

## DEVELOPMENT AND INVITRO EVALUATION OF TRANSFEROSOMAL GEL OF CLOBETASOL PROPIONATE

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### ABSTRACT

Topical delivery of drug means the application of drug to skin for the localized effect, and in transdermal drug delivery system (TDDS) skin is used as a potential route for the delivery of the systemic action of drugs. Transferosomes are the artificial vesicles and the more distort than the standard liposomes. Transferosomes have been delineate to enhance the transdermal delivery of drugs when applied it directly to the surface of skin nonocclusive. Clobetasol propionate is a corticosteroid used to treat skin conditions such as eczema, seborrheic dermatitis, contact dermatitis, and psoriasis. The purpose of this paper was to develop and Invitro Evaluation of Transferosomal gel of clobetasol propionate. The prepare transferosome gel containing clobetasol propionate using different concentration of surfactant and phospholipids. Transferosomes were prepared by thin film hydration technique. This technique is much frequently used owing to its simplicity and practicability and its ability to yield small and uniform vesicles with high drug entrapment efficiency. It was found that the in vitro drug release of formulation CT1G2 (95.10±0.09) was best explained by higuchi model as the plot showed the highest linearity, the value of R<sup>2</sup> found to be highest for the higuchi model.

**Keywords:** Clobetasol propionate, Glucocorticoids, Higuchi Model, Skin infection, Transferosome, Thin film hydration Technique.

### INTRODUCTION

From the last few decades, the considerable attention has been focused on the development of topical delivery of drugs because there are a number of advantages by this route. Topical delivery of drug means the application of drug to skin for the localized effect, and in transdermal drug delivery system (TDDS) skin is used as a potential route for the delivery of the systemic action of drugs. Some potential advantages of transdermal route found over other than conventional routes such as oral route and parenteral route <sup>1</sup>. Transfersomes are the artificial vesicles, and transfersome are the more distort than the standard liposomes. Transfersomes have been delineate to enhance the transdermal delivery of drugs

when applied it directly to the surface of skin nonocclusively<sup>2,3</sup>. Subsequent to having infiltrated through the furthest skin layers, transfersomes arrive at the more profound skin layer, the dermis. From this last skin district, they are typically cleaned out, by means of the lymph, into the blood dissemination and through the last all through the body, whenever applied under appropriate conditions. Transfersomes would thus be able to arrive at all such body tissues that are open to the subcutaneously infused liposomes. The energy of activity of an epicutaneously applied specialist relies upon the speed of transporter entrance just as on the speed of medication<sup>4,5</sup>.

Clobetasol propionate is a corticosteroid used to treat skin conditions such as eczema, seborrheic dermatitis, contact dermatitis, and psoriasis. It is applied to the surface of skin as a form of cream, ointment, or shampoo<sup>6</sup>. It is also used to treat several auto-immune diseases including alopecia areata, lichen planus (auto immune skin nodules), and mycosis fungoides (T-cell skin lymphoma). It is used as first-line treatment for both acute and chronic GVHD of the skin<sup>7</sup>.

Clobetasol propionate is used cosmetically by dark-skinned women for skin whitening, although this use is controversial. Its use should be short term only and only if other weaker corticosteroids are not effective to the skin<sup>8</sup>. Clobetasol propionate is, along with mercury and hydroquinone, "amongst the most toxic and most used agents in lightening products." Many products sold illegally have higher concentrations of clobetasol propionate than is permitted for prescription drugs<sup>9</sup>.

The purpose of this research is to create and characterise a clobetasol propionate transfersosomal gel as a viable topical delivery.

## **MATERIALS AND METHODS**

**Preparation of standard plot of clobetasol propionate in methanol Standard Solution:** Accurately weighed 100mg Drug was dissolved in 100ml of methanol solution to get a solution containing 1mg/ml.

**Dilutions:** Aliquots of standard solution were pipette out into 10ml volumetric flasks. The volume was made upto the mark with methanol solution to produce the concentration ranging from 1-10 µg /ml. The absorbance of each prepared solution was measured at 239 nm in Shimadzu UV-1800 spectrophotometer against an appropriate blank (Methanol solution). All the absorbance were conducted in triplicate (n=3)

## **Preparation of Transfersome**

Clobetasol propionate loaded transfersomes were prepared using a thin film hydration technique. Soya lecithin and surfactant in different ratio were dissolved in an organic phase (20 ml, chloroform: methanol, 2:1 v/v) and subsequently drug was added in the above mixture and then mixture was transferred to a round bottom flask (Table 1). The organic phase was evaporated using a rotary evaporator under reduced pressure. The thin, dried lipid film that was formed on the wall of the flask was then rehydrated with

phosphate buffered saline (pH 7.4, 20 ml). The coarse dispersions so formed were sonicated to produce small vesicles and the final formulations were stored refrigerated <sup>10</sup>.

**Table 1: Composition of different Clobetasol propionate containing Transfersome**

| Formulation<br>code | Clobetasol<br>Propionate<br>(w/w) | Soya Lecithin<br>(%w/w) | Surfactant (%w/w) |         |             |
|---------------------|-----------------------------------|-------------------------|-------------------|---------|-------------|
|                     |                                   |                         | Tween 80          | Span 80 | Tween<br>20 |
| CT1                 | 0.05%                             | 80                      | 20                | --      | --          |
| CT2                 | 0.05%                             | 80                      | --                | 20      | --          |
| CT3                 | 0.05%                             | 80                      | --                | --      | 20          |
| CT4                 | 0.05%                             | 90                      | 10                | --      | --          |
| CT5                 | 0.05%                             | 70                      | 30                | --      | --          |
| CT6                 | 0.05%                             | 60                      | 0                 | --      | --          |
| CT7                 | 0.05%                             | 50                      | 50                | --      | --          |

### **In vitro characterization of Clobetasol propionate loaded transfersomes:**

**Physical Appearance:** Physical appearance of all prepared formulation was determined by using visual observation.

### **Vesicle size, polydispersity index, and zeta potential:**

Before analysis, transfersomes were diluted with filtered phosphate buffered saline. The vesicles size, polydispersity index and zeta potential of transfersomes were measured using a Zetasizer Nano ZS (Malvern Instruments, United Kingdom) at  $25 \pm 1^\circ\text{C}$  <sup>11</sup>

### **Entrapment efficiency determination**

Transfersomes (1ml) were transferred to ependorf tubes and centrifuged for 1 h at 40,000 rpm and  $4^\circ\text{C}$ , using an ultracentrifuge (Optima TM Max-E, Beckman Coulter, Pasadena, CA) <sup>12</sup>. After centrifuged, the supernatant was carefully collected and, after suitable dilution, samples were analyzed by UV spectroscopy. The entrapment efficiency was calculated by using the following equation.

### **Entrapment efficiency (%)**

$$= \frac{(\text{Total amount of drug in the vesicles} - \text{Amount of drug detected only in the supernatant})}{\text{Total amount of drug in the vesicles}} \times 100$$

## Preparation of clobetasol propionate loaded Transfersome gel

The clobetasol propionate loaded transfersome gel was prepared by varying the concentration of carbopol 934 (NF) as 0.5%, 1%, 1.5%, 2%. Carbopol 934 forms very good consistency transparent gel at low concentration. Carbopol gel was prepared accurate amount of carbopol was dipped in sufficient amount of water for 24 hrs and the transfersome formulation was added before neutralization the pH of carbopol gel.

**Table 2: Composition of transfersome containing gel**

| Formulation Code | Clobetasol Propionate<br>Containing transfersome | Carbopol 934<br>concentration (%w/w) |
|------------------|--------------------------------------------------|--------------------------------------|
| CT1G1            | Equivalent to 0.05%                              | 0.5                                  |
| CT1G2            | Equivalent to 0.05%                              | 1                                    |
| CT1G3            | Equivalent to 0.05%                              | 1.5                                  |
| CT1G4            | Equivalent to 0.05%                              | 2                                    |

## Evaluation of clobetasol propionate loaded transfersome gel

**Physical Appearance:** Physical appearance of all prepared transfersomal gel formulation was determined by using visual observation shown in Table no 4.

**pH:** For determination pH of the prepared transfersomal gel, pH meter probe was immersed into the gel for upto stabilization of the pH. Then the pH reading was recorded. The pH meter was calibrated before using it to measure the pH of the gel <sup>13</sup>.

**Percentage drug content:** The percentage drug content of all prepared formulation was determined by solubilizing certain amount of gel in methanol and followed by centrifuged at 5000rpm rpm for 1hrs. Then these samples were analyzed using UV spectrophotometer scanning from 200- 400nm.

**Percentage drug release:** To understand the release behavior of transfersomal gel and further to have an insight on the barrier properties of the SC, in vitro release studies through an artificial cellophane membrane were conducted using fabricated vertical Franz diffusion cells with an effective diffusional surface area of 4.153 cm<sup>2</sup> and 14mL of receptor cell volume. Prior to the study, the dialysis membrane was soaked and the transfersomal gel formulation or control (drug suspended in the same solvent of formulations) equivalent to clobetasol propionate was placed in the donor compartment. The phosphate-buffered saline (pH 7.4) filled in the receptor compartment was maintained at 37± 2°C under constant stirring up to 24 h. To prevent the evaporation of the diffusion medium, the donor chamber and the

sampling port were covered by Parafilm. The samples were withdrawn periodically and replaced with an equal volume to maintain constant receptor phase volume. At the end of the study, the samples were suitably diluted and the amount of drug was quantified by UV spectrophotometer.

### Drug release kinetics

The release kinetic was studied by various kinetic models as zero order plot, first order plot, higuchi plot and korsmeyer-peppas. To study the release kinetics of optimized formulation, data obtained from in vitro drug release studies were plotted in various kinetic models: zero order as cumulative amount of drug releases Vs time, first order as log cumulative percentage of drug remaining Vs time, Higuchi model as cumulative percentage of drug released Vs square root of time. The best fit model was confirmed by the value of correlation coefficient near to 1.

## RESULT AND DISCUSSION

### Preformulation studies

#### Preparation of standard curve of clobetasol propionate in methanol

The calibration curve for clobetasol propionate was obtained by using the 1 to 10 µg/ml solution in methanol. The absorbance was measured at 239 nm. The calibration curve of clobetasol propionate as shows in graph indicated the regression equation  $Y = 0.1002x - 0.0532$  and  $R^2$  value 0.999, which shows good linearity in Figure 1.

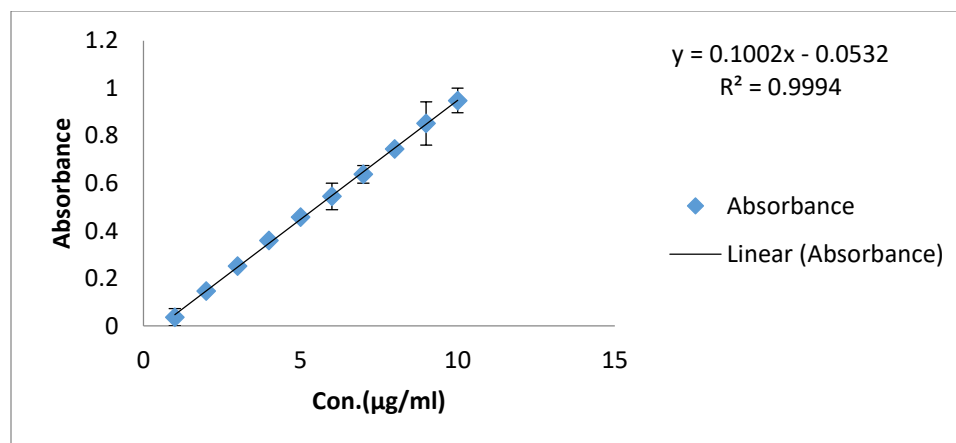


**Figure 1: Standard calibration curve of Clobetasol Propionate in Methanol**

### Partition coefficient and Melting point determination

Partition coefficient was determined as ratio of concentration of drug in octanol to the concentration of drug in phosphate buffer (pH 7.4) and the value were reported as log P. The melting point was observed with the capillary fusion method and shown in Table no 3.

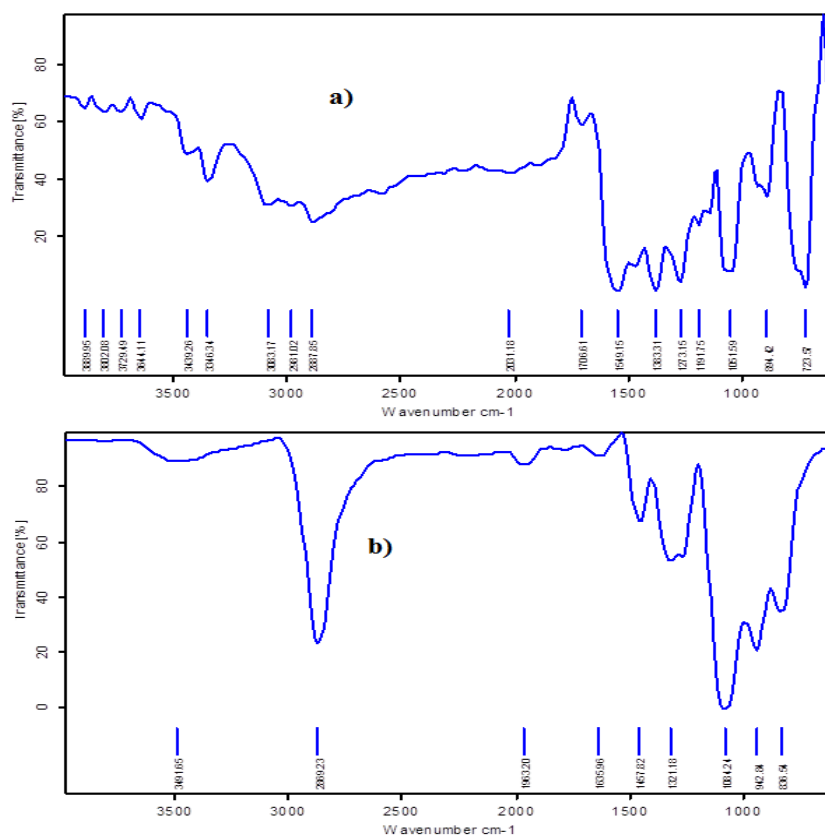
**Table 3: Partition coefficient and observed melting point of Clobetasol propionate Solubility**

| Partition coefficient<br>of drug | Solvent system   | Log p Values* | Observed melting<br>point |
|----------------------------------|------------------|---------------|---------------------------|
| Clobetasol<br>propionate         | Water: n-octanol | 3.24±0.067    | 223-225°C                 |

Clobetasol propionate has maximum solubility in methanol followed dichloromethane. Among different surfactant it has maximum solubility in span 80 as compare to tween.

### FT-IR of Drug and Drug polymer Mixture

Overlay FTIR spectrum of pure drug clobetasol propionate and optimized formulation was shown in figure no 02.


**Figure 2: FT-IR of a) drug and b) optimized formulation**

In FTIR spectrum of clobetasol propionate following peaks was found:

1593: C=C stretching of the aliphatic non-conjugated alkene

1702: C-Cl stretching of chlorine

1257 COO stretching of the ether

942 O-H bending of the alcohol.

In optimized formulation characteristics peak was slightly shift with reduced intensity. This study confirms that drug was encapsulated in phospholipid matrix.

### Preparation of clobetasol propionate containing transfersome

Transfersomes were prepared by thin film hydration technique. This technique is much frequently used owing to its simplicity and practicability, and its ability to yield small and uniform vesicles with high drug entrapment efficiency.

### In vitro characterization clobetasol propionate containing transfersome

**Visual Appearance:** Visual appearance of all prepared formulation was shown in Table no 4. All prepared formulations were uniform, Homogenous, translucent and off-white in color except the formulation CT3 and CT7 formulation were non uniform and having phase separation. Thus both formulations were not selected for further evaluation.

### Determination of pH and Percentage Entrapment efficiency of all prepared formulation

pH all prepared formulation was observed to be in range of  $6.17 \pm 0.047$  to  $6.31 \pm 0.029$  lies close to skin pH.

Percentage Encapsulation of all prepared clobetasol propionate containing transfersome was as given below Table no 4. Percentage Encapsulation Efficacy of all prepared formulation was found to be in a range of  $81.88 \pm 0.17$  to  $90.24 \pm 0.95$ . The entrapment efficiency of the transfersome formulation increase as the concentration increases. Maximum percentage drug encapsulation efficiency was found to be for formulation CT1  $90.24 \pm 0.95$ . On the basis of in vitro characterization parameters CT1 formulation was selected for further evaluation.

**Table 4: Visual appearance, pH and % Entrapment efficiency of all prepared formulation**

| S. No. | Formulation code | Visual appearance                                      | pH               | % Entrapment efficiency |
|--------|------------------|--------------------------------------------------------|------------------|-------------------------|
| 1      | CT1              | Uniform, Homogenous translucent and off-white in color | $6.21 \pm 0.055$ | $90.24 \pm 0.95$        |
| 2      | CT2              | Uniform, Homogenous translucent and off-white in color | $6.28 \pm 0.038$ | $48.59 \pm 0.62$        |
| 3      | CT3              | Phase separation                                       | -                | -                       |
| 4      | CT4              | Uniform, Homogenous translucent and off-white in color | $6.31 \pm 0.029$ | $88.17 \pm 0.31$        |
| 5      | CT5              | Uniform, Homogenous translucent and off-white in color | $6.17 \pm 0.047$ | $85.02 \pm 0.44$        |
| 6      | CT6              | Uniform, Homogenous translucent and off-white in color | $6.20 \pm 0.030$ | $81.88 \pm 0.17$        |
| 7      | CT7              | Phase separation                                       | -                | -                       |

### Particle Size, Polydispersity and Zeta Potential Determination

The particle size analysis of CT1 and revealed that the average particle size measured by laser light scattering method is around 300 to 400 nm, PDI is 0.208 and zeta Potential is -18.19 mv was shown in figure no. 3 & 4.

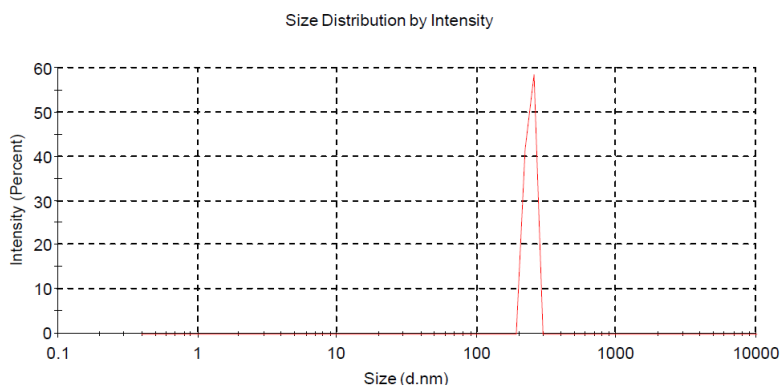


**Figure 3: Particle size distribution of formulation CT1**

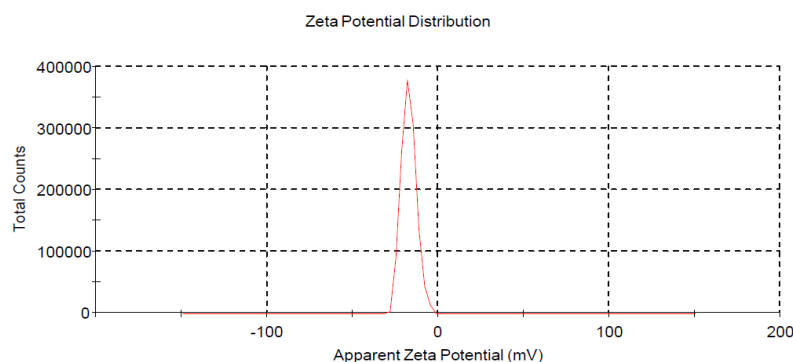


**Figure 4: Zeta Potential of formulation CT1**

### In vitro drug release study

Comparison of In-vitro drug release study of aqueous drug suspension of clobetasol propionate and clobetasol propionate containing transfersome gel CT1G2 was as given below Table no 5.

**Table 5: Comparison of In-vitro drug release study of aqueous drug suspension of clobetasol propionate and clobetasol propionate containing transfersome gel CT1G2**

| S. No | Time (hrs) | % drug release of Pure drug Suspension | % drug release of clobetasol propionate containing transfersome gel CT1G2 |
|-------|------------|----------------------------------------|---------------------------------------------------------------------------|
| 1     | 0          | 0±0                                    | 0±0                                                                       |
| 2     | 0.5        | 1.05±0.25                              | 116.87±0.88                                                               |
| 3     | 1          | 5.67 ±0.36                             | 27.10±0.10                                                                |
| 4     | 2          | 10.39±0.97                             | 49.87±0.37                                                                |
| 5     | 3          | 15.67±0.15                             | 70.17±0.56                                                                |
| 6     | 4          | 21.58±0.85                             | 88.88±0.14                                                                |
| 7     | 8          | 24.69±0.31                             | 91.10±0.31                                                                |
| 8     | 12         | 29.10±0.81                             | 94.89±0.78                                                                |
| 9     | 24         | 32.57±0.10                             | 95.10±0.09                                                                |

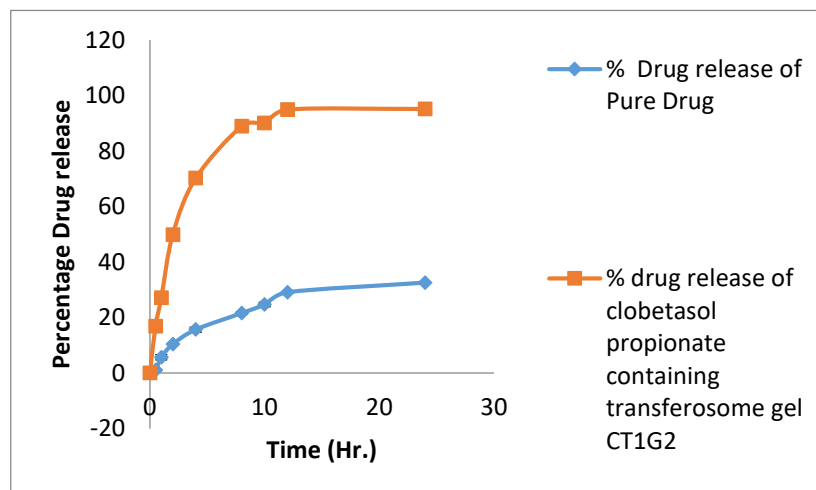


**Figure 5: Comparison of In-vitro drug release study of aqueous drug suspension of clobetasol propionate and clobetasol propionate containing transfersome gel 95.10±0.09**

To know the in vitro release behaviour and the effect of composition on the stability of transfersomal gel artificial cellophane membrane was used. The percentage drug release from drug aqueous suspension and transfersomal gel formulations was represented in Figure no 5. The release of clobetasol propionate from control means drug aqueous suspension was nearly  $32.57 \pm 0.10\%$  within 24 h, which clearly suggests the low permeation and solubilization of drug. A typical biphasic release pattern was observed with transfersomal gel formulations with an initial rapid burst release (4 h), followed by sustained release for a period of 24 h. The high concentration gradient prevailing at the initial time points might have contributed for the rapid drug release and also may be due to the faster release of untrapped drug from the transfersomal gel formulations. The percentage drug release was found to be  $95.10 \pm 0.09$  for formulation 95.10±0.09.

### Drug release Kinetic study

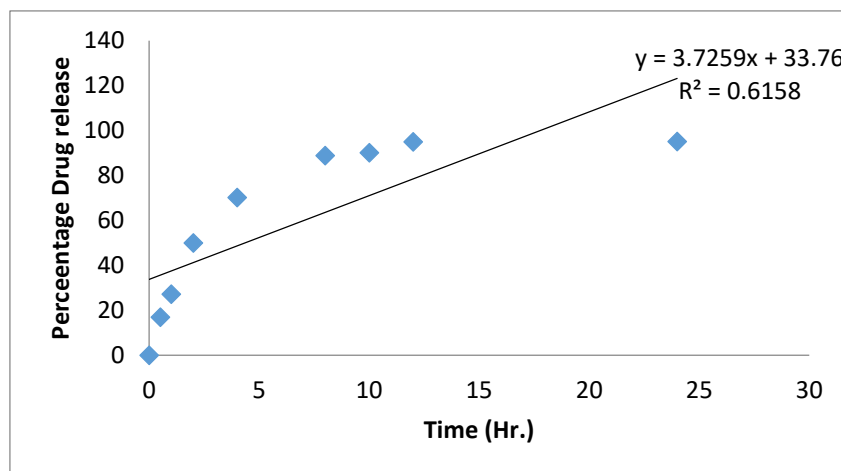


**Figure 6: Zero order Drug Release of CT1G2 formulation**

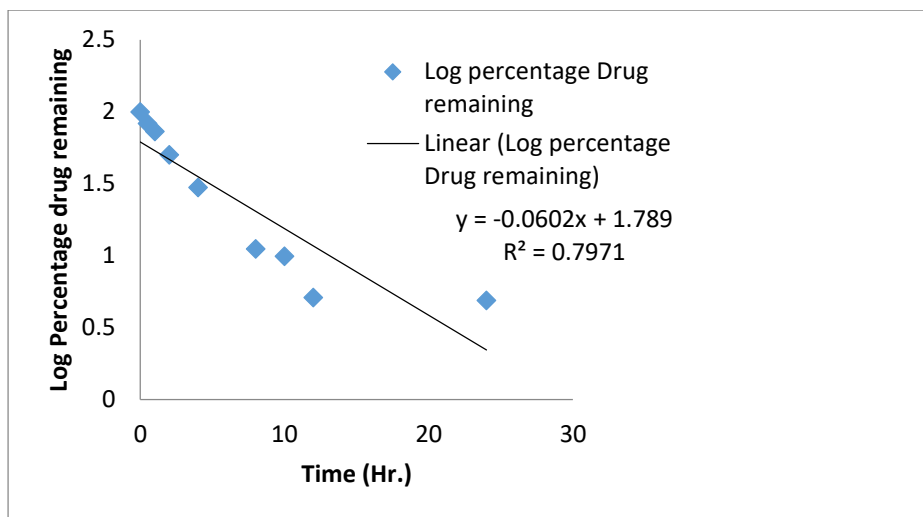


**Figure 7: First order Drug Release of CT1G2 formulation**

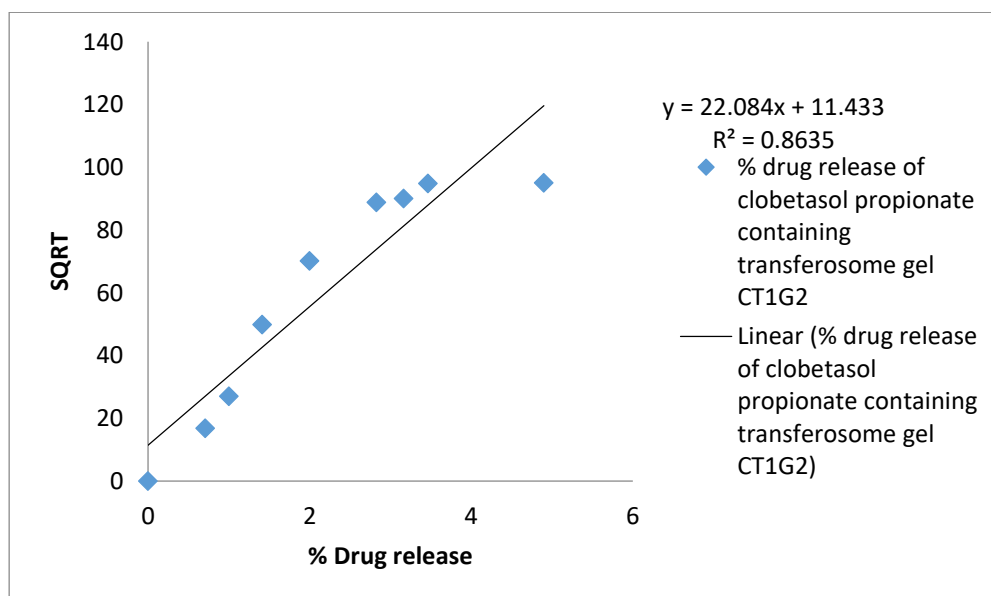


**Figure 8: Higuchi model Drug Release of CT1G2 formulation**

The data obtained for in vitro release shown in Figure 6,7 & 8 were fitted into equation for the zero order, first order and higuchi. The calculated regression coefficients for zero order, first order and higuchi models. it was found that the in vitro drug release of formulation CT1G2 formulation was best explained by higuchi model as the plot showed the highest linearity, the value of  $R^2$  found to be highest for the higuchi model.

## CONCLUSION

In present study, attempt is made to design and evaluate transfersomal gel containing clobetasol propionate for external application. The absorbance of clobetasol propionate was measured at 239 nm. The calibration curve of clobetasol propionate as shows in graph indicated the regression equation  $Y = 0.1002x - 0.0532$  and  $R^2$  value 0.999, which shows good linearity. Transfersomes were prepared by thin

film hydration technique. This technique is much frequently used owing to its simplicity and practicability, and its ability to yield small and uniform vesicles with high drug entrapment efficiency. The entrapment efficiency of the transferome formulation increase as the concentration increases. Maximum percentage drug encapsulation efficiency was found to be for formulation CT190.24±0.95. On the basis of in vitro characterization parameters CT1G2 formulation was selected for further evaluation.

A typical biphasic release pattern was observed with transfersosomal gel formulations with an initial rapid burst release (4 h), followed by sustained release for a period of 24 h. The percentage drug release was found to be 95.10±0.09 for formulation 95.10±0.09. The in vitro drug release of formulation CT1G2 formulation was best explained by higuchi model as the plot showed the highest linearity. The value of R<sup>2</sup> found to be highest for the higuchi model.

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