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Design and Characterization of Etanercept Nanogel for Psoriasis Treatment

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Abstract

Transdermal delivery of drug is promising but challenging system is available for local as well as systemic effect of drug. The prolonged residence of drug formulation in the skin is important for transdermal drug delivery. The present research work was aimed to develop a novel gel for Etanercept (Act) to enhance the drug absorption by the topical application, which overcomes the demerits of oral dosage form and conventional gel system of Etanercept (Act). The objective of the present investigation was to develop a nanogel with reduced particle size in order to improve the bioavailability of the anti-psoriatic drug, Etanercept. The present study is to formulate nanosizes dispersion of Etanercept by emulsion-solvent diffusion method and incorporation of gelling agent to produce nanogel. The formulations are characterized for particle size ranging from 100-400 nm. A drug named Etanercept used Psoriasis diseases. Glycerol: Water (20:80) co-solvent system is selected for preparing Etanercept nanogels using different polymers and has better permeability coefficient than alcohol: water co-solvent. Permeation through cellophane membrane was carried using 0.9% w/v sodium chloride using receptor fluid in Franz diffusion cell (1.74 cm2). Gels containing Etanercept with Eudragit polymer shown better permeability coefficient. Etanercept nanogels formulated using carbopol with permeation enhancer has shown better flux enhancement in comparison with nanogels formulated using HPMC and methyl cellulose. It has been concluded that Etanercept nanogels using carbopol 940 as gelling agent and Eudragit S-100 has shown better flux enhancement with propylene glycol as permeation enhancer.

Keywords: Etanercept, TDDS, Eudragit S-100, Glycerol, Carbopol-940.

Full length article **Corresponding Author*, e-mail: Bandameedi.ramu@gmail.com

1. Introduction

Topical drug delivery system has been the most appropriate and convenient approach over the past two decades [1]. Many conventional semisolid dosage forms such as creams, gels, and lotions found to have problems such as sticky in nature, lesser spreading coefficient, and stability

issues[2]. To overcome such issues, a novel, stable topical drug delivery approach can be used to formulate successful drug delivery for hydrophobic drugs [3]. In recent years, the concept of gel has gained significant interest in the topical drug delivery system [4]. Psoriasis is a chronic T-cell mediated autoimmune inflammatory skin disease with relapsing episodes of inflammation and hyperkeratosis on the skin. It affects millions of population worldwide, with an equal sex distribution [5]. The general characteristics psoriasis are sharply demarcated erythematous (red) papules and plaques with adherent silvery scales which affect the skin and also other parts of the body such as joints, nails, scalp and tendons[6]. Even though it is non-contagious, impacts of psoriasis are analogous to those of cancer, heart disease, diabetes, or depression both physically as well as psychologically [7].Review of literature revealed a prevalence rate of 0.1–8% throughout the world for psoriasis[8]. Although the genetic basis of psoriasis and crucial malfunctions of the innate and adaptive immunity have been emerging as causal factors, therapy is still exclusively symptomatic and a true cure is still elusive [9]. Currently, psoriasis is managed and grounded on the information of its symptoms and affecting factors. Time of incidence, trigger factors, behaviour of disease indifferent individuals, infuriating factors, and effectiveness of the existing drug as well as availability and cost of therapy will have role in its management [10]. Among the different types of psoriasis, pustular psoriasis is highly inflammatory and recalcitrant type [11]. Etanercept (13-cis-trans retinoic acid), the FDA approved systemic retinoid, has been used from the past decades and is found to be very effective for severe psoriasis, especially for the pustular type [12]. But the use is limited due to its severe systemic toxicity such as teratogenecity. So, it is highly essential to develop a topical formulation of Etanercept, which would lower the systemic toxicities associated with the drug by increasing its local availability in the skin [13]. But for formulation scientists, it is a great challenge to develop such a formulation due to the unique problems of the drug such as skin irritation, extremely low solubility and instability in the presence of air, light and heat [14]. In order to overcome the limitations of Etanercept as a topical formulation, numerous efforts have been made and are still under exploration to develop novel topical vesicular system gels [15]. Are favorable and advanced drug delivery systems that can play a vibrant role by addressing these problems associated with the selected drugs [16]. The cationically charged, biodegradable and biocompatible chitin based nanogel system is a good candidate in these aspects, due to its improved skin penetration, enhanced stability and prolonged therapeutic activity [17]. Based on these aspects; we developed carbopol gel system of drugs Etanercept for the topical delivery in psoriasis [18].

2. Materials and method

Etanercept (Act) was procured from Remidex Pharma Private Ltd., Bengaluru. Eudragit S-100 was purchased from Evonik industries, Mumbai. Carbopol 940, Glycerol, Tween 80, propyl paraben, ethanol, and Triethanolamine were purchased from HiMedia Laboratories, Mumbai.

2.1 Method

2.1.1 Preparation of Etanercept Sodium Nanogel

Accurately weighed quantity of Drug, Eudragit S-100 (polymer), and Tween-80 as stabilizer are dissolved in glycerol while stirring. Prepared aqueous phase containing Carbopol-940 dissolved in water with continuous stirring and heat [19]. This drug containing phase is sonicated on Ultra sonic bath sonicator. The drug phase is added drop by drop into the aqueous phase during homogenization to form emulsion. The emulsion converted into nanodroplets by homogenizer which formed O/W emulsion. Homogenization was continued for one hour [20]. Propyl paraben &Triethanolamine added to form the gel with continuous stirring to nanogel. Batch A1, A2, A3 was prepared at highest rpm 8000 with variation in composition. Whereas prototype batches B1, B2, B3 and C1, C2, C3 prepared at different rpm 5000, 6000, 7000 using homogenizer respectively [21] .As shown in Table 1, Table 2, Table 3,Fig:1.

2.2.2 Evaluation Parameters

2.2.2. 1Appearance

The prepared gel bases were inspected visually for clarity, colour and presence of any particles [22].

2.2.2.2 Homogeneity

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates [23].

2.2.2.3 Measurement of particle size of formulation

The mean size of the selected nanogels were determined by using Malvern Mastersizer 2000 MS. The mean particle size was recorded [24].

2.2.2.4 pH measurement

The pH measurement was carried out by using calibrated digital type pH meter by dipping the glass electrode and the reference electrode completely into gel system so as to cover the electrodes [25].

2.2.2. 5 Drug Excipient compatibility studies

The drug polymer and polymer-polymer interaction was studied by the FTIR spectrometer using Shimadzu 8400-S, Japan. Two percent (w/w) of the sample with respect to a potassium bromide disc was mixed with dry KBr. The mixture was grind into a fine powder using an agate mortar and then compressed into a KBr Disc in a hydraulic press at a pressure of 1000psi. Each KBr disc was scanned 16times at 2 mm/sec at a resolution of 4 cm-1 using cosine apodization. The characteristic peaks we rerecorded Table: 4 (Fig: 3, 4, 5) [26].

2.2.2.6 Drug content

For the estimation of the drug in gel, Etanercept was extracted from 1 gm of gel formulation with 50 ml of phosphate buffer pH 5.5 buffer and methanol (7:3) and mixture was filtered through membrane filter (pore size 0.45 μm). From this, 2 ml was pipette out and made up to 10 ml . The absorbance of the sample was determined spectrophotometrically at 354nm. The concentration of Etanercept was estimated from the calibration curve Table: 5 (Fig: 2) [27].

2.2.3 In vitro Release studies

The drug release from the formulation was determined by using the apparatus known as Franz Diffusion Cell, which consist of a cylindrical glass tube which was opened at both the ends [28]. 1 gm of gel equivalent to 10 mg of Etanercept was spread uniformly on the surface of cellophane membrane (previously soaked in medium for 24 hrs) and was fixed to the one end of tube [29]. The whole assembly was fixed in such a way that the lower end of tube containing gel was just touches (1-2 mm deep) the surface of diffusion medium i.e. 100 ml of phosphate buffer pH 5.5 buffers and methanol (7:3) contained in 100 ml beaker [30]. The assembly was placed on thermostatic hot plate with magnetic stirrer and maintained at temperature 37°±2° the contents were stirred using magnetic bar at 100 rpm for a period of 24 hrs, 5 ml of samples were withdrawn at different time intervals [31]. This 5 ml was diluted up to 10 ml of fresh phosphate buffer (phosphate buffer pH 5.5 buffer and methanol (7:3) and sample were analyze at 354 nm in UV-Vis spectrometer for Etanercept (Fig: 7, 8, 9) [32]. Skin irritation test: Test for irritation was performed on human volunteers. For each gel, four volunteers were selected and 1.0 g of formulated gel was applied on an area of 2 square inch to the back of hand. The volunteers were observed for lesions or irritation [33].

2.2.4 Spreadability

Spreadability is determined by apparatus suggested by Mutimer. It consists of wooden block, which is provided by a pulley at one end. By this method, Spreadability is measured on the basis of "Slip" and "Drag". A ground glass slide is fixed on this block [34]. A sample of 0.1 g of nanogel under study is placed on this ground slide. The gel is fixed on the beach formula was pressed between two slides and a 1 kg weight is placed on the top of two slides and left for about 5 min to expel air and to provide a uniform film of the nanogel between two slides. Excess of the gel is scrapped from edges [35]. The top plate is then subjected to pull the weight. With help of string attaches to the hook and the time required by top slide to cover the distance is noted [36]. A shorter interval indicates better Spreadability **(**Fig: 6) Spreadability was calculated by using the formula,

S=M.L/T,

Where,

S=Spreadability,

L=Length of glass slide,

M=weight tied to upper slide,

T=Time taken to separate the slides.

2.2.5 Extrudability

It is a usual empirical test to measure the force required to extrude the material from tube. The method applied for determination of applied shear in the region of the Rheogram corresponding to a shear rate exceeding the yield value and exhibiting plug flow [37]. The method adopted for evaluating nanogel formulation for Extrudability is based upon the quantity in percentage of nanogel and nanogel extruded from lacquered aluminium collapsible tube on application of weight in grams required at least 0.5cm ribbon of nanogel in 10 sec [38]. The measurement of Extrudability of each formulation shows the triplicate and averages value is presented [39].

Extrudability = Applied weight to extrude the nanogel from tube (in gm)/ Area (in cm2).

2.2.6 Rheological Studies

Brookfield viscometer was used for the studies. First, the spindle was dipped into the gel till the notch on the spindle touched the gel surface [40]. 3gm each of gel I and gel II (Stability chamber and Room temperature) was used in the study. The spindle no.61, 63, 64 was selected based on viscosity of gel [41]. The dial readings were taken at 50, 100, 150, 250 rpm and viscosity was measured [42] From the evaluation parameter performed for the three prototype batches, the result for the batch A-1 was found to be satisfactory in all attributes and hence selected for trial batches [43]. As shown in Table 7, Table 8, Table 9.

The evaluation parameter was performed on marketed product as shown in (Table: 11).

2.2.7 UV Spectroscopy

After studying the UV spectra of Etanercept, it was found that drug shows absorbances at 354 and 354 nm but maximum absorbance was at 354 nm when solution is prepared in distilled water [44]. So, 354nm was considered as λmax. UV Etanercept is shown in (Fig: 2).

2.2.8 Effect of change in pH on λ_{max}

λ max of drug was observed by making its solution in different pH to check the effect of pH on λmax. Result of the same is given in Table 5. There was no significant change in λmax of Etanercept at different pH [45]. So calibration plot can be constructed by using distilled water and can be used for quantitative evaluation purpose; though the medium of evaluation of release is phosphate buffer pH 5.5 buffers and methanol (7:3) (Table:6) [46] .

2.2.9 Calibration curve of Etanercept

The calibration curve for Etanercept in phosphate buffer pH 5.5 buffers and methanol (7:3) is shown in Fig: 2 and its observation values in (Table: 5) [47]. The graph of absorbance vs. concentration was found to be linear in the concentration range of 4-24 µg/ml at 276 nm. The R2 of the calibration curve was found to be 0.999 [48].

2.2.10 Stability batches evaluation

The stability studies were carried out on optimized formulation. The samples were stored at 40oC±2oC and75%±5% relative humidity for three months as per ICH guidelines. After 1, 2 and 3 months samples were with- drawn and tested for appearance, pH, particle size, Drug Content, Spreadability, Extrudability, viscosity.

2.2.11Stability data of Optimized Formulation

The evaluation parameter performed for the trial batches (B and C) are the same as done for the above prototype batch (A) and they are appearance, homogeneity, particle size measurement, pH measurement, drug entrapment efficiency, drug content, *in vitro* drug release, skin irritation study, Spreadability, Extrudability, rheological study, stability batches.

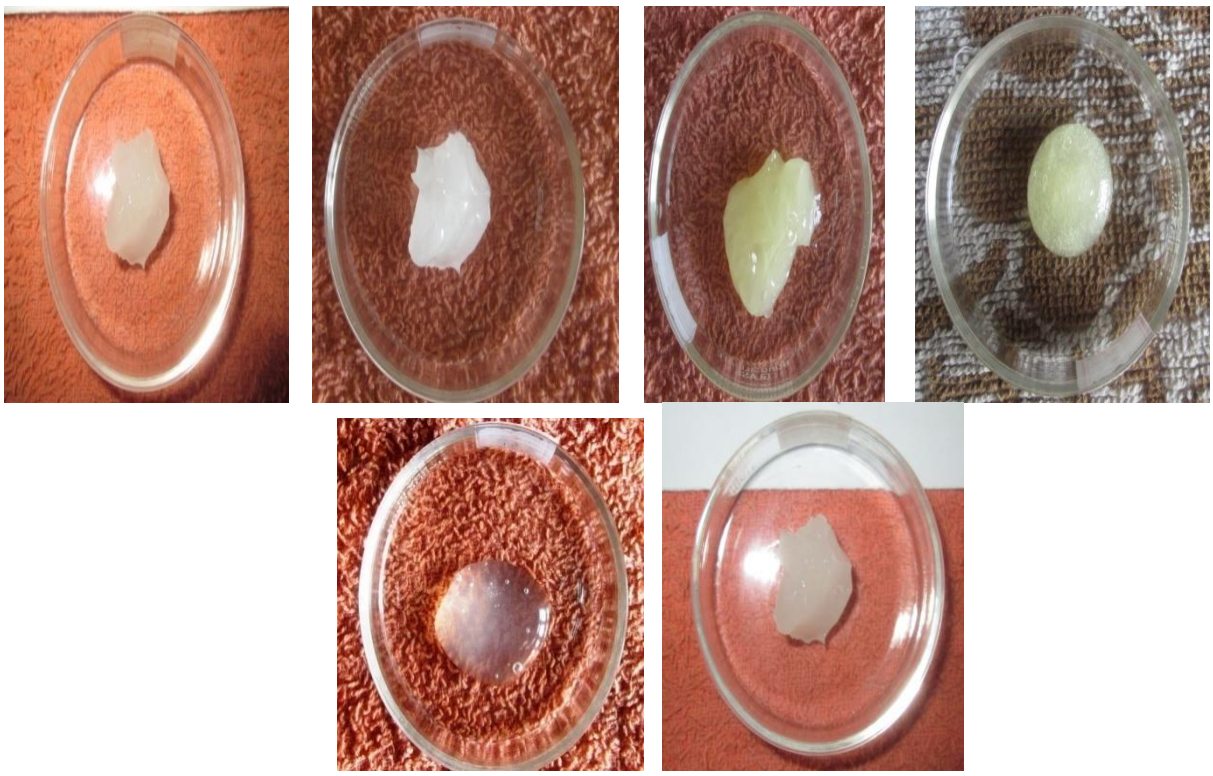

Fig: 1 Formulation of Etanercept Nanogels

Table 1: Etanercept Nanogel (Composition of Batch A)			
Compositions	**A-1**	**A-2**	**A-3**
Etanercept (g)	0.5	0.5	0.5
Eudragit S-100 (g)	0.15	0.2	0.25
Tween-80 (ml)	0.1	0.3	0.5
Glycerol (ml)	5	10	15
carbopol 940 (g)	0.5	0.1	0.3
Water (ml)	70	30	50
Propyl Paraben	0.1	0.2	0.3
Triethanolamine (ml)	2	3	4

Table 2: Etanercept Nanogel (Composition of Batch B)			
Composition	**B-1**	**B-2**	**B-3**
Etanercept (g)	0.5	0.5	0.5
Eudragit S-100 (g)	0.15	0.15	0.15
Tween-80 (ml)	0.1	0.1	0.1
Glycerol (ml)	5	5	5
carbopol 940 (g)	0.1	0.1	0.1
Water (ml)	30	30	30
Propyl Paraben	0.1	0.2	0.3
Triethanolamine (ml)	2	2	2

Table 3: Etanercept Nanogel (Composition of Batch C)			
Composition	**C-1**	**C-2**	**C-3**
Etanercept(g)	05	0.5	0.5
Eudragit S-100 (g)	0.15	0.15	0.15
Tween-80 (ml)	0.1	0.1	0.1
Glycerol (ml)	5	5	5
carbopol 940 (g)	0.1	0.1	0.1
Water (ml)	30	30	30
Propyl paraben(mg)	0.1	0.2	0.3
Triethanolamine (ml)	2	2	2

Table 4: Interpretation of FTIR spectrum of pure Etanercept	
Peaks cm-1	**Groups**
3651	O-H
1248	C-O
2936	Aliphatic C-H
1079	Asymmetric C-O-C
1012	Symmetric C-O-C

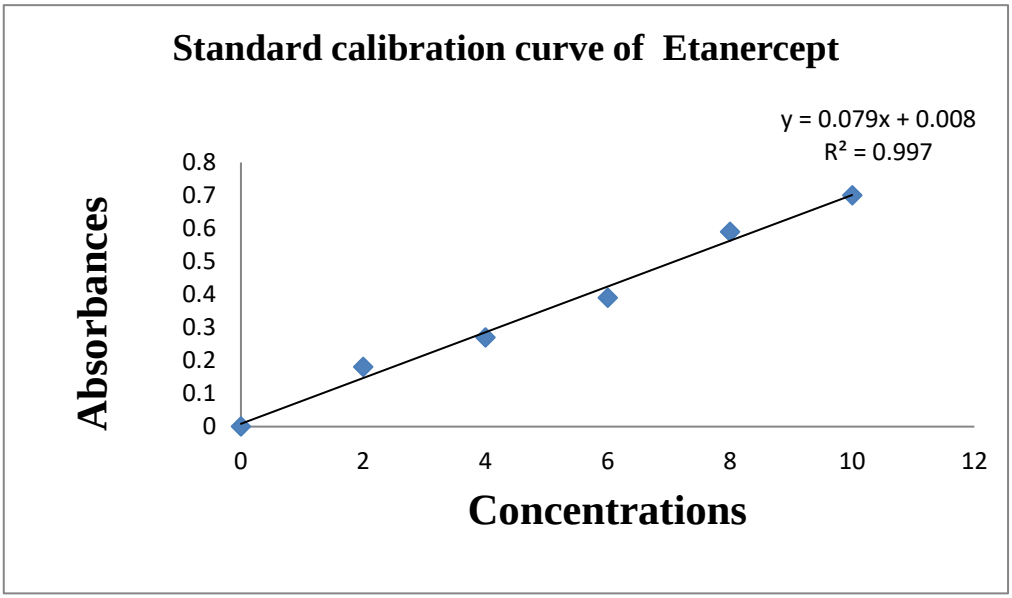

Fig 2: Standard calibration curve of Etanercept

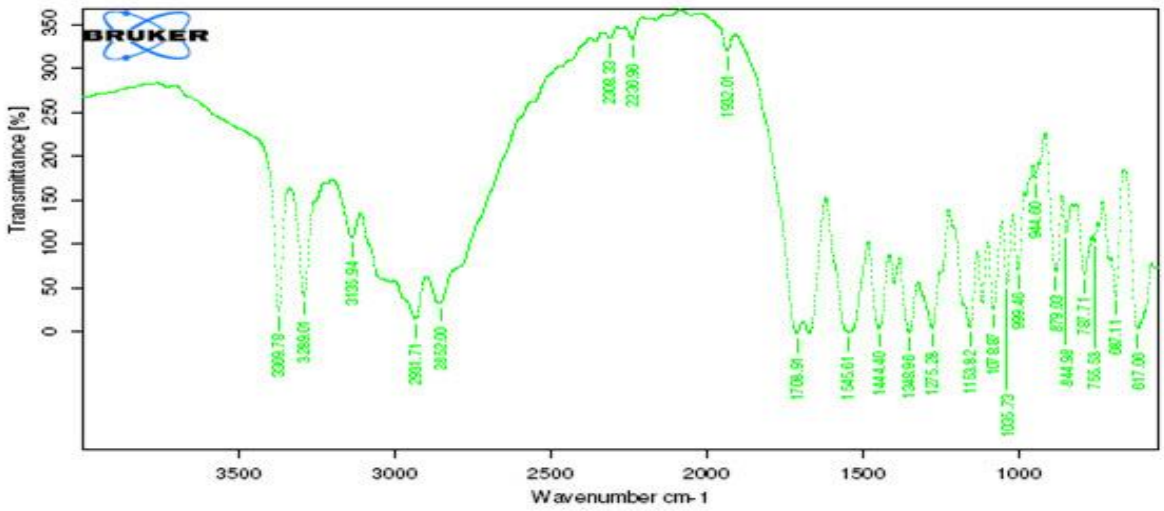

Fig 3: FTIR Spectra of Etanercept

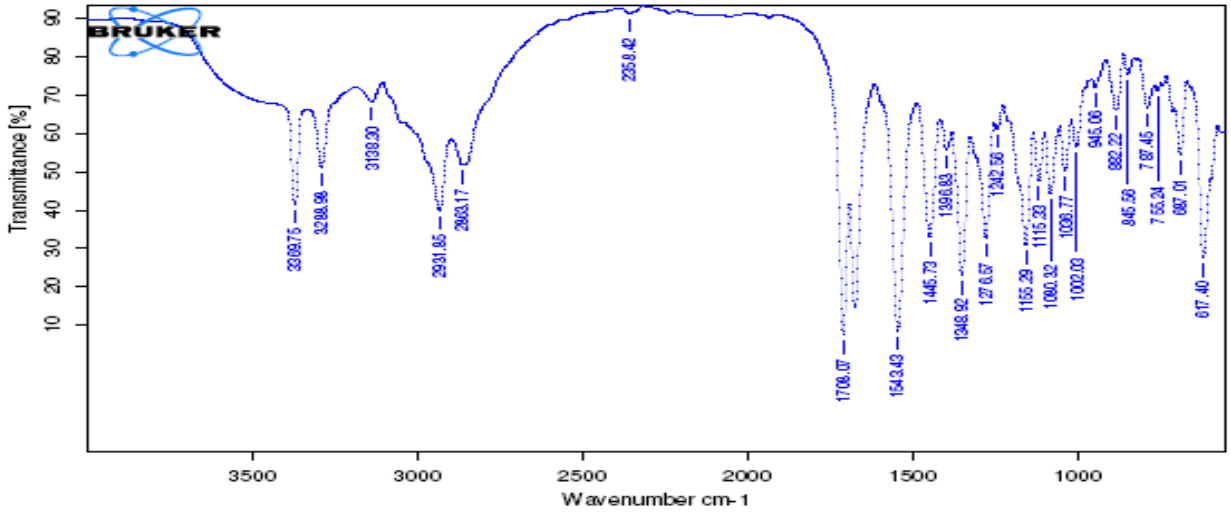

Fig 4: FTIR of Carbopol 940

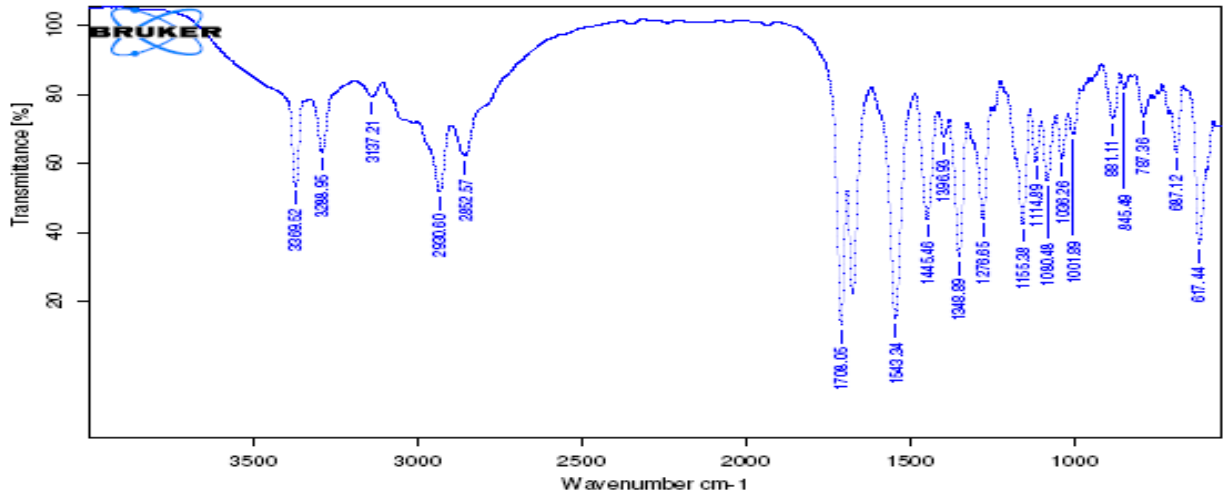

Fig 5: FTIR of Eudragit S-100

Figure 6: Spreadability test for Act gels

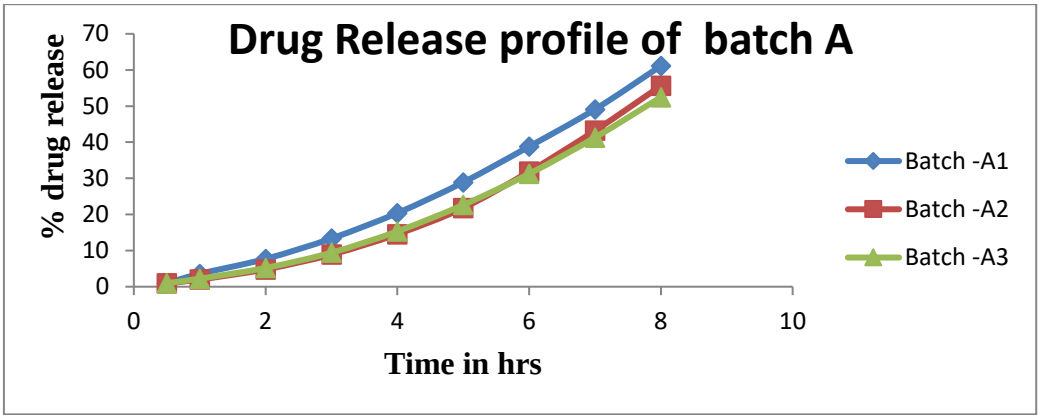

Figure 7: Drug release profiles of batch A

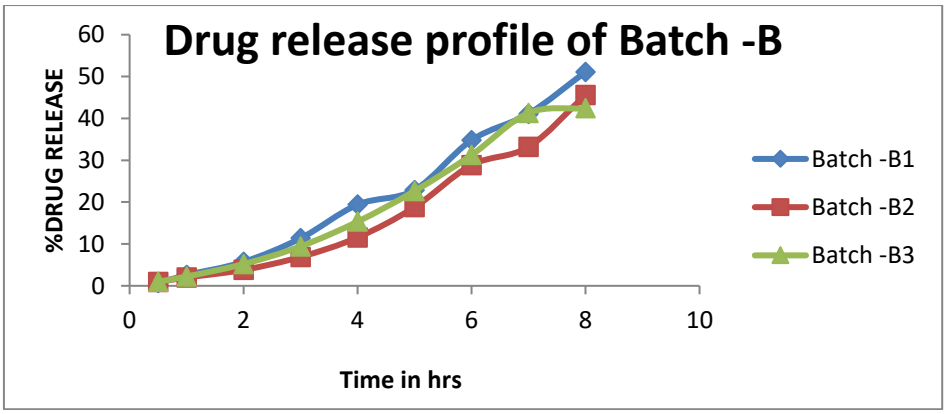

Figure 8: Drug release profiles of batch-B

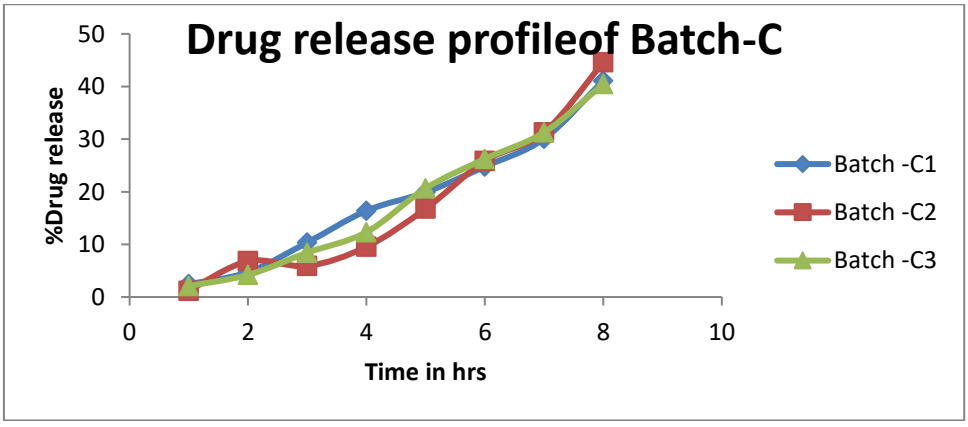

Figure 9: Drug release profiles of batch-C

Table 5: Calibration curve of Etanercept		
S. N	**Concentration (µg /ml)**	**Absorbance**
1.	2(µg/ml)	0.15
2.	4 (µg/ml)	0.31
3.	6(µg/ml)	0.38
4.	8(µg/ml)	0.59
5.	10(µg/ml)	0.68

Table 6: Effect of pH on Λ max	
Drug solution in pH	**Λ max**
5.5	354nm
5.5 buffer and methanol (7:3)	354nm

Fig10: Diffusion study optimized Etanercept -Batch-A1

Table 7: Evaluation parameters for Batch A			
Evaluation parameters	**A-1**	**A-2**	**A-3**
Appearance	Clear	Clear	Clear
Homogeneity	Homogeneous	Homogenous	Homogenous
Particle size (nm)	152	169	210
Ph	6.9 ± 0.00	6.4 ± 0.02	6.1 ± 0.20
Drug content ± SD	97.8 ± 0.02	95.5 ± 0.02	97.8 ± 0.04
In vitro drug release (%)	96.72 ± 0.0784	94.75 ± 0.963	92.78 ± 0.77
Skin irritation test	No irritation	No irritation	No irritation
Spreadability (g.cm/s)	5.3 ± 0.5	5.7 ± 0.6	5.4 ± 0.6
Extrudability (g)	269 ± 0.7	258 ± 0.5	244 ± 0.7
Viscosity in cp at 50 (rpm)	9463	8562	7000

Table 8: Evaluation parameters for Batch B			
Evaluation parameters	**B-1**	**B-2**	**B-3**
Appearance	Clear	Clear	Clear
Homogeneity	Homogenous	Homogenous	Homogenous
Particle size (nm)	161	166	218
pH	5.2 ± 0.00	5.7 ± 0.02	5.5 ± 0.20
Drug content± SD	91.8 ± 0.02	94.5 ± 0.02	95.85± 0.04
In vitro drug release (%)	92.72 ± 0.0784	91.75 ± 0.963	93.78 ± 0.77
Skin irritation test	No irritation	No irritation	No irritation
Spreadability (g.cm/s)	6.6 ± 0.5	6.5 ± 0.6	6.7 ± 0.6
Extrudability (g)	269 ± 0.7	250 ± 0.5	261 ± 0.7
Viscosity in cp at 50 (rpm)	8863	8582	7888

Table 9: Evaluation parameters for Batch C			
Evaluation parameters	**C-1**	**C-2**	**C-3**
Appearance	Clear	Less clear	Clear
Homogeneity	Homogeneous	Homogeneous	Homogeneous
Particle size (nm)	165	160	160
Ph	5.5 ± 2	5.7 ± 1	5.2 ± 2
Drug content ±SD	90.2 ± 0.029	93.6 ± 0.04	91.5 ± 0.072
In vitro drug release (%)	92.25 ± 0.903	90.72 ± 0.861	91.3 ± 0.85
Spreadability (g.cm/s)	6.3	6.4	6.3
Extrudability (g)	251	233	214
Viscosity in cp at 50 (rpm)	9385	95288	8400

Table 10: Stability data of optimized formulation		
Time period	**Particle size(nm)**	**Total drug content (%)**
Initial	165	98.2 ± 0.029
After storage **(**40°C ± 2°C and 75% ± 5%RH)		
1 Month	162	95.72 ± 0.861
2 Month	160	94.3 ± 0.85
3Month	164	93.25 ± 0.903

Table 11: Evaluation parameter of marketed product	
Evaluation Parameters	**Market product ((Losone)**
Appearance	Clear
Homogeneity	Homogenous
Particle size (nm)	168
pH	5.5 ± 0.2
Drug content ±SD	96.8 ± 0.02
In vitro drug release (%)	95.72 ± 0.0784
Skin irritation test	No irritation
Spreadability (g.cm/s)	6.6 ± 0.5
Extrudability (g)	275 ± 0.7
Viscosity in cp at 50 (rpm)	9400

From the evaluation parameter result of trial batches we found Batch-A1 as the optimized batch and further experimental design is formulated. Figure 5. The evaluation parameter performed for the trial batches (B and C) are the same as done for the above prototype batch (A) and they are appearance, homogeneity, particle size measurement, pH measurement, drug entrapment efficiency, drug content, in vitro drug release, skin irritation study, Spreadability, Extrudability, rheological study, stability batches From the evaluation parameter result of trial batches we found Batch-A1 as the optimized batch and further experimental design is formulated. As shown in Table 10.

4. Conclusion

Nanogel formulation containing Etanercept was successfully prepared and shows effective as well as better carrier for the transdermal topical preparation. The formulated nanogel was optimized for homogeneity, particle size, pH, drug content, *in vitro* drug release, skin irritation test, Spreadability, Extrudability, and viscosity. Administration of this through dermal route bypass the disadvantages of which overcomes the demerits of oral dosage form and conventional gel system of Etanercept (Act). The initial release rate from each formulation was very rapid, this may be due to incomplete gel formation in the earlier time period, but the release became slow in latter period after complete gel formation. The release profiles exhibited an inflection point, which indicated gel formation on the diffusion membrane in donor compartment of diffusion cell. During gel formation, formulation got converted into the gel phase and thus drug release became slow. The results showed that the formed gels had the ability to retain Etanercept for the duration. The production of the formulation is also proved to be better and cost effective in comparison with oral dosage forms. Etanercept Has Been Used As Anti-Psoriatic Drug For Its Effect In Oral Route Causes Which Overcomes The Demerits Of Oral Dosage Form. Nanogel System Is A Good Candidate In These Aspects, Due To Its Improved Skin Penetration, Enhanced Stability And Prolonged Therapeutic Activity. Based On These Aspects Nanogel Combines The Advantage Of Hydrogel Inhering The Property Of Nanoscale Size. Nanogel Network Provide High Specific Form Can Host And Protect Drug Molecules. The Release Of The Drug Molecules Can Be Incorporated By Providing High- Affinity Functional Groups. Etanercept Nanogel Which Provides Prolonged Release.

Conflict of interests
All authors have none to declare.

Source of support
Nil

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Abbreviation used

nm: Nanometer; **GIT:** Gastrointestinal tract; **gm:** Gram; **ml:** Milliliter; **kg:** Kilogram; **cm:** Centimeter; **rpm:** Revolutions per minute; **ICH:** International Conference on Harmonization; **µm:** Micrometer; **mm:** Milimeter; **sec.:** Second; **µg/ml :** Microgram per milliliter; **g.cm/s:** Gram centimeter per second; **RH:** Relative Humidity.

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