



Formulation and Evaluation of Acitretin Transdermal Gel for The Treatment of Psoriasis

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ABSTRACT

The present research work was aimed to develop a novel gel for Acitretin (Act) to enhance the drug absorption by the topical application, which overcomes the demerits of oral dosage form and conventional gel system of Acitretin (Act). The gels were prepared with carbopol 934 as a gelling agent used in six different concentrations. Span 20 and Tween 20 were included as emulsifying agents in two different concentrations. Liquid paraffin was used as an oil phase, and methyl and propyl paraben were included as preservatives. Ethanol was used to dissolve the drug for preparing the aqueous phase and Triethanolamine was added at the end of preparation, as quantity sufficient for pH adjustment. All the formulated nanogels were screened for the parameters, namely, appearance, pH, viscosity study, Spreadability, swelling index, drug content, and in vitro drug release studies. The optimized formulation ActG-4 showed 81.95% of drug release up to 8 h, and the particle size analysis reported good size range, and the gel was found to be nonirritant and nontoxic which was confirmed by HET CAM test. Acitretin (Act) can be successfully formulated as gel for better-sustained effect and can be a suitable alternative approach to the oral dosage forms for the management of Psoriasis.

Key words: Acitretin (Act), carbopol 934, viscosity.

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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].

Acitretin (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 teratogenicity. So, it is highly essential to develop a topical formulation of Acitretin, 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 Acitretin 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 Acitretin for the topical delivery in psoriasis [18]

MATERIAL AND METHODS

Acitretin (Act) was procured from Remidex Pharma Private Ltd., Bengaluru. Carbopol 934, liquid paraffin, span 20, tween 20, methylparaben, propyl paraben, ethanol, and Triethanolamine were purchased from HiMedia Laboratories, Mumbai.

Methodology

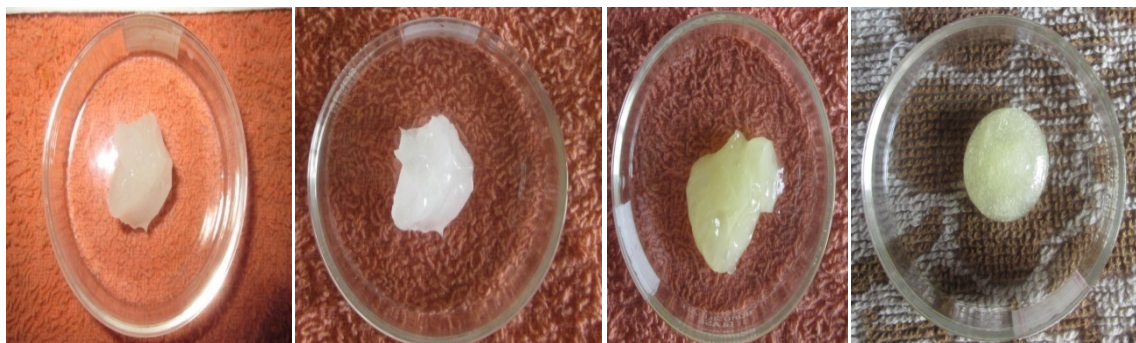
Preparation of Acitretin gel

The Act gel phase and emulsion phase were prepared separately. First, the gel phase was prepared by dispersing the different concentrations of carbopol 934 in distilled water and mixed by a mechanical stirrer [19]. The emulsion phases were prepared by the addition of varying amounts of span 20 in varying quantities of liquid paraffin followed by mechanical stirring [20]. The aqueous phase of gel was prepared by incorporating tween 20 in distilled water with continuous stirring, then methyl and propyl paraben were added in propylene glycol⁽²¹⁾, and of Acitretin (0.5 g) was dissolved in ethanol, and both the solutions were mixed with water phase of the emulsion [22]. Both the water and oil phases were heated at 70–80°C for 20 min. Later, the oily phase was added to the aqueous phase by gentle stirring and allowed to cool. Finally, the prepared emulsion was mixed with gel base in a 1:1 ratio by manual stirring to get a clear gel of Acitretin. The pH of all the prepared gels was adjusted by drop wise addition of Triethanolamine [23].

A quantity of 100 g of Acitretin was prepared for all the six formulations and the formulation composition of aceclofenac gels is shown in Table 1.

Table 1: Formulation design of Acitretin gels

Ingredients	Formulation code					
	Act-1	Act-2	Act-3	Act-4	Act-5	Act-6
Acitretin(g)	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g
Carbopol934(%w/w)	0.5%	1%	1.5%	2%	2.5%	3%
Liquid Paraffin (ml)	5 ml	5 ml	8 ml	8 ml	10 ml	10 ml
Span 20(%w/w)	0.2%	0.5%	0.2%	0.5%	0.2%	0.5%
Tween 20(%w/w)	0.2%	0.5%	0.2%	0.5%	0.2%	0.5%
Methylparaben(mg)	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%
Propyl paraben(mg)	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%
Ethanol (ml)	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
Distilled water(ml)	q.s	q.s	q.s	q.s	q.s	q.s
Triethanolamine(ml)	q.s to adjust pH	q.s to adjust pH	q.s to adjust pH	q.s to adjust pH	q.s to adjust pH	q.s to adjust Ph



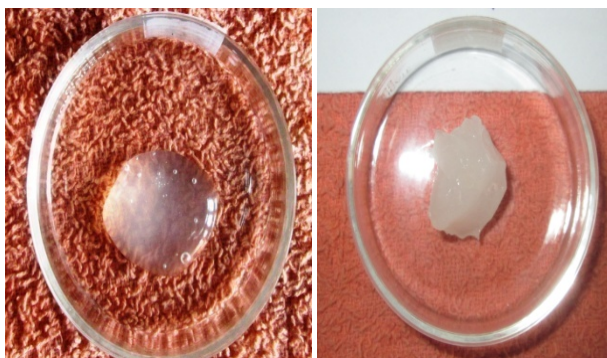


Fig: 1 Formulation of Acitretin gels. (Act-1 to Act-6)

Characterization of Acitretin gels:

About six formulations i.e. Act gel-1 to-6 were conducted. Gels were evaluated for their appearance, pH, viscosity, Spreadability, Extrudability, skin irritation test, percentage drug content, *in-vitro* diffusion studies, *in-vitro* drug release kinetic study, *ex-vivo* permeation studies using rat abdominal skin and stability studies by using standard procedure. All studies were carried out in triplicate and average values were reported [24].

Appearance: All the formulated Acitretin Act gels were visually inspected for color, clarity, and homogeneity [25] [Table 02].

Surface pH

2.5 gm of gel was accurately weighed and dispersed in 25ml of distilled water. The pH of the dispersion was determined by using digital pH meter [26]. The results are shown in [Table 02].

Viscosity

Viscosity was determined by using Brookfield viscometer. Viscosity measurements were carried out at room temperature (25- 27°C) using a Brookfield viscometer (Model RVTDV II, Brookfield Engineering Laboratories, Inc, Stoughton, MA) [27]. The results are shown in [Table02].

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 [28]. The results are shown in [Table 03]

Consistency

The estimation of consistency of the prepared gels was done by dropping a cone attached to a holding rod from a fixed distance of 10cm in other way that it should fall down on the centre of the glass cup was filled with the gel [29]. The penetration by the cone was accurately measured from the surface of the gel to the tip of the cone inside of the gel. The distance traveled by cone in the period was noted down after 10sec. The results are shown in [Table 03]

Extrudability

Extrudability test was carried out by using Pfizer hardness tester. 15gm of gel was filled in collapsible aluminium tube [30].The plunger was adjusted to hold the tube properly the pressure of 1kg/cm² was applied for 30 sec. The quantity of the gel extruded was weighed. The procedure was repeated at three equidistance places of the tube. The test was carried out in triplets. The results are shown in [Table 03].

Spreadability

An ideal topical Act gel should possess a sufficient spreading coefficient when applied or rubbed on the skin surface. This was evaluated by placing about 1 g of formulation on a glass slide. Another glass slide of the same length was placed above that, and a mass of 500 g was put on the glass slide so that the gel gets sandwiched between the two glass slides and spreads at a certain distance the [31]. Time taken for the gel to travel the distance from the place of its position was noted down. Spreadability was determined by the following formula [32] [Table 02].

$$S = \frac{M \times L}{T}$$

Where, S-Spreadability, g.cm/s

M-Weight put on the upper glass

L-Length of glass slide

T-Time for spreading gel in sec.

Swelling index

It was measured by placing 1 g of formulation in a porous aluminum foil and was placed in a 50 ml beaker containing 10 ml of 0.1 N Sodium hydroxide. The samples were removed from beakers at different time intervals and put on the dry place for some time and reweighed[33] [Table 02].

The swelling index of gels was calculated using the following formula.

Swelling index (SW) % = $[(W_t - W_0)] / W_0 \times 100$

Where, (SW) % = Equilibrium percent swelling

W_t = Weight of swollen emulgel after time t ,

W_0 = Weight of emulgel before swelling at zero time, t .

Particle size analysis

This study was done for optimized formulation of Act gel. The procedure involves dilution of 1 g of Act gel with distilled water which was observed under high resolution Biovis Particle Size Analyzer and the average size of the particles were measured in microns [34].

Scanning electron microscopy (SEM): The Act gels surface and shape characteristics were determined by using the gold sputter technique. ESEM (QUANTA-200-3D, FEI, USA) at 20.0 KV in environmental mode used for the identification and morphology of Gels[35].

Entrapment Efficiency: It was expressed by knowing the percentage of the drug trapped after formulation to that of an added drug. EE and loading efficiency of gel formulations were determined by separating the un-encapsulated drug by centrifugation using an AmiconUltra-15 30 K tube (Millipore, Germany) (at 5000 rpm for 30 min) and then measured the concentration of free drug in the lower chamber [36]. The contents in the upper chamber of the AmiconUltratube were rinsed three times by hydroalcoholic solution to remove unloaded drug and were used for the subsequent experiments. Finally, the percent amount of drug was determined by spectrophotometer [37].

Drug-polymer compatibility by Fourier transforms infrared (FTIR) study

This study was carried out by FTIR spectroscopy to verify whether the drug and polymer are compatible with one another or not. It was evaluated by obtaining the IR spectral data of Acitretin and physical mixture of Acitretin with carbopol 934 using ATR-Bruker FTIR spectrophotometer. The interaction study was concluded from the interpretation of IR spectra [38].

Drug content

To determine the drug content of Act gel, 1 g of the formulation was diluted with 10 ml of phosphate buffer pH 5.5 buffer and methanol (7:3).The volumetric flask was shaken well followed by bath Sonication for 2 min and the solution was filtered and scanned at 354 nm spectrophotometrically and the absorbance was noted [39]. The amount of drug present in the gels was determined from the standard plot of Acitretin [Table 02].

Stability studies

The optimized formulation F4 was subjected to a stability testing for the period of three months as per ICH norms at a temperature of $25 \pm 2^\circ\text{C}$ with relative humidity RH= $60 \pm 5\%$ and $40 \pm 2^\circ\text{C}$ with relative humidity RH= $75 \pm 5\%$. The optimized formulation F4 was analyzed for the changes in appearance, pH, percentage of drug content and *in-vitro* diffusion study by procedure stated earlier. The results are shown in [Table 06]. [40].

Drug release kinetic studies

Various models were tested for explaining the kinetics of drug release. To analyze the mechanism of the drug release rate kinetics of the dosage form, the obtained data was fitted into zero-order, first order, Higuchi and Korsmeyer Pappas release model, to study the drug release from the dosage form⁽⁴¹⁾. The results are shown in [Table05].

Zero order release rate kinetics:-

To study the zero-order release kinetics the release rate data are fitted to the following equation.

$$F = K_0t$$

Where 'F' is the drug release, 'K' is the release rate constant and 't' is the release time. The plot of % drug release versus time is linear.

First-order release rate kinetics:-

The release rate data are fitted to the following equation.

$$\text{Log}(100 - F) = kt$$

A plot of log % drug release versus time is linear.

Higuchi release model:-

To study the Higuchi release kinetics, the release data were fitted to the following equation.

$$F = kt^{1/2}$$

Where 'k' is the Higuchi constant.

In Higuchi model, a plot of % drug release versus square root of time is linear.

Korsmeyer-Pappas release model:-

The release rate data were fitted to the following equation.

$$M_t/M_\infty = Kt^n$$

Where, M_t/M_∞ is the fraction of drug released, 'K' is the release constant,

't' is the release time's' is diffusion exponent. If $n = 0.89$, the release is zero order. If $n = 0.45$ the release is best explained by Fickian diffusion, and if $0.45 < n < 0.89$ then the release is through anomalous diffusion or non Fickian diffusion (Swallowable & Cylindrical Matrix). In this model, a plot of $\log (M_t/M_\infty)$ versus \log (time) is linear.

The drug release data of optimized tablet were fitted to Zero-order, First-order, and Higuchi and Korsmeyer- Pappas model to study the kinetics of drug release[42].

In vitro drug release study

Release study of the Act gels was performed using modified Franz diffusion six cell apparatus which has donor and receptor compartment with a linear end for the solution withdrawal. A dialysis membrane, which was soaked overnight in phosphate buffer pH 5.5 buffers and methanol (7:3), was tied on the upper surface of the donor compartment. An amount of 12 ml of freshly prepared phosphate buffer pH 5.5 buffer and methanol (7:3).was put in receptor chamber. The dialysis membrane was sandwiched between the donor and receptor compartment⁽⁴³⁾. A magnetic bead was placed inside the receptor compartment by operating at 50 rpm and the apparatus assembly was maintained at $37 \pm 0.5^\circ\text{C}$. 500 mg of Act gel was placed on the dialysis membrane, which was mounted on the donor compartment. Aliquots of 1 ml were withdrawn at time intervals of every 30 min and diluted to 10 ml with phosphate buffer pH 5.5 buffer and methanol (7:3).The study was done for a time period of 6 h. All the solutions were scanned at 354 nm using UV Spectrophotometer. The amount of drug released was estimated, and the percentage cumulative drug release of the Act gels was calculated [44].

In vitro skin irritation study

For checking the skin irritation, an *in vitro* OECD recommended test was used known as Hen's Embryo Test-Chorioallantoic Membrane (HET-CAM test). In this method, hen eggs which are freshly layed were used and were embryonated to check the irritation on the developed chick embryo. Three groups were made, each containing three eggs [45].

Negative control: Here, the eggs were treated with 0.9% NaCl as a standard.

Test group: In this group, eggs were tested with the optimized formulation.

Positive control: In this, eggs were treated with 1% SDS (Sodium dodecyl sulfate) as an irritant for comparison with negative control and test.

Methods

The collected hen's eggs were placed on a metal tray which was kept in an incubator at temperature of $37 \pm 0.5^\circ\text{C}$ and relative humidity of $58 \pm 2^\circ\text{C}$ required for the embryo development in the eggs [46]. During incubation, the eggs were hand rotated 5 times in a day and this process was continued for 8 days. On 8th day, the incubated eggs were observed for the embryo growth and after confirmation the eggs were placed back to the incubator[47]. On 9th day, the eggs were removed from the incubator and on the top surface; hole was drilled on the air sac of egg shell without harming the embryo. After making the hole, all the groped eggs were treated with respective solutions and observed for the signs of hemorrhage, coagulation, and Lysis of blood vessels for a time period of 300 s (5 min) [48].

The irritation effect was confirmed by getting the mean irritation score from the formula.

The irritation score (IS) formula is given below, followed by irritation score value with inference in

$$IS = \frac{301-H}{300} \times 5 \pm \frac{301-L}{300} \times 7 \pm \frac{301-C}{300} \times 9$$

Where, H- Hemorrhage.

L- Lysis of blood vessels.

C- Coagulation.

RESULTS AND DISCUSSION

Appearance and Surface pH

All the formulated Act gels were found to be white-colored translucent gels with good homogeneity. The pH of the gels was found to be in the range of 6.4–7.0 and was found to be satisfactory. The results of appearance and pH of the Act gels are discussed.

Viscosity study

The viscosity of the Act gels was found to be in the range of 438.6–622.4 cps. Results were reported in Table 3. From the study, it was observed that viscosity of the formulated Act gels was dependent on the concentration of carbopol 934. As the concentration of carbopol 934 was increased, the viscosity of Act gels was also increased.

Homogeneity:

All developed Act gels (1-6) showed good homogeneity with absence of lumps. The developed preparations were much clear and transparent.

Consistency

All formulations showed good Consistency when applied between the horizontal plates. Consistency of these formulations was acceptable and smooth when applied.

Extrudability:

The extrusion of the gel from the tube is an important during its application and in patient acceptance. Gels with high consistency may not extrude from tube whereas, low viscous gels may flow quickly, and hence suitable consistency is required in order to extrude the gel from the tube. Extrudability of Carbopol 934P gel i.e. AG-4 formulation was found to be Excellent when compared to other formulations.

Spreadability

The Spreadability of all the Act gels was ranging from 11.54 to 42.24 g.cm/s. It was observed that formulations AG-4, AG-5, and AG-6 showed higher Spreadability, which may be due to an increased concentration of carbopol 934. The Spreadability test results are interpreted in Table 3, and Spreadability test for Act gels is depicted in Figure 3.

Parameter	Formulation code					
	Act-1	Act-2	Act-3	Act-4	Act-5	Act-6
Appearance	White translucent gel	White translucent gel	White translucent gel	White translucent gel	White translucent gel	White translucent gel
Ph	7.0	6.7	6.4	6.5	6.5	6.7
Spreadability (g.cm/s)	21.25	14.16	11.54	40.43	42.24	36.8
Viscosity (Cps)	438.6	504.8	552.4	568.2	611.6	622.4
Swelling index (%)	28.44	23.58	27.77	30.08	15.92	20.53
Drug content (%)	91.18	93.80	94.37	96.32	95.22	92.46

Swelling index

Aceclofenac Act gels showed swelling index ranging from 15.92 to 30.08%, which was found to be satisfactory. The results of the swelling index are reported in Table 3.

Particle size:

Surfactant greatly influenced the particle size distribution which resulted in greater stability. The size of nanoparticles may be the reason for drugs having enhanced solubility.

Scanning Electron Microscopy (SEM): The images of SEM for optimized formulation (AG1-4) were shown in Fig 4. As per SEM results, particles were in circular shapes, which indicated the drug was circularly encapsulated into the lipid matrix.

Entrapment efficiency: Among the all AG1-6 formulations, the AG-4 was found as 91.3±12% of EE. The EE was proportionally increasing with an addition of drug and incubation time, which indicates the influence of these two factors. From the graph of the concentration-depth profile, it is clear that the drug retention for the drug-loaded nanogel system is more in the dermal and epidermal layers to that of the control drug solution. Which indicated the retention is much in the dermal layer of skin Fig 5.

FTIR spectroscopy

From the FTIR interpretation, it was observed that the peaks that are found both in the IR spectra of Acitretin and physical mixture of aceclofenac with carbopol 934 were found to be the same, and hence there was no interaction between the drug and polymer used. The FTIR spectral images of Acitretin and physical mixture of drug and polymer are shown in Fig-6-7.

Drug content

The drug content of all the formulated Act gels was in the range of 91.18–96.32%, and formulation Act-4 showed the highest drug content among the other five formulations. The results of the drug content are shown in Table 4.

Stability Studies:

Accelerated stability studies was conducted in best formulation AG-4, according to ICH guidelines i.e. 25°±2°C/60±5%RH for first 30 days and 40°±2°C/75±5%RH up to 90 days. The results indicate that there was no so much change in appearance, pH, and drug content and in-vitro drug release studies. The results are shown in [Table 05].

In vitro drug release study

From the drug release study, it was observed that formulations Act-1, Act-2, Act-3, and Act-4 showed the drug release from 72.54 to 93.13 up to 6 h. This might be due to the increase in the concentration of carbopol 934 from 0.5 to 2% along with increase in the amount of emulsifying agents added. The Act gels

Act-5 and Act-6 showed drug release of 87.26 and 84.33 up to 6 h which may be due to the fact that increased concentration of carbopol 934 (2.5%) in Act-5 and Act-6 (3%) was led to increasing the viscosity of these formulations which in turn makes the diffusion of drug through the dialysis membrane slower. Among all the six Act gels formulated, formulation Act-4 containing 2% of carbopol 934 and 8% of liquid paraffin showed highest drug release of 91.43% and was optimized as the best and was subjected for particle size analysis and *in vitro* skin irritation study. The drug release profile of Act gels is depicted in Figure 7.

Table 3: Results of Homogeneity, Consistency, Extrudability of Act gels

Formulation Code	Homogeneity	Consistency	Extrudability
AG-1	Satisfactory	----	+
AG-2	Good	----	++
AG-3	Good	----	++
AG-4	Excellent	No change	+++
AG-5	Good	Change is observed	+
AG-6	Good	Change is observed	++

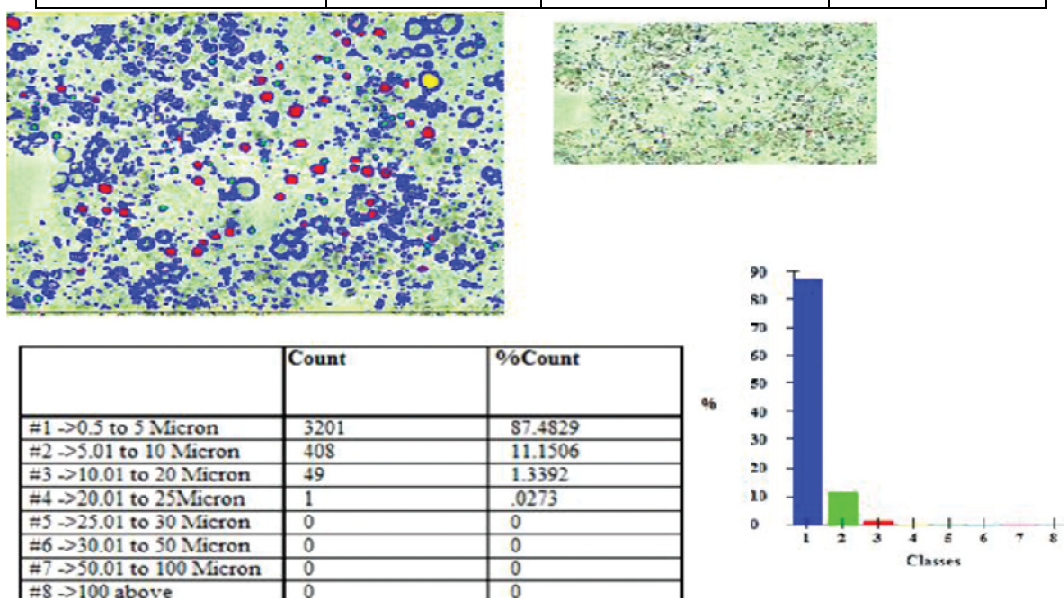


Figure 2: Particle size data for optimized Act gel formulation Act-4



Figure 4: Spreadability test for Act gels

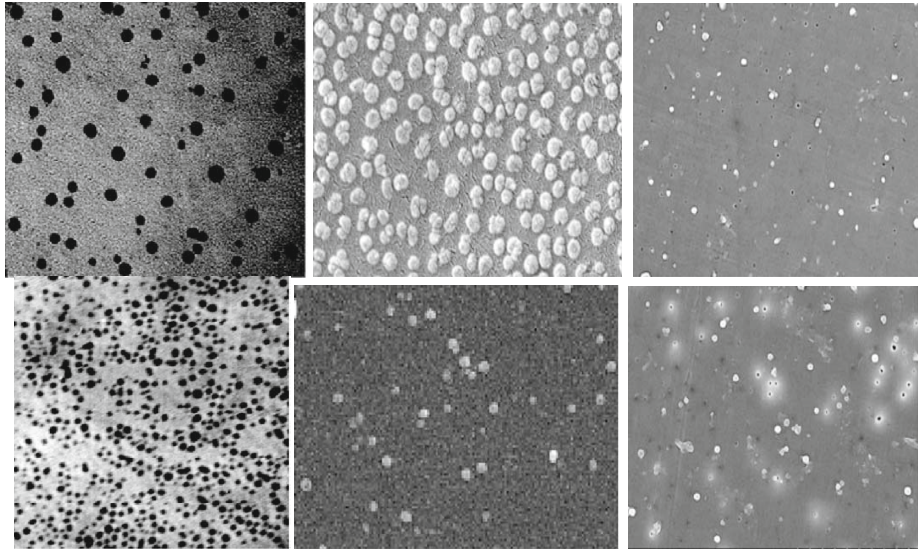


Figure: 4 SEM images of the Act gels (Act-1 to Act-6) nanoparticles

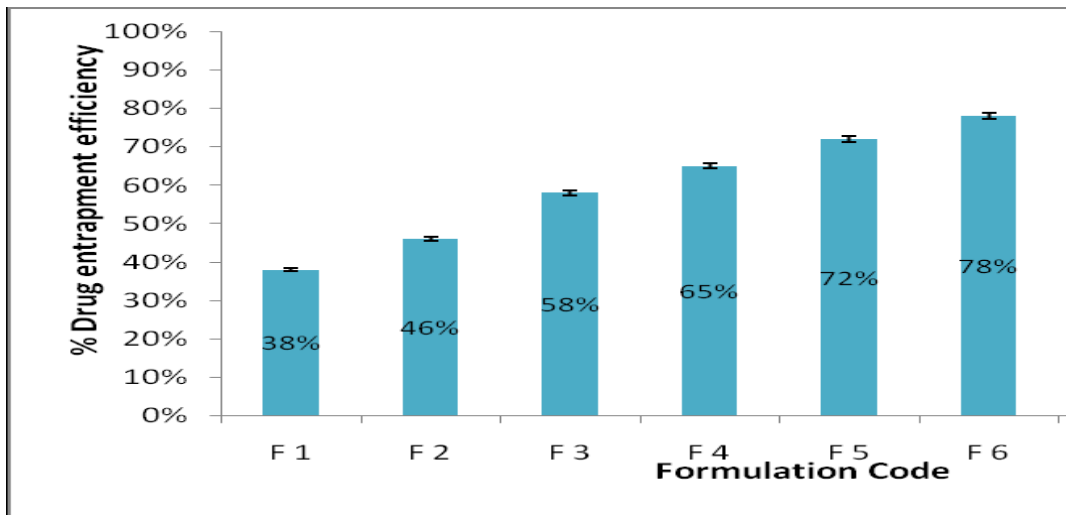


Figure 5: Drug entrapment efficiency of Act Gels (Act-1 to Act-6)

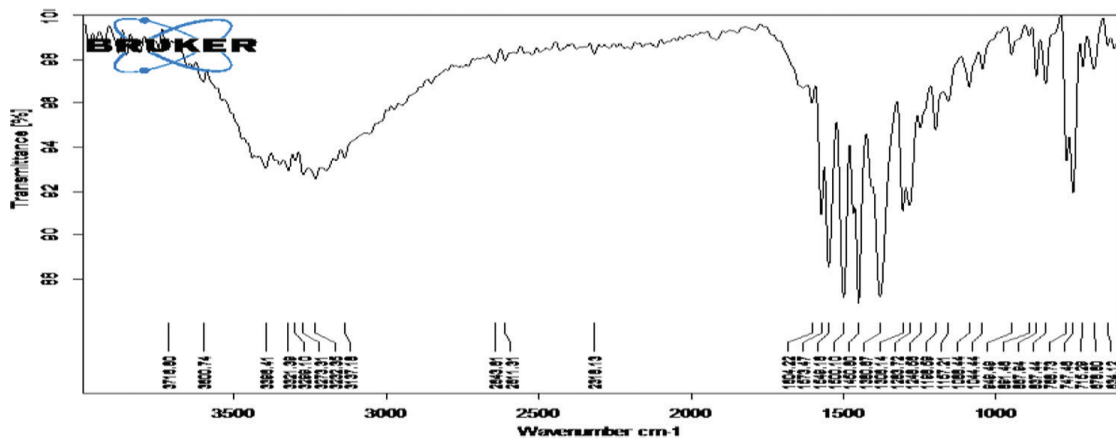


Figure 6: Infrared spectrum of pure Acitretin drug

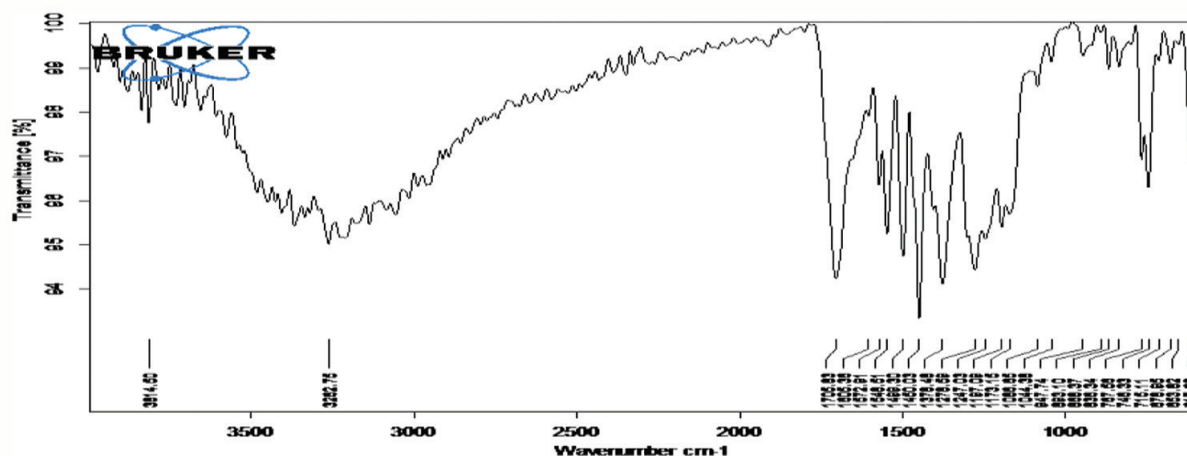


Figure 7: Infrared spectra of Acitretin and carbopol 934 physical mixture

Table 4: Act gel % drug release

Time (hr)	Act Gel-1 (% CR)	Act Gel-2 (% CR)	Act Gel-3 (% CR)	Act Gel-4 (% CR)	Act Gel-5 (% CR)	Act Gel-6 (% CR)
0.5	0.97±0.01	0.94±0.0	1±0.05	0.92±0.05	0.96±0.05	0.94±0.05
1	3.57±0.27	1.99±0.2	2.22±0.50	6.33±0.18	1.92±0.20	2.99±0.20
2	7.71±0.55	4.81±0.0	5.20±0.80	14.95±0.85	4.31±0.03	6.81±0.03
3	13.36±0.24	8.91±0.4	9.43±0.85	27.77±0.50	11.91±0.4	10.91±0.4
4	20.40±1.29	14.56±0.7	15.33±0.7	37.37±1.60	13.16±0.7	12.56±0.7
5	28.90±1.05	21.80±0.3	22.65±0.4	48.81±0.35	24.80±0.3	22.80±0.3
6	38.80±2.02	31.86±0.9	31.25±0.2	55.65±0.30	30.86±0.9	29.86±0.9
7	49.10±2.20	43.20±0.1	41.30±0.9	69.50±0.15	41.20±0.1	40.20±0.1
8	61.11±0.28	55.60±0.2	52.45±0.0	81.95±0.10	56.60±0.2	51.60±0.2

Table 5: Drug release kinetics of all the formulations (AG-1 – AG-6)

Formulation code	Zero order	First order	Korsmeyer-Pappas		Higuchi
R ²	R ²	R ²	N	R ²	R ²
AG-1	0.989	0.899	0.996	0.783	0.955
AG-2	0.990	0.871	0.997	0.780	0.955
AG-3	0.989	0.870	0.990	0.7765	0.951
AG-4	0.990	0.932	0.997	0.784	0.953
AG-5	0.990	0.922	0.993	0.788	0.951
AG-6	0.990	0.908	0.995	0.789	0.952

Table 6: Stability studies of formulation AG-4

Formulation	Days	Temperature And Relative Humidity	Appearance	PH	Drug Content	In-Vitro Drug Release
AG-4	0	25°±2°C/60±5% RH	Clear	6.27	101.3	81.95
AG-4	15	25°±2°C/60±5% RH	Clear	6.25	101.1	81.78
AG-4	30	25°±2°C/60±5% RH	Clear	6.20	99.8	81.64
AG-4	60	40°±2°C/75±5% RH	Clear	6.18	99.5	81.55
AG-4	90	40°±2°C/75±5% RH	Clear	6.15	99.2	81.50

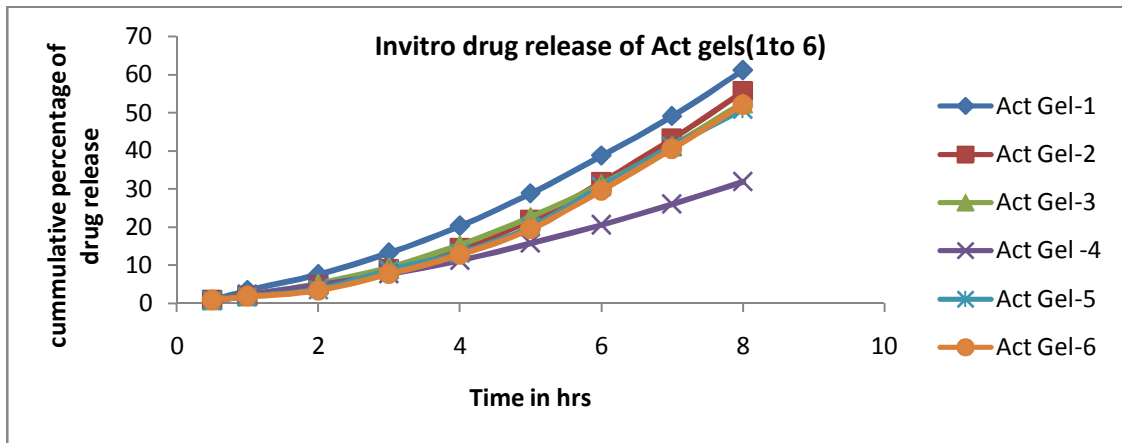


Figure 7: Drug release profile of Act gels (Act-1 to Act-6)

In vitro skin irritation

From the HET-CAM test, it was observed that chick embryo treated with 1% SDS caused Lysis of blood vessels and hemorrhage with mean irritation score of 16.21 indicating severe irritation whereas there were no signs of irritation found with 0.9% NaCl and the optimized formulation, Act-4 showed mean irritation score of 0.04 with no signs of blood vessels Lysis, hemorrhage, and coagulation after a time period of 5 min in HET-CAM test when compared with positive control and negative control, confirming that the optimized emulgel was nonirritant and nontoxic in nature [Table . The images of the HET-CAM test are depicted in Figure 8.

Table 7: Scoring Scheme for Irritation Testing with the HET-CAM Test Method

Effect	Score		
	0.5 min	2 min	5 min
Lysis	5	3	1
Hemorrhage	7	5	3
Coagulation	9	7	5

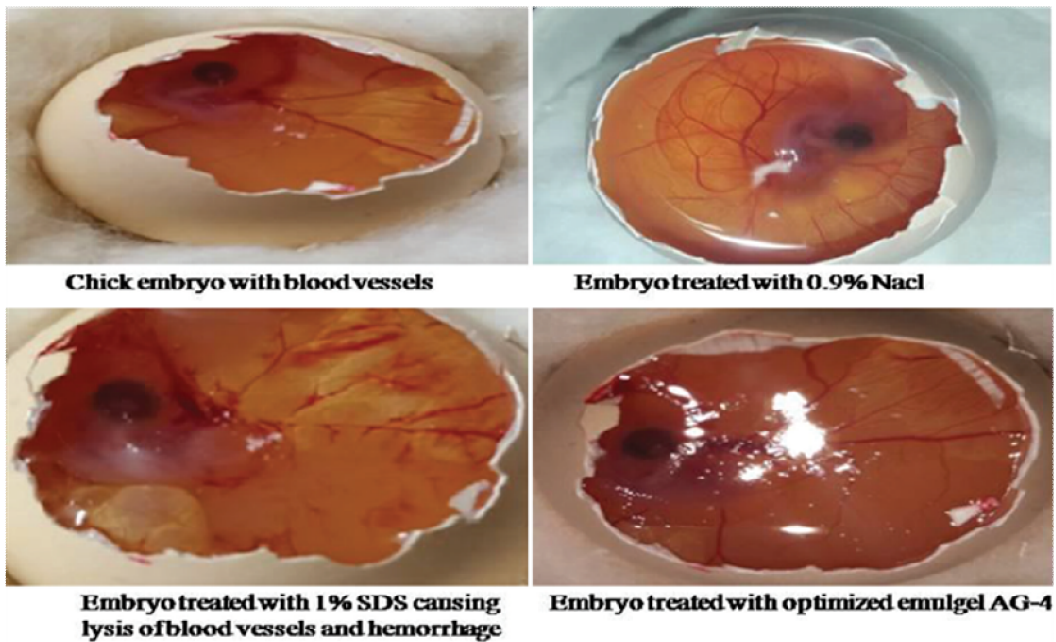


Figure 8: HET-CAM test for *in vitro* skin irritation study.

Release kinetics

Data obtained from *in vitro* release studies of the Acitratin from various gel formulations were fitted to various kinetic equations such as Zero order, First order, Higuchi model and Korsmeyer-Peppas model

and the results are presented in Figure 5. The release of Acitratin from the gel was First order diffusion as indicated by higher R2 values in First order kinetics and Higuchi model. (Fig 9-12)

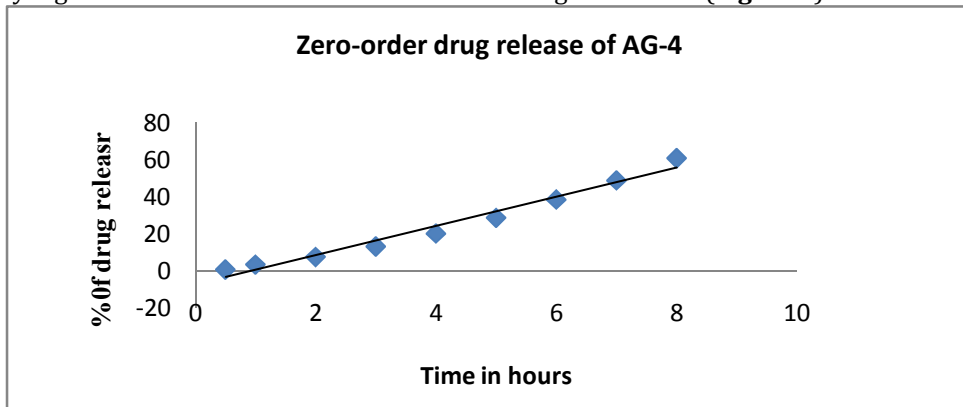


Fig 9: Zero-order drug release of AG-4

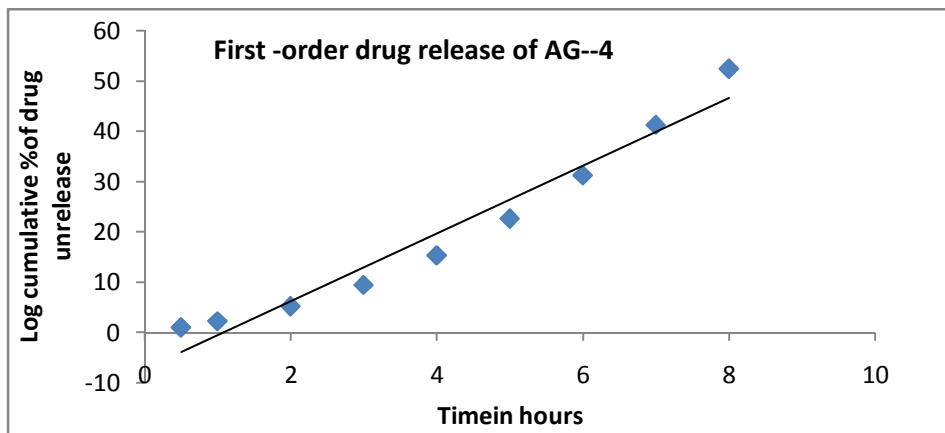


Fig 10: First-order drug release of AG-4

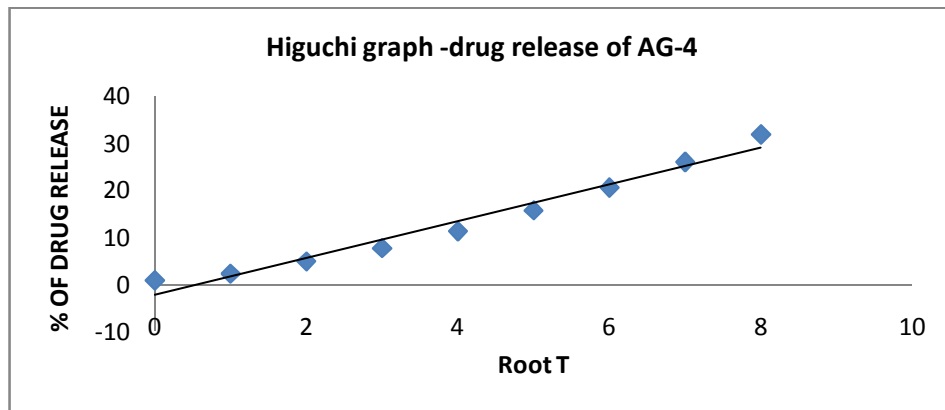


Fig11: Higuchi graph of drug release of AG-4

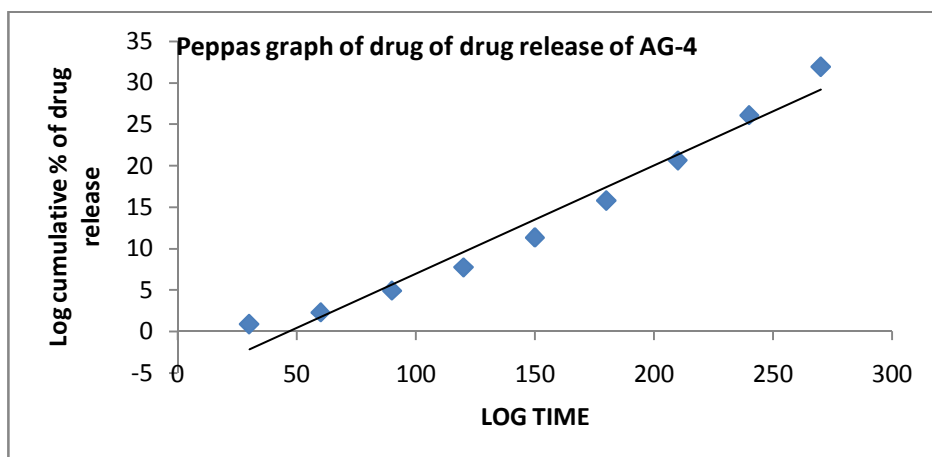


Fig 12: Peppas graph of drug release of AG-4

CONCLUSION

Act gels were prepared using carbopol 934 as gelling agent with the aid of liquid paraffin as oily phase and span 20 and tween 20 as emulsifying agents. The prepared Act gels were evaluated for formulation parameters, and from the drug release study, the formulation Act-4 was optimized as best with higher drug release, and the formulation showed acceptable mean particle size with no signs of skin irritation that was confirmed by the HET-CAM test. Based on the results obtained with the current research, it can be concluded that Act gels will be better promising drug delivery approach for Acitretin to enhance and achieve controlled drug release in comparison to its oral dosage forms and conventional gels.

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

REFERENCES

1. Yadav SK, Mishra MK, Tiwari A, Shukla A.(2017). A new approach for enhanced topical drug delivery. *Int J Curr Pharm Res*; 9:15-9.
2. Joshi B, Gurpreet S, Rana AC, Seema S.(2021). Development and characterization of clarithromycin emulgel for topical delivery. *Int J Drug Dev Res*; 4:310-23.
3. L. Flatz, C. Conrad, (2013). Role of T-cell-mediated inflammation in psoriasis: pathogenesis and targeted therapy, *psoriasis, Targets Ther.* 3, 1-10.
4. M. Pradhan, D. Singh, and M.R. Singh, (2013)., Novel colloidal carriers for psoriasis: current issues, mechanistic insight and novel delivery approaches, *J. Control. Release* 3, 380-395.
5. D.S. Dubois, R. Pouliot, (2013). Promising new treatments for psoriasis, *Sci. World J.*980419.
6. P. Gisondi, G. Malara, M. Ardigo, (2011). The psoriatic patient profile for infliximab, *Eur. Rev. Med. Pharmacol. Sci.* 15; 1445-1451.
7. R.K.H. Mak, C.Hundhausen, F.O.Nestle, (2009). Immune pathogenesis of psoriasis, *Acta dermo-sifiliograficas* 100, 2-13.
8. P.B. Liao, R. Robinson, R. Howard, G. Sanchez, I.J. Frieden, Annular pustular psoriasis-most common form of pustular psoriasis in children: report of three cases and review of the literature, *Pediatr. Dermatol.* 19 (2002) 19-25.
9. E.A. Brezinski, A.W. Armstrong, (2014). Strategies to maximize treatment success immoderate to severe psoriasis: establishing treatment goals and tailoring of biologic therapies, *Semen. Cut an. Med. Surg.* 33 ; 91-97.
10. K.M. Varman, N. Namias, C.I. Schulman, L.R. Pisano, (2014). Acute generalized pustularpsoriasis, von Zumbusch type, treated in the burn unit. A review of clinicalfeatures and new therapeutics, *Burns* 40, 35-39.

11. C. Surber, K.P. Wilhelm, D. Bermann, H.I. Maibach, (1993). In vivo skin penetration of acitretin in volunteers using three sampling techniques, *Pharm. Res.* 10,1291–1294.
12. K. Lauren Dunn, R. Laini Gaar, A. Brad Yentzer, L. Jenna, R.F. Steven, (2011). Acitretin in dermatology: a review, *J. Drugs Dermatol.* 10, 772–782.
13. P. Marta, K. Andrzej, (2011). Acitretin, a systemic retinoid for the treatment of psoriasis-current state of knowledge, *Postep. Dermatol. Alergol.* 4, 285–292.
14. Hsiu-Mei Chiang, Lin Ya-Tze, H. Pei-Ling, S. Yu-Han, T. Hui-Ting, W. Kuo-Ching, (2012). Determination of Marked Components-aloin and aloe-emodin in Aloe vera before and after hydrolysis, *J. Food Drug Anal.* 20, 646–654.
15. X.Y. Dai, W. Nie, Y.C. Wang, Y. Shen, Y. Li, S.J. Gan, (2012). Electro spun emodin poly(vinyl pyrrolidone) blended nanofibrous membrane: a novel medicated biomaterial for drug delivery and accelerated wound healing, *J. Mater. Sci. -Mater. Med.* 23, 2709–2716.
16. T.A. Syed, S.A. Ahmad, A.H. Holt, S.A. Ahmad, S.H. Ahmad, M. Afzal, (1996). Management of psoriasis with Aloe vera extract in a hydrophilic cream: a placebo-controlled, double-blind study, *Trop. Med. Int. Health* 1, 505–509.
17. J. Gibson, (2011). An evaluation of the effects on ProZ92 on proliferation of skin cells in culture, <www.proz92.com/psoriasis/research> (Cited 16.01.14).
18. A. Yogeeta, C.P. Kailash, K.S. Krutika, (2010). Development, evaluation and clinical studies of Acitretin loaded nanostructured lipid carriers for topical treatment of psoriasis, *Int. J. Pharm.* 401, 93–102.
19. A. Sharma, T. Garg, A. Aman, K. Paschal, R. Sharma, S. Kumar, T. Markandeywar, (2016). Nanogel—an advanced drug delivery tool: current and future, *Artif. Cells Nanomed. Biotech Mol.* 44, 165–177.
20. R. Jayakumar, N. Amrita, R.N. Sanoj, S. Maya, S.V. Nair, (2012). Doxorubicin-loaded pH responsive chitin nanogels for drug delivery to cancer cells, *Carbohydr. Polym.* 87, 2352–2356.
21. R.N. Sanoj, R. Ranjusha, B. Avinash, M. Nishil, R. Jayakumar, (2014). Gold–chitin–manganese dioxide ternary composite nanogels for radio frequency assisted cancer therapy, *RSC Adv.* 4, 5819–5825.
22. H. Tamura, H. Nagahama, S. Tokura, (2006). Preparation of hydrogels under mild conditions, *Cellulose* 13, 357–364.
23. M. Sabetha, R.N. Sanoj, N. Amrita, L. Vinothkumar, S.V. Nair, R. Jayakumar, (2012). Curcumin loaded chitin nanogels for skin cancer treatment via the transdermal route, *Nanoscale* 4, 239–250.
24. R.N. Sanoj, N. Amrita, M. Sabetha, K.P. Chennazhi, H. Tamura, S.V. Nair, R. Jayakumar, (2012). Synthesis, characterization and in vitro cytocompatibility studies of chitin nanogels for biomedical applications, *Carbohydr. Polym.* 93, 936–942.
25. K.T. Smitha, A. Anitha, T. Furuike, H. Tamura, S.V. Nair, R. Ajaikumar, (2013). In vitro evaluation of paclitaxel loaded amorphous chitin nanoparticles for colon cancer drug delivery, *Colloids. Surf. B. Biointerfaces* 104, 245–253.
26. R.K. Farag, R.R. Mohamed, (2012). Synthesis and characterization of carboxymethyl chitosan nanogels for swelling studies and antimicrobial activity, *Molecules* 18, 190–203.
27. P.P. Shah, P.R. Desai, A.R. Patel, M.S. Singh, (2012). Skin permeating nanogel for the cutaneous co-delivery of two anti-inflammatory drugs, *Biomaterials* 33 ;1607–1617.
28. B.P. Ravi, S. Luis, W. Hanping, K. Tianyi, A.E. Agata, (2010). Effect of injection site on in situ implant formation and drug release in vivo, *J. Control. Release* 147; 350–358.
29. D. Ashish, C.M. Jithin, V. Sreeja, H. Tamura, S.V. Nair, R. Jayakumar, (2010). Novel carboxymethyl chitin nanoparticles for cancer drug delivery applications, *Carbohydr. Polym.* 79; 1073–1079.
30. J. Smith, E. Wood, M. Dornish, Effect of chitosan on epithelial cell tight junctions, *Pharm. Res.* 21 (2004) 43–49.
31. L. Hao, J. Chan, T. Ying, W. Fude, M.Y.Y. Chunwang, (2014). Cytotoxicity of silicic acid nanoparticles on HaCaT cells, *J. Appl. Toxicol.* 34 ; 367–372.
32. S. Daoud-Mahammed, P. Couvreur, R. Gref, (2007). Novel self-assembling nanogels: stability and lyophilisation studies, *Int. J. Pharm.* 332, 185–191.
33. J.R. Laxmi, R. Karthikeyan, B.P. Srinivasa, R.V.V. Narendra Babu, (2013). Formulation and evaluation of antipsoriatic gel using natural excipients, *J. Acute Dis.* 2; 115–121.
34. A. Vijayalakshmi, V. Ravichandiran, V. Malarkodi, S. Nirmala, M. Anusha, S. Jayakumari, K. Masilamani, (2013). Anti-psoriatic activity of Smilax China Linn. Rhizome, *Ind. J. Pharm. Edu. Res.* 47; 82–89.
35. S. Manmohan, K. Niraj, L. Cassia tora L. (2012). Creams inhibit psoriasis in mouse tail model, *Pharm. Crop* 3, 1–6.
36. A. Nagle, A.K. Goyal, R. Kesarla, R.S.R. Murthy, (2011). Efficacy study of vesicular gel containing methotrexate and menthol combination on parakeratotic rat skin Model, *J. Liposome Res.* 21; 134–140.
37. S. Khurana, N.K. Jain, P.M. Bedi, (2015). Nanostructured lipid carriers based nanogel for meloxicam delivery: mechanistic, in-vivo and stability evaluation, *Drug Dev. Ind. Pharm.* 8, 1368–1375.
38. A. Inmaculada, M. Marian, H. Ruth, P. Ines, M. Beatriz, A. Niuris, G. Gemma, H. Angeles, (2009). Functional characterization of chitin and chitosan, *Curr. Chem. Biol.* 3; 203–230.
39. A.E. Stuck, C.J. Brindley, A. Busslinger, F.J. Frey, (1989). Pharmacokinetics of acitretin and its 13-cis metabolite in patients on haemodialysis, *Br. J. Clin. Pharmacol.* 27; 301–304.
40. M. Chaitanya, B. Babajan, M. Naveen, P. Madhusudana, C.M. Anuradha, K.C. Suresh, (2013). Design and evaluation of new chemotherapeutics of aloe-emodin (AE) against the deadly cancer disease: an in silico study, *J. Chem. Biol.* 6, 140–153.
41. T.R. Arunraj, R.N. Sanoj, N. Ashwin Kumar, R. Jayakumar, (2014). Bio-responsive chitin poly(L-lactic acid) composite nanogels for liver cancer, *Colloids Surf. B. Biointerfaces* 113, 394–402.

42. P. Desai, R.R. Patlolla, M. Singh, (2010). Interaction of nanoparticles and cellpenetratingpeptides with skin for transdermal drug delivery, *Mol. Membr.Biol.* 27, 247-259.
43. L. Malpezzi, G.A. Magnone, N. Masciocchi, A. Sironi, (2005). Single crystal and powder diffraction characterization of three polymorphic forms of Acitretin, *J. Pharm.Sci.* 94, 1067-1078.
44. S. Wang, T. Chen, R. Chen, Y. Hu, M. Chen, Y. (2012). Wang, Emodin loaded solid lipidnanoparticles: preparation, characterization and antitumor activity studies,*Int. J. Pharm.* 430, 238-246.
45. N. Sanoj Rejinold, M. Muthunarayanan, V.V. Divyarani, P.R. Sreerekha, K.P.Chennazhi, S.V. Nair, H. Tamura, R. Jayakumar, (2011). Curcumin-loaded biocompatible thermo responsive polymeric nanoparticles for cancer drugdelivery,*J. Colloid Interface Sci.* 360, 39-51.
46. S.B. Murray, A.C. Neville, (1998). The role of pH, temperature and nucleation in the formation of cholesterol liquid crystal spherulites from chitin and chitosan, *Int.J. Biol. Macrogol.* 22,137-144.
47. W. Xu, P.A. Ledin, F.A. Plamper, C.V. Synatschke, A.H.E. Müller, V.V. Tsukruk, (2014). Multi responsive microcapsules based on multilayer assembly of starpolyelectrolytes, *Macromolecules.* 7858-7868.
48. G.M.G. Chiara, F. Silvia, P. Sara, C. Emanuela, V. Giulio, M. Luisa, M. Paola, C.Francesco, (2016). Skin penetrating peptide as a tool to enhance the permeation of heparin through human epidermis, *Biol. Macromol.* 17, 46-55.

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