

## Design and Development of Clobetasol Propionate Topical Gel Thickened with Novel Copolymer

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### ABSTRACT

Topical delivery of clobetasole propionate (CP) offers several formulation related problems due to poor water solubility and photo degradation property. In the present investigation, topical gel of CP was formulated using Acrylamide/ Sodium Acryloyldimethyl taurate copolymer (SEPINEO™ P 600) as a gelling agent and evaluated with respect to different physicochemical parameters such as pH, viscosity, bio-adhesivity, spreadability, in vitro drug release and photo stability. Permeation of CP gel was studied using freshly excised pig ear skin for 24 h. The cumulative permeation of drug through excised rat skin was  $3.0 \pm 1.2$  mg cm<sup>-2</sup> with the corresponding flux value of  $0.24 \pm 0.09$  mg cm<sup>-2</sup> h<sup>-1</sup>. The *in vitro* release studies showed 101.43±1.12 % drug release over 10 h. The selected formulation was found to be effective with respect to percent drug content, permeation characteristics, pH, viscosity, and photostability. Therefore, CP gel could be very promising alternative for the topical drug delivery.

**Keywords:** Minoxidil, Nanostructured lipid carriers, Hair follicle.

### INTRODUCTION

Clobetasol propionate (CP) is the most potent currently available topical corticosteroids<sup>1</sup>. Its clinical effectiveness in the treatment of various disorders including psoriasis, atopic dermatitis, vitiligo and alopecia areata is related to its vasoconstrictive, anti-inflammatory, immunosuppressive, and antiproliferative effects<sup>2,3</sup>. Prolonged therapy of topical CP formulations leads to dermal side effects such as skin atrophy, acne, peri-lesional hypo-pigmentation and allergic contact dermatitis<sup>4</sup>. However, CP presents a number of challenges from formulation point of view owing to its low water solubility and significant photodegradation under UV light exposure<sup>5</sup>. Over the years, research has focused on strategies to improve the benefit-risk ratio of CP by developing novel drug delivery. Several attempts have been made to decrease the adverse effect CP by new application schedules, special vehicles and newly synthesized agents<sup>6</sup>. Gels have been tested as effective carriers of a variety of drugs for topical therapy of skin diseases. These are natural or synthetic polymer forming a three dimensional matrix entrapment a large amounts of aqueous or hydro-alcoholic liquid and form thin film of entrapped drug over skin after evaporation of liquid<sup>7</sup>. Recent studies had been investigated to the use of novel polymer as a medium to obtained gel. SEPINEO™ P 600, a concentrated dispersion of Acrylamide/ Sodium Acryloyldimethyl taurate copolymer/ Isohexadecane/ Polysorbat 80, having different gelling and thickening property and is able to emulsify and stabilize many lypophilic drug without addition of a conventional emulsifier<sup>8</sup>.

The aim of present work was to develop novel polymer gel containing CP; to evaluate its physicochemical properties and stability.

### MATERIALS AND METHODS

#### Materials

CP was obtained as a gift sample from Mahima Life Sciences Pvt. Ltd. (Sonepat, India). SEPINEO™ P 600 (concentrated dispersion of acrylamide/sodium acryloyldimethyl taurate copolymer in isohexadecane) was received as a kind gift sample from Seppic (India). Propylene glycol was purchased from Sigma-Aldrich, Mumbai, India. Dialysis membrane (Molecular weight cut off 14000 Da) was purchased from Hi-Media, Mumbai, India. Double distilled water was used throughout the study. All other chemical reagents and solvents used were of analytical grade.

#### Preparation of topical gel formulation

Initially, CP was dissolved into the required mass of propylene glycol later gels were then processed as follows: Blank gel (B-gel) was formulated using different amount of gelling agent (1%, 2% and 3% w/v) in de-ionised water with continuous mechanical stirring (5 min at 200 rpm; Eurostar Digital, IKA Labortechnik). For preparation gel containing CP (CP-Gel), was used. Required quantity of gelling agent and drug solution were weighted and dispersed in a small quantity of distilled water to form a gel as stated above.

#### Evaluation of gel

##### Drug content and pH

To ensure the homogeneity of drug content in the the gel, a specific amount (500 mg) of developed gel was taken in

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Table 1: Regression coefficient ( $R^2$ ) obtained from various kinetics models.

Formulation	Zero Order Kinetics	Higuchi Kinetics	Korsmeyer Peppas Kinetics	First Order Kinetics
CP-Gel	0.923	0.974	0.874	0.929

volumetric flask followed by dissolved in 100 ml of phosphate buffer of pH 7.4 for 2 h on mechanical shaker in order to get complete solubility of drug. The solution was filtered through 0.45  $\mu\text{m}$  membrane filter and it was diluted appropriately and analyzed by the high-performance liquid chromatography (HPLC) method described below. The pH of gel formulation was determined by using digital pH meter (Model MK-VI, Kolkata, India) in triplicate<sup>9,10</sup>.

#### Viscosity and spreadability Measurement

Brookfield digital viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) was used to measure the viscosity (cps) of prepared gel formulation. The spindle no. 6 allowed moving freely into the gel and the reading was noted at 10 rpm at  $25 \pm 1$  °C. Three readings ( $n = 3$ ) were recorded to obtain an average viscosity for gel<sup>11</sup>.

Spreadability is represented by the extent of area to which gel readily spreads on application. Glass slide method was used to measure the spread ability of gel; 500 mg of formulation was placed within a circle of 1 cm diameter pre-marked on a glass plate (10  $\times$  10 cm) followed by covered with second glass plate. Weight of 200 g was allowed to place over the upper glass plate for 5 min and measures the variation in the area of gel as a function of weight as response factors<sup>12</sup>.

#### Skin adhesion study

The modified two arm balance method was used to measure the adhesiveness of the prepared gel. The principle of measurement is the work required to overcome the attractive forces between the sample (gel) and the pig ear skin which comes in contact with it gradually. One arm of balance was tied with one glass slide having the skin and other glass slide with skin was fixed on the wooden block. Both the pan was balanced by adding extra weight to the right pan. 500 mg of the prepared gel was sandwiched between these two slides and pressed them to remove air bubbles and kept for 5 minutes. Weight was added to the other pan in increasing order at 100 mg/ min till the patch detached from the skin surface. Noted down the weight (gram force) required detaching the gel from the skin surface. Bioadhesive strength was measured using the formula<sup>13</sup>.

Bio – adhesive Strength

= Weight required (in gms) / Area (cm<sup>2</sup>)

#### In vitro drug release studies

In vitro release studies were conducted using a Franz diffusion cell method. The test formulation was applied on dialysis membrane having molecular weight cut-off between 12,000- 14,000 was mounted between donor and receptor compartment of the diffusion cell. Phosphate buffer saline (pH 7.4) was used as a dissolution media being maintained at  $32 \pm 0.5$ °C temperature by circulating water jacket. The surface area of the release membrane was 3.14 cm<sup>2</sup>. This whole assembly was kept on a magnetic stirrer and the solution was stirred continuously using a

magnetic bead. At predetermined time intervals, sample (2 ml) was withdrawn and replaced with equal amounts of fresh dissolution media. Samples were analyzed HPLC at 236 nm and the cumulative % drug release was calculated. To evaluate the mechanism of drug release from prepared gel, the drug release data to various dissolution kinetic model equations such as the zero order (1), first order (2), Higuchi matrix (3), and Korsmeyer-Peppas (4) given below<sup>14,15</sup>:

$$Q_t = Q_o + K_0 \quad (1)$$

$$Q_t = \ln Q_o - K_1 t \quad (2)$$

$$Q_t = K_H t \quad (3)$$

$$K_p t^n \quad (4)$$

Where  $Q_0$  is the initial drug amount,  $Q_t$  the amount of drug remaining at a specific time,  $k$  the rate constant;  $t$  is the time;  $n$  is release exponent.

#### Ex vivo skin permeation study

The skin penetration study of selected formulation was carried out pig ear skin. The experiment was performed using Franz diffusion cell. For the preparation of section of skin, fresh hairless pig ear obtained from local slaughter house was cleaned with water followed by dry them with tissue paper. The full thickness of ear skin was isolated from ears was placed in the space between the donor and receptor compartment of the Franz diffusion cell, keeping the *stratum corneum* upward. Specified amount of test formulation (0.05% w/w) was applied on donor area and receptor compartment was filled with 6.0ml of phosphate buffer saline (pH 7.4), thermoregulated at 37 °C and magnetically stirred at 400 rpm. Aliquot (1.0 ml) of receptor fluid was withdrawn at an interval of 0.5, 1, 2, 3, 6, 12, 18 and 24 h and replaced with equal amounts of fresh medium to maintain sink condition. Each sample was filtered through a 0.45- $\mu\text{m}$  filter paper (Sartorius AG, Germany) and then determined for CP content by HPLC method<sup>16</sup>. Permeation parameters were calculated using equation given below. Graph of percentage of drug permeated through the skin ( $\mu\text{g}\cdot\text{cm}^{-2}$ ) was plotted as a function of time (h). The steady state flux ( $J_{ss}$ ) was calculated by dividing the slope of linear portion of the curve with the diffusion cell area ( $\text{mg cm}^{-2} \text{h}^{-1}$ )<sup>17</sup>.

$$Q_t = VrCt + \sum_{i=0}^{t-1} VsCi \quad (5)$$

Where  $C_t$  is the drug concentration of the receiver solution at each sampling time,  $C_i$  the drug concentration of the  $i$ th sample, and  $V_r$  and  $V_s$  are the volumes of the receiver solution and the sample, respectively

#### HPLC Method

Clobetasol propionate was assayed by HPLC method consisted of a Shimadzu LC-20A system (Kyoto, Japan) equipped with a model LC-20AT pump, an SPD-M20A PDA detector. The quantification was performed by RP-18 Gemini column (250 mm  $\times$  4.60 mm, 5 $\mu\text{m}$  particle size, 110 Å pore diameter) with a mixture of methanol-acetonitrile-water (50:15:35 v/v) as mobile phase, a flow

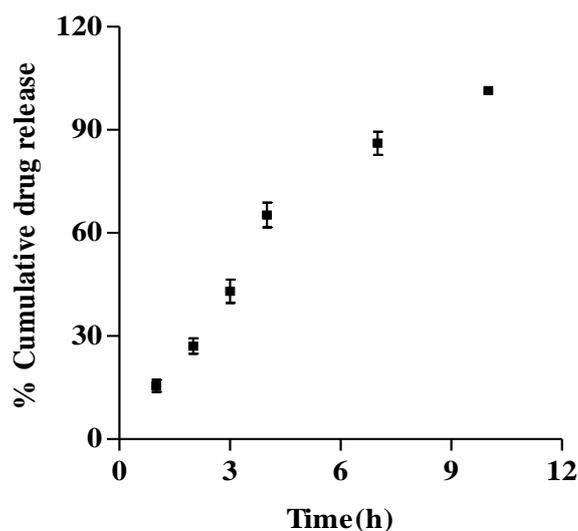


Figure 1: *In vitro* cumulative % drug release from CP-Gel (mean  $\pm$  SD, n = 3).

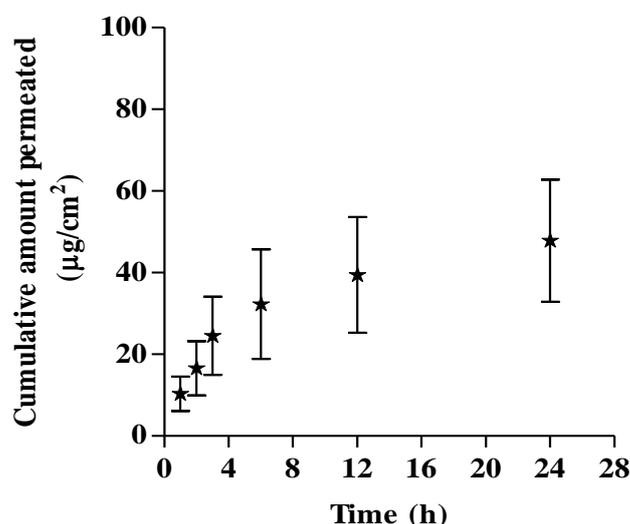


Figure 2: *Ex vivo* skin permeation of CP through pig ear skin (mean  $\pm$  SD, n = 3).

rate of 1.2 ml/min, oven temperature of 30°C, injection volume of 50  $\mu$ l and detection at 240 nm<sup>18</sup>.

#### Photo stability studies

The prepared gel was stored at photo stability chamber containing near UV and visual light testing with fluorescent lamps (UV, 320–400 nm) for a period of 3 months to access the photo stability as per ICH guidelines (Q1B)<sup>19</sup>. Samples were withdrawn at 30 day time intervals and evaluated for pH and drug content.

#### Statistical Analysis

The CP-Gel was tested for significance by using Student's *t*-test using Graph Pad Prism software 5.0 version (Graph Pad Software Inc., San Diego, CA, USA) and the *p* values < 0.05 were considered.

## RESULTS AND DISCUSSION

#### Preparation of CP-Gel

The Gel was formulated by the use of 1%, 2% and 3% w/w SEPINEO™ P 600. CP-Gel (2% w/w) was found to be

showed an optimum viscosity. Whereas CP-Gels containing 1.0 % and 3.0% w/w of Sepineo P 600 form a very thin gel and more sticky gel respectively that could create problem during handling.

#### Evaluation of Gel

##### Drug content and pH

The drug content of the CP-Gel was found to be 98.80  $\pm$  0.82% and pH ranges between 6.1  $\pm$  0.02, which could easily be tolerated on skin without irritation.

##### Viscosity and spreadability study

Rheological behavior of topical drug delivery applications is directly dependent on the polymeric content of the formulation and influence their physical properties, the contact time on the application area, and their drug release rate. Viscosity has inverse relationship with rate of drug release i.e. viscosity increases the rate of drug release decreases<sup>20</sup>. The viscosity of the CP-Gel was found to be 561  $\pm$  0.57 cps. The spreading ability of gel determines the area to which the gel readily spreads. The CP-Gel

spreading diameter was  $6.1 \pm 0.06$  cm indicating a good spreadability<sup>21</sup>.

#### Skin adhesive measurement

Bioadhesion defines the ability of some synthetic, biological, or hydrocolloidal macromolecules to stick to biological tissues or application area. It is the significant characteristic of gel results in the prolongation of drug residence time with the epithelial barrier in the treatment of skin disorders<sup>22</sup>. The bioadhesive strength of final gel was  $1.28 \pm 0.10$  gm/cm<sup>2</sup>.

#### In vitro release of CP-Gel

The study of drug release from gel gives an idea of the amount of free active drug available for partitioning into the *stratum corneum*. Percentage drug release from CP-Gel was found to be  $101.43 \pm 1.12$  % of drug over a period of 10 h. Table 1 shows results of *in vitro* release data fitted to the different models. The final formulation followed Higuchi kinetics ( $R^2 = 0.974$ ). This indicates that the test product follows matrix diffusion based release kinetics.

, complete amount of drug was released in the dissolution medium could be attributed to the presence of propylene glycol in the formulation which was used to solubilize the drug completely before incorporation into the polymer base.

#### Ex vivo skin permeation study

Skin permeation of CP from gel was evaluated using pig ear skin. Drug penetration into different layers of the skin can be achieved as a consequence of high concentration gradient across the skin due to presence of propylene glycol which served the purpose of not only drug solubilization but also act as permeation enhancers<sup>23</sup>. About 23 % of the applied amount of drug was permeated across the skin. The corresponding flux was  $1.634 \pm 0.69$  mg cm<sup>-2</sup> h<sup>-1</sup>.

#### Stability study

The selected formulation, CP-Gel, was subjected to photo stability studies for three months as per ICH guidelines. No significant change was found in pH and drug content of

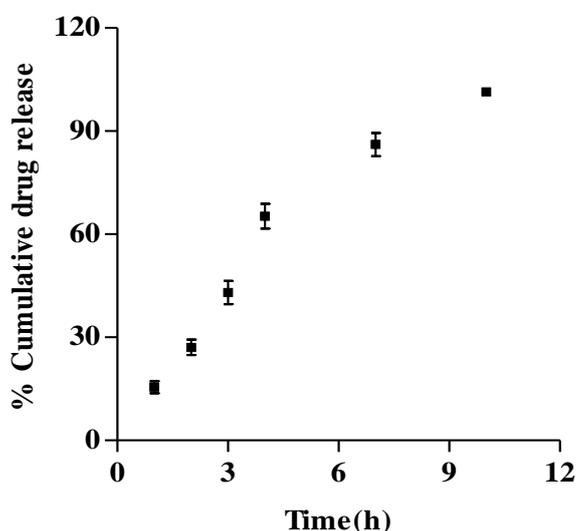


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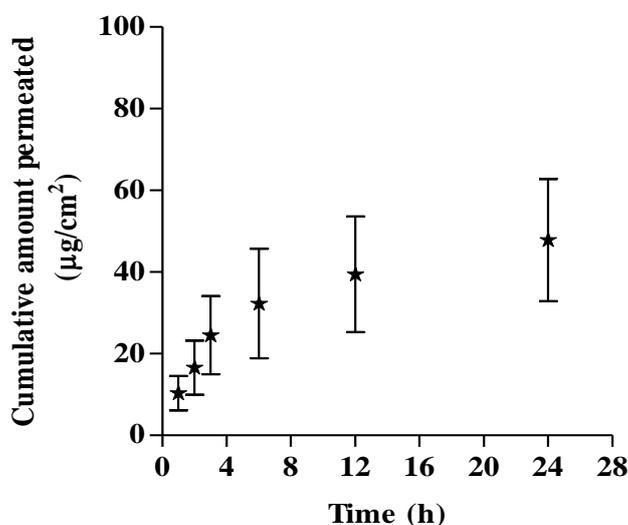


Figure 2: *Ex vivo* skin permeation of CP through pig ear skin (mean  $\pm$  SD, n = 3).

formulation. This shows that the prepared formulation is stable at the studied temperatures.

## CONCLUSION

In the present study, CP was successfully incorporated into the SEPINEO™ P 600 to obtain a gel, which was subjected to physicochemical studies such as rheological, spreadability, bioadhesion strength, *in vitro* drug release and *ex vivo* skin permeation. The outcomes of these studies indicate that prepared gel was to be safe, stable, and therapeutically efficacious in the treatment of skin disorders.

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## CONFLICT OF INTEREST

The authors declare that no competing interests.

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