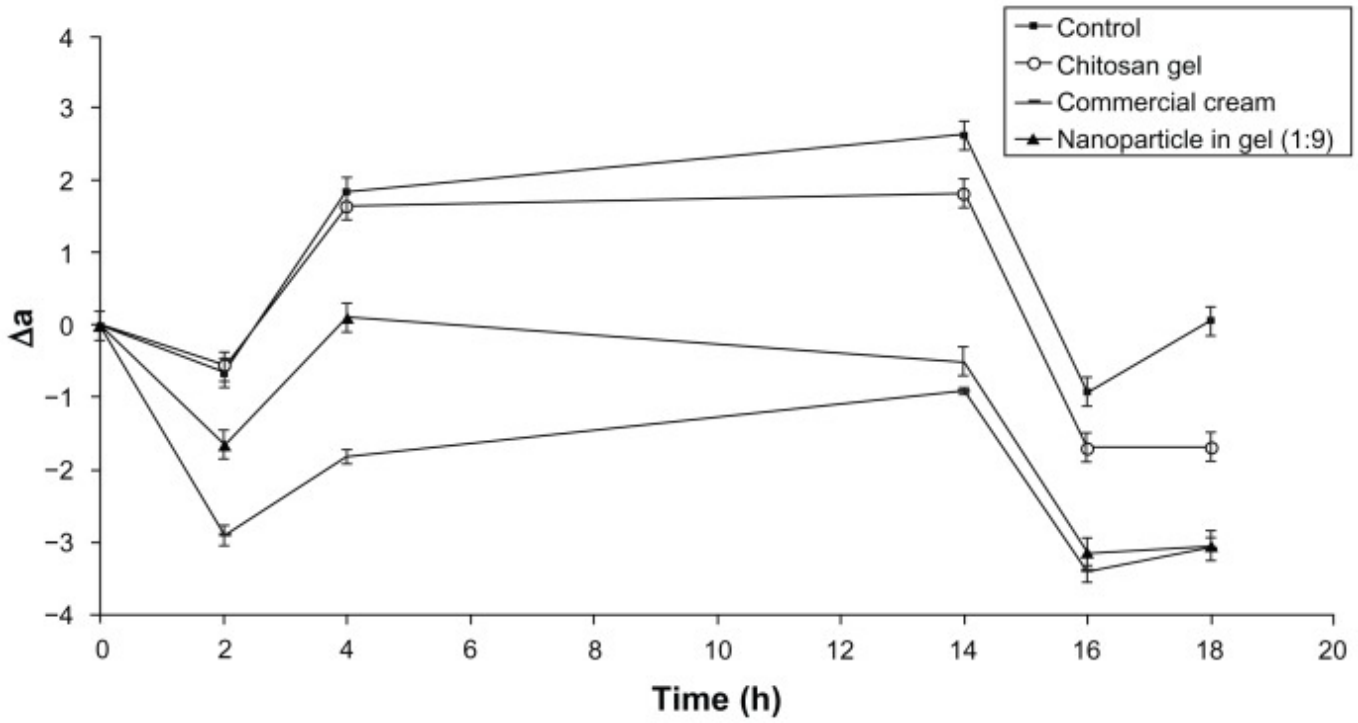



**Figure 11:** Plot for effect of bleaching action of different creams on rat skin at different time periods