Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2017, 9(22):29-37
(http://www.derpharmachemica.com/archive.html)

Formulation and Evaluation of Topical Solid Lipid Nanoparticulate System of Clobetasole Propionate

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ABSTRACT

In the Present study Propionate (CP) Solid Lipid Nanoparticles (SLN) have been formulated, to increase drug stability and to improve skin retention properties. The nanoparticles were prepared using lipid extrusion method followed by high pressure homogenization at speed of 15000 rpm for 30 min. Six formulations were prepared using variable ratio of the lipids, keeping rpm, time, concentration of surfactants, and active ingredient constant. Formulations were prepared using bees wax, carnauba wax, cetyl alcohol as oil phase, lecithin soya and tween 20 as emulsifying agent. The SLNs were characterized for visual appearance, drug content, particle size analysis, zeta potential, Scanning Electron Microscopy (SEM), Differential Scanning Calorimetry (DSC). Six formulations were subjected to in vitro release characteristics. The formulation F6 with highest drug content and encapsulation efficiency was incorporated into carbopol gel base. The particle size was ranged between 46.33-301.2 nm and zeta potential ranged between 18.5 mV-29.9 mV. The particle size was found to be increased with increase in the concentration and ratios of lipids. The ex vivo skin permeation studies on rat skin showed an appreciable increase in drug permeation in comparison to marketed formulation. The short term stability studies indicated no considerable change in drug content, loading and release profiles.

Keywords: Solid lipid nanoparticles, Skin permeation, *In vitro* release profiles, Clobetozole propionate, Gel formulation

INTRODUCTION

Topical drug delivery is used to deliver drugs to surface of the skin as well as underneath the skin. The main advantages of topical drug delivery system is that it bypasses the first-pass metabolism, avoids effect of gastric pH, effect of enzymes and also effect of gastric emptying, etc. [1,2].

Phenomenon of skin permeation consisting of a series of steps in sequence: such as sorption of a penetrant molecule onto the surface layer of stratum corneum, diffusion through the viable epidermis, finally through the papillary layer of the dermis and then molecule will be taken up into the microcirculation for subsequent systemic distribution [3,4]. For effective topical treatment, the drug must be sufficiently permeable into the skin to reach the desired location of infection. Nanoparticles made from lipids attract major attention as novel colloidal drug carrier for various applications as they have been proposed as an alternative particulate carrier system [5].

The Solid lipid nanoparticles (SLNS) are submicron colloidal carriers have size range of 50-1000 nm, composed of physiological lipids dispersed in water or in an aqueous surfactant solution. The SLN combine the advantages of physical stability, protection of incorporated labile drugs from degradation, controlled release, excellent tolerability, and scalability to large-scale preparations and excellent biocompatibility in comparison to traditional colloidal systems [6,7]. SLN can improve permeability properties; retention properties and it also decrease irritation potential due to entrapment [8].

Psoriasis is an autoimmune disease, which is characterized by patches of itchy and painful abnormal skin. In psoriasis, body begins to make new skin cells more quickly than normal and these build up on the skin in raised patches, this is because the immune system triggers a reaction even though there is no infection or wound to heal [9,10].

Clobetosole propionate has anti-inflammatory, antipruritic, and vasoconstriction activity, used for the local treatment of mild to moderate psoriasis. The mechanism of the anti-inflammatory activity of the topical steroids, in general, is unclear. However, corticosteroids are thought to act by the induction of phospholipase A_2 inhibitory proteins, collectively called lipocortins. It is postulated that these proteins control the biosynthesis of potent mediators of inflammation such as prostaglandins and leukotriene's by inhibiting the release of their common precursor, arachidonic acid [11]. So an attempt is made to prepare solid lipid nanoparticles of clobetasol propionate with an intension to increase the permeation and skin retention properties of drug using natural lipids.

MATERIALS AND METHODOLOGY

Materials

Clobetozole propionate was obtained from Dr. Reddy's labs, Hyderabad India as a gift sample. Carnauba wax, bees wax, cetyl alcohol, tween 80 etc., were purchased from Sigma Aldrich ltd. All reagents used were of analytical grade.

Analytical method

UV scanning was done for clobetasole propionate at 200-400 nm in methanol and in phosphate buffer pH 6.8. Accurately weighed 50 mg CP was dissolved in the methanol/Phosphate buffer pH 6.8 and volume was made up to 100 ml. From stock, different dilutions were prepared in the concentration range of 5, 10, 15, 20, 25 and 30 µg/ml using methanol/phosphate buffer pH 6.8. The absorbance of these solutions was measured against methanol/phosphate buffer pH 6.8 as blank in UV spectrophotometer at 240 nm.

Compatibility studies

Fourier Transform Infra-Red (FTIR) spectroscopy

FTIR spectra of pure clobetasol, physical mixtures are carried out to check if there was any interaction between the drug and formulation components using IR spectrometer Shimadzu 8400 series. Pinch of the solid sample was taken on a spatula mixed with KBr. The pellets were prepared at 5000-10000 psig. The pellets were carefully removed and placed in a FTIR sample holder and a spectrum was recorded.

Differential Scanning Calorimetry (DSC)

The DSC (Mettler-Toledo star 822e system, Switzerland) was carried out to check any further interaction between the drug and excipients.

Preparation of SLN by lipid extrusion technique

SLN was prepared using carnauba wax, beeswax, and cetyl alcohol as lipid phase, lecithin soya as surfactant, tween 20 as co surfactant and distilled water as aqueous phase. In this method, both aqueous phase and lipid phase were heated to 70-75°C. When lipid phase was heated to desired temperature, the drug was dispersed in it and lipid phase is added in to aqueous phase by drop wise using a syringe under the magnetic stirring for 30 min. This respective pre-emulsion was subjected to high-shear homogenization using Polytron PT 1600E homogenizer [12,13]. Formulation design is shown in Table 1.

Table 1: Formulation of solid lipid nanoparticles

	F1 (mg)	F2 (mg)	F3 (mg)	F4 (mg)	F5 (mg)	F6 (mg)
Clobetasol propionate	25	25	25	25	25	25
Bees wax	500	600	800	1000	1500	2000
Carnauba wax	250	300	600	1000	1500	2000
Cetyl alcohol	250	300	400	600	800	800
Lecithin soya	200	200	200	200	200	200
Tween 20	250	250	250	250	250	250
Distilled water (ml)	50	50	50	50	50	50

Evaluation of SLN

Particle size and Zeta potential measurement

Zeta potential of the selected formula was done by dynamic light scattering technology using Malvern Instruments at Aimil Ltd. Bangalore. The incident laser beam passes through the centre of the sample cell, and the scattered light at an angle of about 13° is detected. When an electric field is applied to the cell, any particles moving through the measurement volume will cause the intensity of light detected to fluctuate with a frequency proportional to the particle speed and this information is passed to the digital signal possessor and then to a computer [14]. Zetasizer software produces a frequency spectrum from which the electrophoretic mobility hence the zeta potentials calculated.

Scanning Electron Microscopy (SEM)

Surface morphology of the specimen was determined using a SEM. The samples were dried thoroughly in vacuum desiccators before mounting on brass specimen studies, using double sided adhesive tape. Gold-palladium alloy of 120°A Knees was coated on the sample sputter coating unit (Model E5 100 Polaron U.K) in Argon at ambient of 8-10 with plasma voltage about 20 mA. The sputtering was done for nearly 5 min to obtain uniform coating on the sample to enable good quality SEM images. The SEM was operated at low accelerating voltage of about 15 KV with load current about 80 mA. The condenser lens position was maintained between 4.4-5.1. The objective lens aperture has a diameter of 240 microns and working distance WD=39 mm.

Drug content

The formulation equivalent to 100 mg of drug was taken and dissolved in small quantity of methanol. The solution was filtered and subsequently diluted to Beer's concentration range. Absorbance was measured at 240 nm.

In vitro release studies for SLN

In vitro release studies were carried out by modified Franz diffusion cell. SLN formulation consists of 10 mg drug was placed on a cellophane membrane between donor and receptor compartment of diffusion cell assembly. The donor compartment was wetted by 0.5 ml of phosphate buffer. The receptor compartment was filled with 50 ml phosphate buffer pH 6.8 maintained at 35°C. The study was carried out for 24 h and the sample was withdrawn at 30 m time interval and same volume was replaced with fresh phosphate buffer. The content of clobetasol propionate from withdrawn sample was measured UV spectrophotometrically after suitable dilution at 240 nm [15].

Preparation of carbopol gel

Required quantity of Carbopol 934 (1g) was taken hydrated in sufficient quantity of water (100 ml) for 24 h. Further, the hydrated gel was stirred for 4 h. The gel was neutralized by triethanolamine (added drop by drop) until a clear transparent gel was obtained.

Preparation of SLN incorporated gels

About 50 mg drug equivalent weight of SLN was incorporated in to gel by mechanical mixing.

Rheological studies

Viscosity measurements were carried out using a Brookfield viscometer (T –bar spindle). The SLN based gel formulation was kept in 100 ml beaker and dial readings was noted at 10, 12, 20, 30, 50 and 60 rpm.

Ex vivo permeation studies for SLN incorporated gel

Albino rats were selected for the *ex-vivo* studies owing to its structural similarities to human skin. The rats were sacrificed and full thickness abdominal skin was excised. A specific portion of the skin was cut and used for the permeation study after washing it with distilled water. *Ex vivo* release studies were carried out using modified Franz diffusion cell. Approximately 10 mg drug equivalent weight of SLN was incorporated into gel and placed on to rat abdomen skin between donor and receptor compartment of diffusion cell assembly. The donor compartment was filled with 50 ml phosphate buffer pH 6.8, which was magnetically stirred. The receptor fluid was collected at regular time intervals and analyzed for the drug content spectrometrically at 240 nm [16,17].

Stability studies

A one month accelerated study was conducted for optimized formulations F6 at 40 ± 2°/60% ± 5% RH.

RESULTS AND DISCUSSION

Analytical method

The standard graph of clobetasol propionate was prepared in both methanol and phosphate buffer pH 6.8 The linearity was found at the concentration range of 5-25 µg/ml in methanol and 5-30 µg/ml in phosphate buffer pH 6.8 at 240 nm.

Compatibility studies

Physical mixture of drug and lipids was characterized by FTIR spectral analysis for any physical as well as chemical alteration of the drug characteristics. The principal peaks of the clobetasol propionate were found to be unaltered in the spectra of the drug -lipid mixture as shown in Figures 1 and 2. The interpretations of the peaks are given in the Table 2.

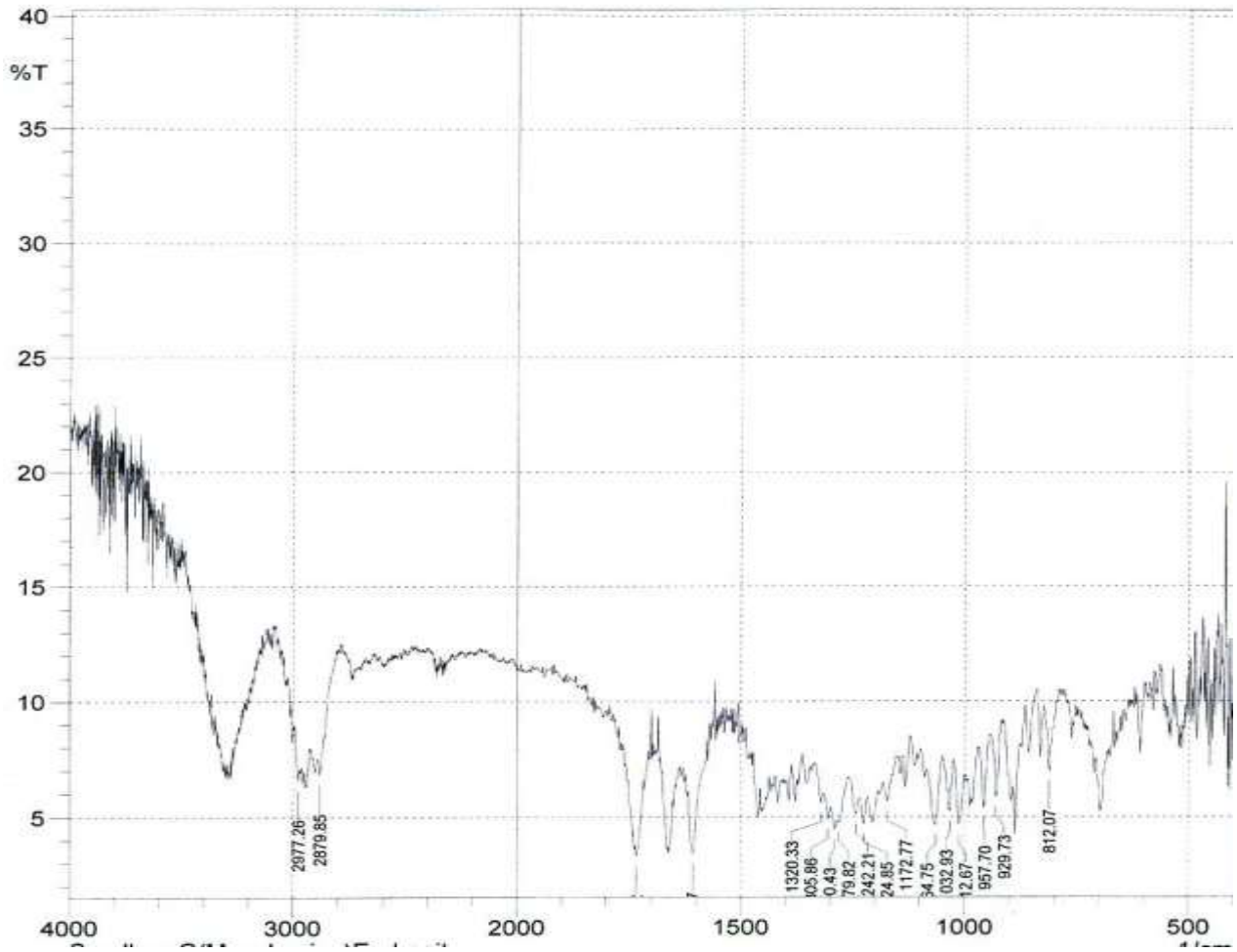

Figure 1: IR Spectra of clobetasol propionate

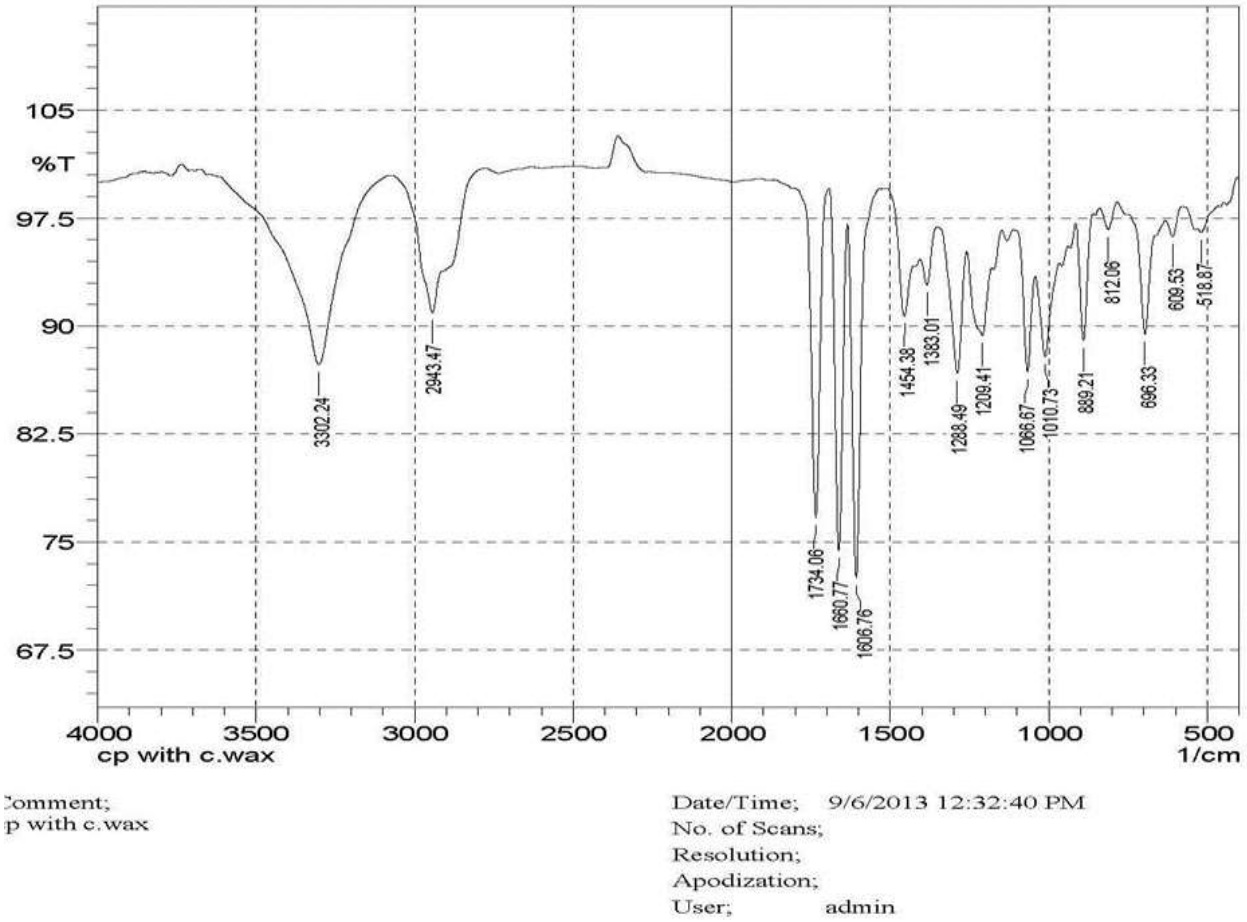

Figure 2: IR Spectra of clobetasol propionate with carnauba wax

Table 2: IR graph of clobetasol propionate

Band position (cm^{-1})	Functional group	Interpretation
1666	C=C	stretching of the aliphatic non-conjugated alkene
1612	C=O	stretching of the ketone
1724	C-Cl	stretching of chlorine
1063	COO	stretching of the ether
1010	O-H	bending of the alcohol

DSC

The thermal behavior of the clobetasol and drug loaded solid lipid nanoparticles studied using DSC. The DSC thermogram of the clobetasol exhibits an endothermic peak at 196.17^{o}. In the case of selected formulation F6, endothermic peak shifted towards 175.5^{o}, indicates the lipids (Beeswax and carnauba wax) and surfactants may have decreased the melting point of the clobetasol and there could be a chance for formation of the rigid solid lipid nanoparticles of clobetasol. As given in the Figures 3 and 4.

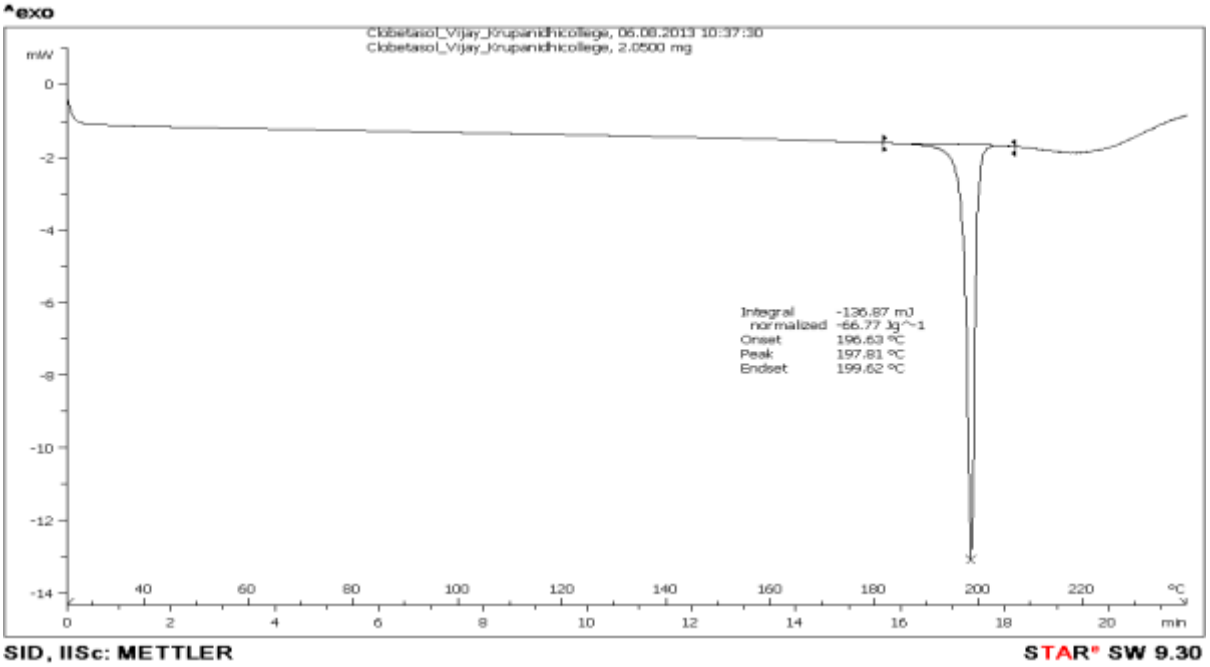

Figure 3: DSC thermogram of clobetasol propionate

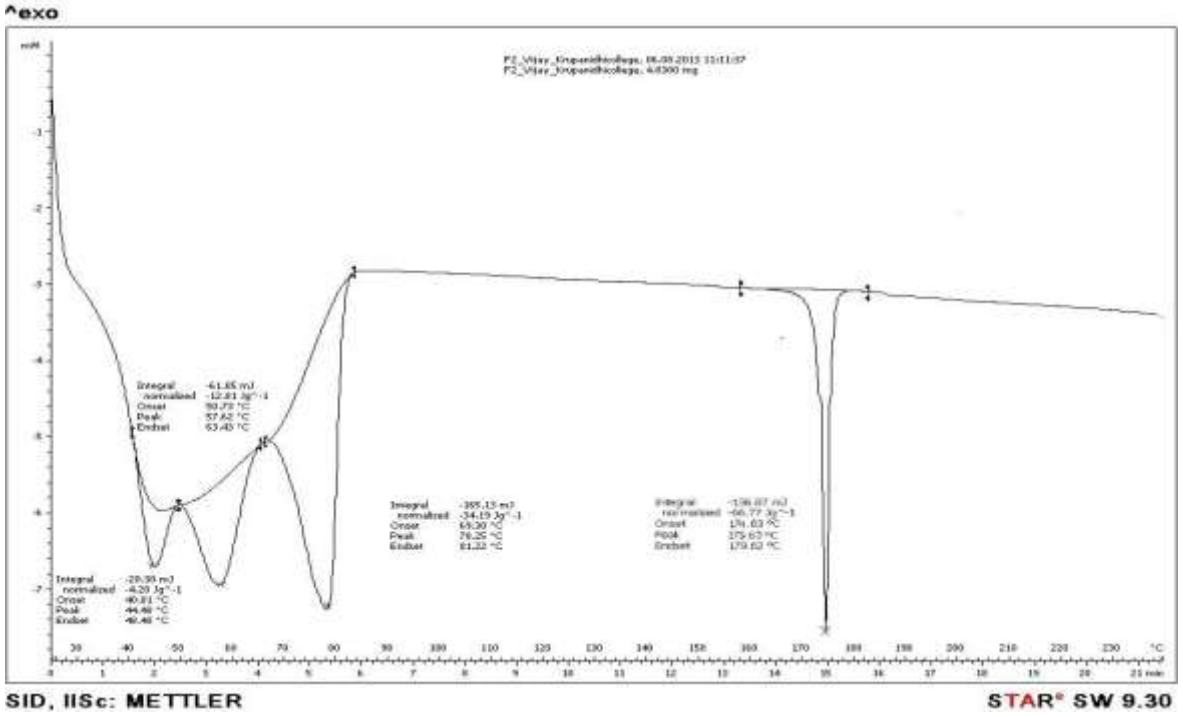

Figure 4: DSC thermogram of selected formula F6

Drug content

The drug content of formulations F1, F2, F3, F4, F5 and F6 was 85.87 ± 0.25, 86.41 ± 1.10, 88.54 ± 0.48, 87.56 ± 0.67, 89.86 ± 0.76, 94.23 ± 0.98 respectively is given in Table 3. It was seen that as the lipid concentration is increased, the drug content was found to be increased.

Table 3: Drug content and drug loading of nanoparticles

S. No.	Formulation code	Drug content in %	Entrapment efficiency %
1	F1	85.87 ± 0.25	41.2 ± 0.23
2	F2	86.41 ± 1.10	48.4 ± 0.34
3	F3	88.54 ± 0.48	59.76 ± 0.43
4	F4	87.56 ± 0.67	65.3 ± 0.65
5	F5	89.86 ± 0.76	83.45 ± 0.65
6	F6	94.23 ± 0.98	92.23 ± 0.87

Particle size analysis

The particle size analysis was done for all the formulations using zeta sizer. The mean particle size of formulations F1, F2, F3, F4, F5, F6, was found to be 46.33 nm, 63.65 nm, 93.85 nm and 116.6 nm, 287.9 nm, 302.2 nm respectively. As the lipid concentration was increased, there was considerable increase in the particle size. The average particle size of formulation F6 was found to be 302 nm and poly dispersibility index of 0.028 is given in Figure 5. Good particle size distribution is an indication of suitability of the method of preparation and processing condition for the preparation of nanoparticles.

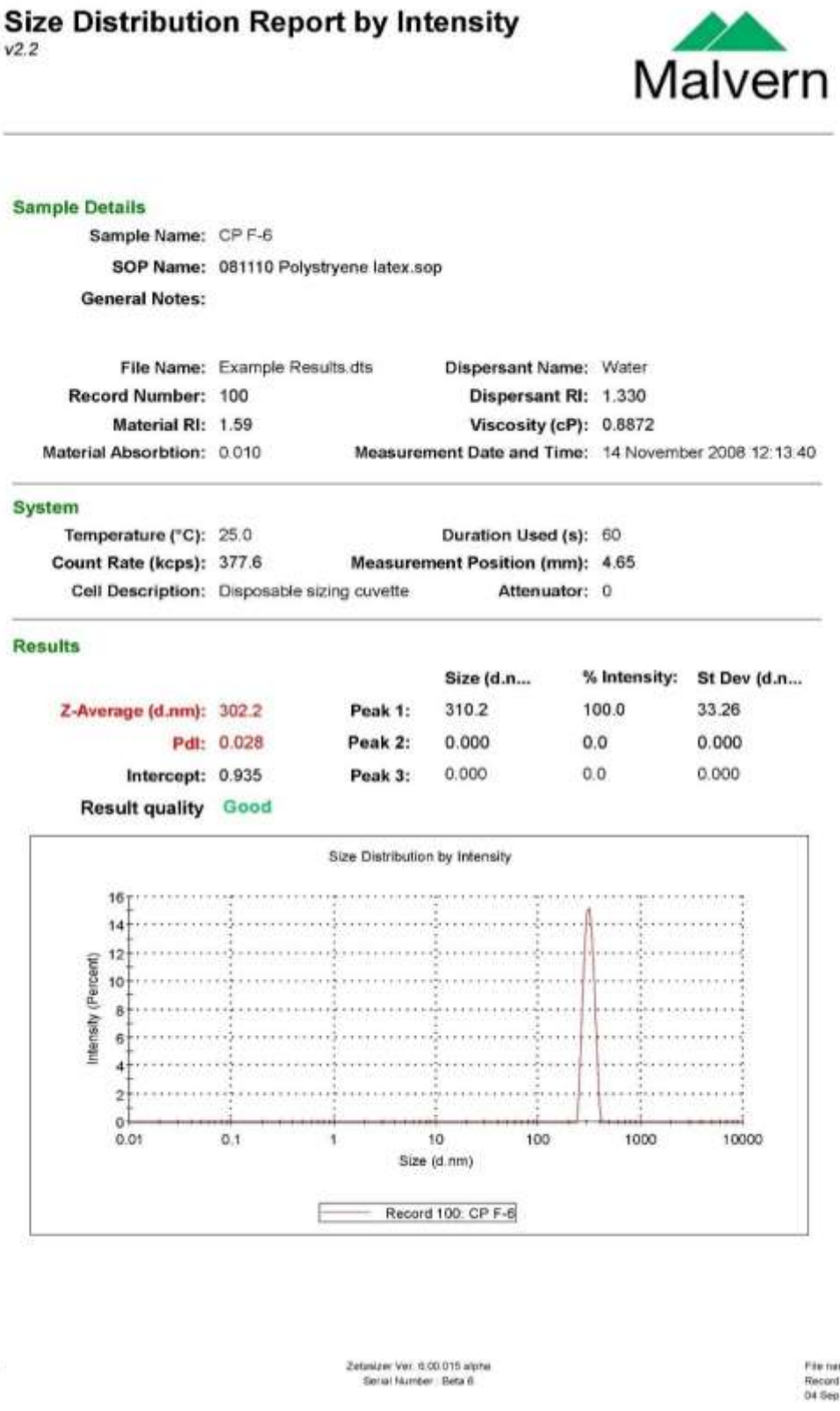

Figure 5: Particle size analysis of the selected formula F6

Zeta potential

Zeta potential of all the formulations were found to be -18.5, -15.6, -33, -23.1, -19.7, -29.9 mV respectively, indicated a considerable stability of prepared nanoparticles. As given in the Figure 6, the formula F3 exhibited, zeta potential of -33 indicates good stability of the nanoparticulate system.

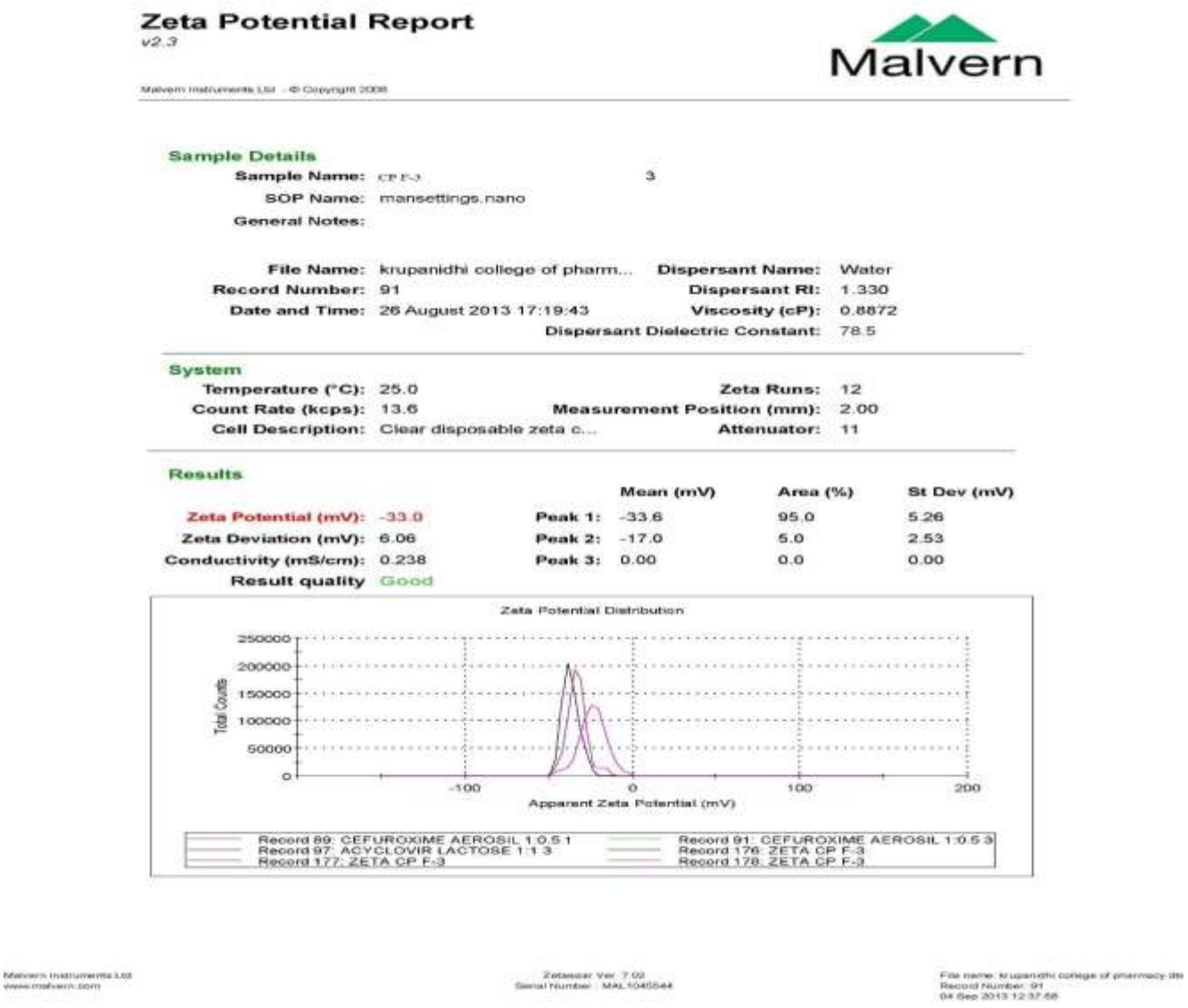

Figure 6: Zeta potential of the selected formula F6

SEM

SEM of the selected formulations F6, given in Figure 7, shows particle with uneven surfaces.

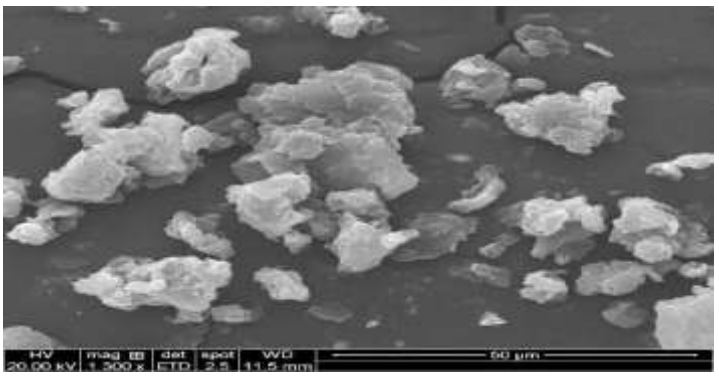

Figure 7: Scanning Electron Microscopy (SEM) of the selected formula

***In vitro* release studies**

Formulations F1-F6 were subjected to *in vitro* release studies in phosphate buffer of pH 6.8 The results revealed that, there was gradual and slow release drug from the nanoparticles and the release was found to be concentration dependent. The release profile is given in Figure 8.

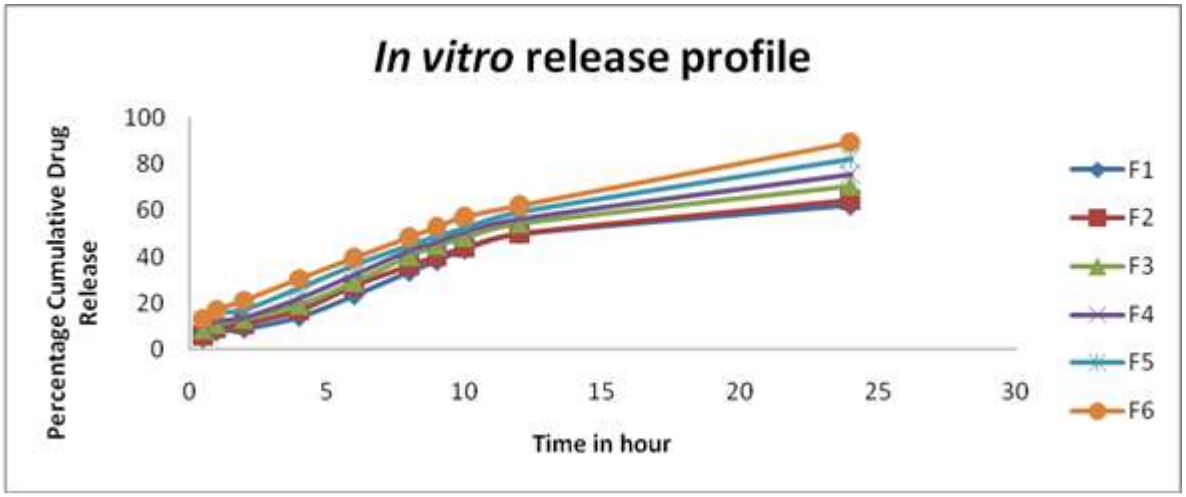

Figure 8: *In vitro* release profile of F1-F6

Viscosity of SLN gel

The viscosity of the SLN gel was 3160 cps using Brookfield viscometer.

***Ex vivo* permeation studies**

The *ex vivo* studies of nanoparticulate gel system showed an increased skin permeation in comparison to the marketed formula. This may be due to the fact that SLN could have permeated through the skin layers, due to its particulate nature and also, since they are lipid based preparation, they could easily permeate through skin Figure 9.

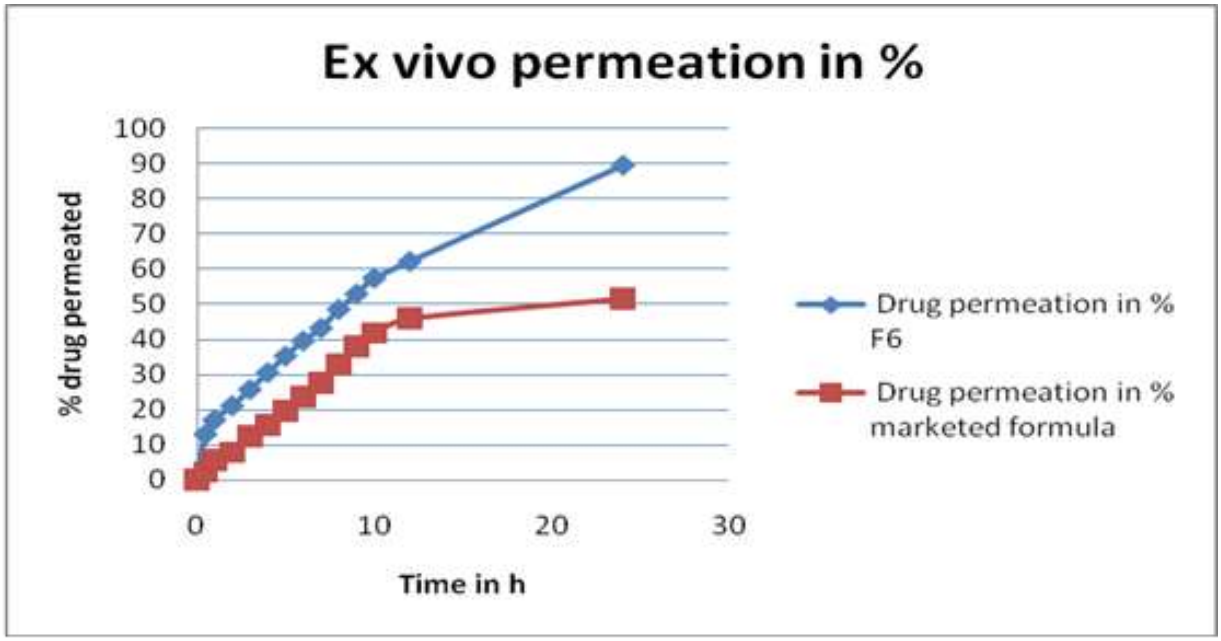

Figure 9: *Ex vivo* permeation studies

Table 4: Stability studies

Formulation code	Temperature and humidity	Appearance in weeks	Drug content after 4 weeks
F6	40 ± 2°C/75% ± 5% RH	No change	90.22%

The selected formulation was subjected to short term stability studies for a period of four weeks at 40 ± 2°C/75% ± 5% RH. Both physical and chemical changes were observed during the study. Physical stability was analyzed by morphological appearance and chemical stability was analyzed by the change in the drug content. The results revealed that no much changes in morphological appearance as well as in the drug content and release. The results of stability studies are given in Table 4.

CONCLUSION

SLNs is promising colloidal drug delivery systems for skin applications due to their advantageous effects on skin such as its suitability for inflamed or damaged as they are prepared of non-toxic and non-irritant lipids. The SLN formulation can be economically manufactured from relatively cheap raw materials such as beeswax, carnauba wax, carbopol-934, tween-20 etc. The release profile of Clobetasol from the SLNs is amenable to slow and increased delivery of the drug compared to marketed product. The developed SLNs offers the advantage of high drug-lipid ratio, drug loading, minimal particle size and size-distribution and a good zeta potential. The nanoparticulate colloidal drug delivery system of clobetasol prepared from beeswax, carnauba wax, tween-20 and soya lecithin is expected to provide a new choice of an economical, safe and efficient regimen in the management of skin infections.

ACKNOWLEDGEMENT

We thank Krupanidhi College of Pharmacy for the support given for conducting the experimental studies.

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