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RESEARCH ARTICLE

FORMULATION AND EVALUATION OF TOPICAL SPRAY OF ANTIACNE AGENT

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Abstract: The aim of present work was to develop a topical spray formulation of anti acne agent, which would form a clear transparent thin film at the site of application, effectively delivering the drug without pain or irritation. Aerosol solution contained Eudragit E100 as a polymer, propylene glycol (PG) as a plasticizer, ethanol as a solvent and Isopropyl alcohol (IPA) as a co-solvent. Solutions for topical sprays were filled in aluminium containers fitted with continuous spray valve. Evaluations for the Adapalene topical spray included determination of delivery rate, delivery amount, pressure test, drug content, minimum fill, leakage test, flammability, spray patterns, particles size, thickness of formed film and diffusion release profile etc. Glass containers were used to study physical incompatibility between the aerosol concentrate and propellant (LPG) due to the ease of visible inspection. Formulation with eudragit E100 (0.5%), IPA (1.5%) and PG (2.0%) concentration was found to give higher release profile. Accelerated stability studies were conducted as per ICH guidelines at 40°C ± 2°C temperature and 75% ± 5% RH for 20 days and indicated that optimized formulations were stable. Skin irritation studies of optimized formulations were performed using rabbit as an animal model for 24 h and no erythema and edema were recorded after 24 h. The result obtained shows that topical spray of Adapalene can be used as effective topical system for treatment of Acne.

KeyWords: Topical spray, Adapalene, Aerosol, Eudragit E100.

Introduction:

Acne affects mostly skin with the densest population of sebaceous follicles; these areas include the face, the upper part of the chest, and the back. Severe acne is inflammatory, but acne can also manifest in noninflammatory forms. To overcome the limitations of the conventional dosage forms, spray or aerosol dosage form for topical application have been formulated. Topical aerosols are products that are packaged under pressure. The active ingredients are released in the form of fine liquid droplets upon activation of an appropriate valve system. The aim of this investigation was to prepare and evaluate topical spray of adapalene. The topical spray dosage form would deliver the bioactive compound directly to the infected area and produced a thin film that would cover the infection and act as reservoir for the bioactive drug. This would also minimize the pain irritation during application. The objective of the research work to development and characterization of topical spray solution for characteristics like pH, viscosity, drug content, film formation, spray pattern, particle size distribution etc, optimization of aerosol package and evaluation of topical spray system.

MATERIALS AND METHODS

Materials:

Adapalene was obtained as a gift sample from Videv intermediates, Surat. Ethanol, Acetone was purchased from Chemdyes corporation, Ahmedbad, India. Eudragit E100, PVP K30, HPMC 100 LV, carbopol 934, Isopropyl myristate, Propylene glycol, glycerin was procured from Chemdyes corporation, Ahmedbad, India. Butane gas (LPG) was purchased from Vimsons aerosol. Isopropyl alcohol was procured from Sisco research lab., Mumbai.

METHODS

Calibration curve of Adapalene in isopropyl alcohol (IPA)

The stock solution was prepared by accurately weighing 5 mg of Adapalene, which was transferred to a 50 ml volumetric flask, and then it was dissolved in 50 ml of the isopropyl alcohol to obtain the working standard of 100µg/ml (stock solution).

Calibration curve of Adapalene in isopropyl alcohol (IPA) & phosphate buffer pH 6.8 (80:20)

The stock solution was prepared by accurately weighing 5 mg of Adapalene, which was transferred to a 50 ml volumetric flask, and then it was dissolved in 50 ml of the IPA and phosphate buffer pH 6.8 (80:20) to obtain the working standard of 100µg/ml (stock solution).

Calculation of dose of Adapalene

Conventionally, Adapalene is available as cream and gel in concentration of 0.1% w/w, which is prescribed once a day.

1 gm cream/gel = 1 mg Adapalene

and 1 mg of Adapalene is required for 24 h.

So, 20 mg of Adapalene required for preparation of 20 ml solution for spray

METHOD OF PREPARATION OF TOPICAL SPRAY OF ADAPALENE

Selection of solvent and plasticizer.

50mg of Adapalene was taken in capped vial containing 2 ml of each of the screened vehicle. After sealing, the mixture was heated in water bath at 40°C for 30 minutes to facilitate solubilisation of drug. Then mixing of systems was performed using magnetic stirrer at room temperature for 48 h. Mixing of the systems was performed at 50 RPM. These systems were centrifuged at 5000 rpm for 15 min and then analysed for Adapalene by UV-VIS spectroscopy[26, 27].

Selection of polymer.

100mg of Polymer was taken in capped vial containing 2 ml of each of the screened vehicle. After sealing, the mixture was heated in water bath at 40°C for 30 minutes to facilitate solubilisation of polymer. Then mixing of systems was performed using magnetic stirrer at room temperature for 48 h. Mixing of the systems was performed at 50 RPM by using magnetic stirrer. These systems were centrifuged at 5000 rpm for 15 min and then analysed for Adapalene by UV-VIS spectroscopy[26, 27].

Physical compatibility of polymers with solvents: - Solutions were kept at laboratory bench for a week and observed for precipitation and crystal growth to check the compatibility.

FORMULATION OF TOPICAL SPRAY PREPARATION

Preparation of solution for topical spray

Film forming agent or polymer (Eudragit E 100 and PVP K30) was added in ethanol. IPA was added into above preparation. Finally drug and IPM were mixed with solution containing solvent & co-solvent to obtain 0.1% w/v concentration. Solution is stirred with the help of magnetic stirrer at 45°C to ensure uniform mixing. The weights of the solutions were made up to 100 % with the addition of ethanol.

Preparation of preliminary batches: Batches were prepared by using polymers like Eudragit E100, PVP K30 and combination of Eudragit E100 and PVP K30 (1:1). All the variables like concentration of drug, PG, IPA and IPM were kept constant. These batches were studied for drug release.

Optimization of batches based on preliminary study: From the preliminary study eudragit E100 was selected as the polymer due to maximum release of drug from diffusion study. Further batch optimization was done according to table 2. These batches were studied for drug release. Selections of batches for evaluation were depending on greater release profile.

Table 2: - Concentration of Adapalene & Excipients.

Drug	Polymer	IPA	PG	IPM	Ethanol
20mg	100-200mg	0.5-1.5 ml	1-2 ml	0.5 ml	Upto 20 ml

Table 3: - Optimization of batches based on preliminary study.

Batch code	Drug (mg)	Eudragit E100 (mg)	IPA (ml)	PG (ml)	IPM (ml)
F1	20	100	0.5	1.0	0.5
F2	20	100	0.5	1.5	0.5
F3	20	100	1.0	2.0	0.5
F4	20	100	1.0	1.0	0.5
F5	20	100	1.5	1.5	0.5
F6	20	100	1.5	2.0	0.5
F7	20	150	0.5	1.0	0.5
F8	20	150	0.5	1.5	0.5
F9	20	150	1.0	2.0	0.5
F10	20	150	1.0	1.0	0.5
F11	20	150	1.5	1.5	0.5
F12	20	150	1.5	2.0	0.5
F13	20	200	0.5	1.0	0.5
F14	20	200	0.5	1.5	0.5
F15	20	200	1.0	2.0	0.5
F16	20	200	1.0	1.0	0.5
F17	20	200	1.5	1.5	0.5
F18	20	200	1.5	2.0	0.5

Filling of aerosol solution

Batches were selected based on showing good results mainly release of drug from the spray solution. These batches were filled in aluminium canisters by pressure filling method. Pressure filling method was used to fill LPG (butane) propellant (50%-60%) in aluminium canisters containing aerosol concentrate. Weight of LPG was 50-60% to the weight of net content.

Figure 1: - Aerosol crimping and filling machine.

Approximately 20ml of the solution was filled into each of 50 ml aluminium canister (container).

Evaluation of Topical Spray

***In vitro* drug release studies**

The *in vitro* drug release studies were performed by using Franz diffusion cell with cellophane membrane. The water jacketed recipient compartment had total capacity of 25 ml and it had 2 arms, one for sampling and another for thermometer. The donor compartment had internal diameter of 2 cm. The donor compartment was placed in such a way that it just touches the diffusion medium in receptor compartment. The receptor compartment contained IPA and phosphate buffer pH 6.8 (80:20) that was maintained at 32^0C ± 1^0C. The membrane was equilibrated before application of the equivalent to 1 mg of drug onto the donor side. Samples were periodically withdrawn from the receptor compartment, replacing with the same amount of IPA and phosphate buffer 6.8 (80:20), and assayed by UV spectrophotometer at 268 nm.

Physical compatibility of aerosol concentrates with propellants.

Glass containers containing concentrate of aerosol and specified propellant of optimized batches were kept at room temperature on the laboratory benches for 15 days but not protected from light. The contents were examined visually from time to time to detect any physical change like precipitation and crystal growth indicating incompatibility[12].

Delivery rate

The delivery rate of Topical spray was evaluated according to procedure stated in USP. Not fewer than four aerosol containers were selected. Each valve was actuated for 5 seconds at a temperature of 25^0C (accurately timed by use of a stopwatch). Each container was weighted accurately and immersed in a constant-temperature bath until the internal pressure was equilibrated at a temperature of 25°C. The containers from the bath were removed. Excess of moisture was removed by blotting with a paper towel. Again each valve was actuated for 5 seconds at a temperature of 25^0C and weighted accurately. Process was repeated for three times[31].

Delivery amount

The delivery amount of Adapalene Topical spray was determined by using not fewer than four containers of aerosol according to procedure stated in USP. The valves were pressed continuously for 5 seconds each time until no more spray emerged. Sufficient time was allowed between each actuation to avoid significant canister cooling. The total weight loss was calculated from each container as the deliverable amount[31].

Minimum fill

Ten filled containers were selected and weighed individually. The contents were removed from each container. The packages were opened or dismantled and any residue was removed by washing with suitable solvents and rinsed with a few portions of methanol. The container, the valve, and all associated parts were collected and heated to dryness at 100°C for 5minutes and

cooled. The weight of each container together with their corresponding parts was determined. The difference between the weight of the filled container and the weight of the corresponding empty container was the net weight of the content. The requirements are met if net weight of contents of each of the ten containers is not less than the labeled amount[31].

Pressure test

The pressure of Adapalene Topical spray containers was determined according to the method in USP, at a temperature of 25°C. Not fewer than four containers of aerosol were selected. Caps and covers of containers were removed and immersed in until internal pressure is constant at a temperature 25°C. Containers from bath were removed and shaked. Water from containers was removed. Actuators were removed from valve stem. Each container was placed in an upright position to the pressure gauge. The gauge was pressed to actuate the valve and the pressure exerted by the propellant was noted for each aerosol container. Reading from pressure gauge was measured[31].

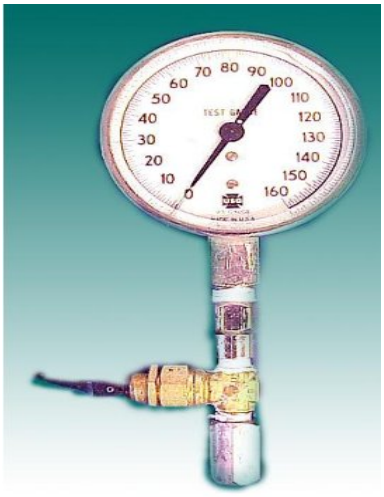


Figure 4.2:- Figure of pressure gauge.

Flammability[3]

The flammability of Adapalene Topical spray was determined to check the flame extension. The formulation was sprayed for 4 second into a flame. Depending on the nature of the formulation flame was extended and the exact length was measured with the help of a ruler. The sample is classified as flammable if ignition occurs at a distance equal or greater than 15 cm but less than 75 cm^3.

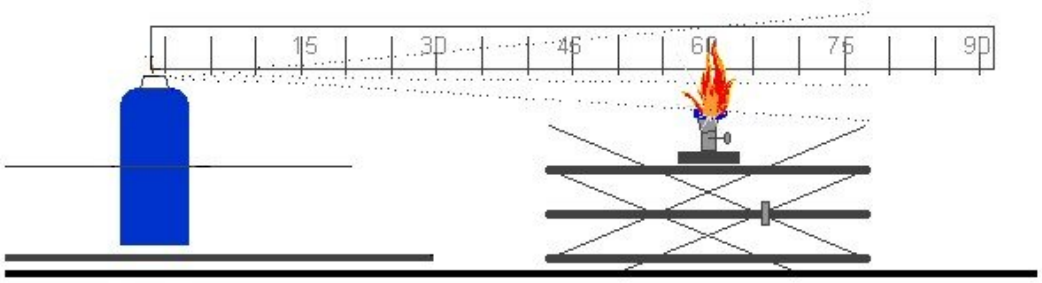


Figure 4.3: - Flammability testing of Aerosol.

Spray patterns

The spray pattern was assessed by delivering the spray through the topical spray of Adapalene onto a glass plate containing activated silica gel. The formulation was held at a distance of 4.5-5.0 cm from glass plate containing activated silica gel. The spots formed as a result of spray testing were observed under UV light and their diameters were measured[3, 10].

Leakage test

Four batches were selected to check crimping dimension and effective sealing. Containers were kept in 25°C before operation. Leakage test was carried out by passing the containers in water bath at 55°C. Defective crimping or leakages were found by this method[3].

Drug content studies

Remove all the content from container actuating the valve. 1 ml spray for solution was taken in 10 ml volumetric flask containing 5 ml IPA and diluted upto 5ml with the same solvent. From the above solution, 1 ml was further diluted with 10 ml IPA. The resultant solution was filtered through Whatman filter paper and absorbance of the solution was measured at 233 nm using UV spectrophotometer.

Particle size of Topical spray

The particle size solution for spray of Adapalene was determined by optical microscopy. Microscopic method is generally employed for measurement of particle size in range of 0.2 to 100. The particle sizes of optimized formulations were estimated. The formulation was sprayed on a clean glass slide and atleast sizes of 100 particles were measured under an optical microscope[32].

***Ex vivo* skin permeation study**[10, 41]

Ex vivo skin permeation study was performed by using Franz diffusion cells with an effective diffusion area of 2 cm^2. The excised skin samples (dorsal side) of Albino wistar rat (250gm-300gm) was clamped between the donor and the receptor compartment of Franz diffusion cells with the Stratum corneum facing the donor compartment. Then, 1 ml of Topical spray containing 1% (w/w) Adapalene was applied on the donor compartment. The receptor compartment was filled with IPA and phosphate buffer pH 6.8 and maintained at 37°C with stirring at 50 rpm. At predetermined time intervals, 1 ml receptor medium was withdrawn and the same volume of pure medium was immediately added into the receptor compartment. The procedure was repeated upto 24 h. All samples were filtered through Whatman filter paper and analyzed by UV spectrophotometer at 268 nm.

Skin irritation study [23]

As the formulation was intended for dermal application, skin irritancy should be tested. Skin irritation tests were conducted at Albino rabbits (New Zealand white variety) to determine to determine irritancy after single application of Topical spray. The room temperature was maintained at 22±3°C. Skin was prepared by removing hair of the rabbit (backside) twenty four

hour before the control and test (Topical spray) application. The optimized formulation was sprayed on the patch of preshaved skin ($3cm^2$) for 2 second and occluded with adhesive tapes and resulting reactions such as erythema and edema were scored after 24 h. The patch was removed after 24 h and treatment sites were cleaned with wet gauze to remove any residual test substance. Exposed skin was graded for formation of edema and erythema. Based on the scoring, the formulation was graded as 'non-irritant', 'irritant' and 'highly irritant'. The irritation scores of the test area were obtained by judging the extent of erythema and edema. Erythema and edema were graded as 0 for no visible reaction, 1 for just present reaction, 2 for slight reaction, 3 for moderate reaction and 4 for severe reaction. Eventually, the total scores for irritation test in each formulation were calculated using the following equation.

Table 4: - Number of animals.

Species	**Weight**	**Gender**	**Numbers to be used**
Albino Rabbit	1.5-2.5kg	Female	2

Table 5: - Groups of animals

Group no.	**Name of Group**	**Treatment**
1	Group -1: No application (control)	Vehicle for 24 hr
2	Group-2: Topical spray of Adapalene	For 24 hr

$$\text{Primary irritation index} = \frac{(\text{Erythema reaction scores} + \text{Edema reaction scores})}{\text{Time interval (h)}}$$

Table 6 : - Table indicating score of Erythema and Edema formation.

Erythema and Edema formation	Score
No	0
Very slight	1
Well defined	2
Moderate to severe	3
Severe	4

Table7: - Evaluation of primary irritation index.

Index	**Evaluation**
0.00	No irritation
0.04-0.99	Irritation barely percipitable
1.00-1.99	Slight irritant
2.00-2.99	Mild irritation
3.00-5.99	Moderate irritation
6.00-8.00	Severe irritation

Results & discussions

Solubility study for selection of solvent, plasticizer and polymer.

Selection of solvent and plasticizer

Table 7: - Solubility of adapalene in various solvents.

Sr No.	Solvent	Solubility (mg/ml)*
1	**Ethyl alcohol**	**6.7 ± 0.13**
2	Acetone	4.2 ±1.01
3	Ethyl alcohol + Acetone (2:1)	5.1 ±0.59

Table 8: - Solubility of Adapalene in various Plasticizers.

Sr No.	Plasticizer	Solubility (mg/ml) *
1	**Propylene Glycol**	**5.7±0.54**
2	Glycerin	3.2±1.21

*** Mean ± SD; n=3**

Selection of solvent and plasticizer were based on solubility of Adapalene in solvent and plasticizer. From the screened solvents and plasticizers ethanol as a solvent and propylene glycol as a plasticizer were selected due to greater solubility of Adapalene in ethanol and PG.

Selection of Polymers

Table 9 : - Solubility of polymer in various solvents.

Sr No.	Polymers	Solvents	Solubility (mg/ml) *
1	Eudragit E100	Ethanol	45.32±0.16
		Acetone	23.23±2.43
		Ethanol: Acetone (2:1)	36.4±2.03
2	PVP K30	Ethanol	35.5±1.44
		Acetone	39.06±0.45
		Ethanol: Acetone (2:1)	37.45±0.59
3	Carbopol 943	Ethanol	10.43±1.85
		Acetone	14.66±3.56
		Ethanol: Acetone (2:1)	18.21±2.34
4	HPMC 100 LV	Ethanol	5.3±2.89
		Acetone	8.6±1.19
		Ethanol: Acetone (2:1)	7.3±2.67

*** Mean ± SD; n=3**

Eudragit E100 and PVP K30 were selected on the basis of greater solubility and compatibility in ethyl alcohol. Preparation containing carbopol 943 and HPMC 100LV were rejected due to particle size and crystal growth in ethyl alcohol, acetone and ethyl alcohol with acetone.

Calibration curve of Adapalene in isopropyl alcohol (IPA):

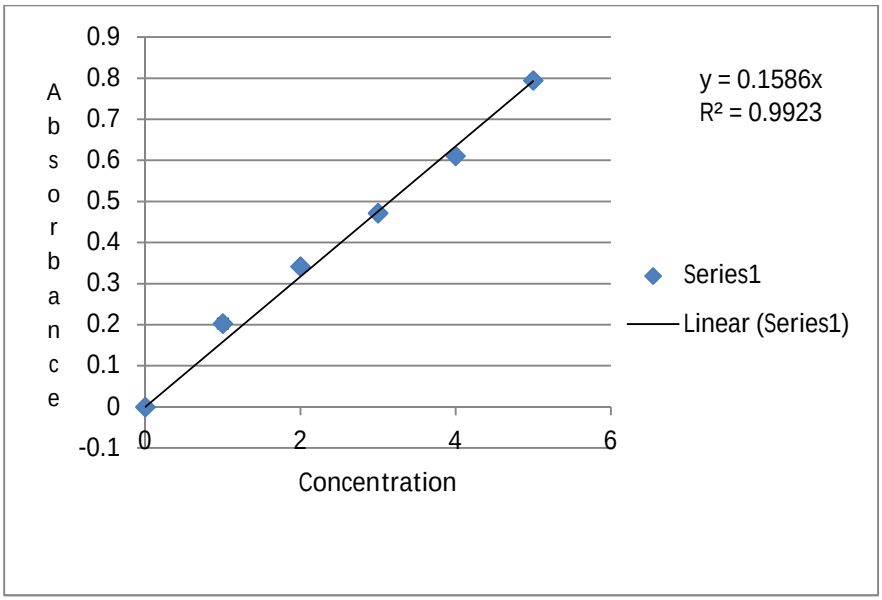


Figure 2: - Calibration curve of Adapalene in IPA.

Regression co-efficient was found to be 0.998 indicating linearity in the range of 1 to 5µg.

Calibration curve of adapalene in IPA and Phosphate buffer pH 6.8 (80:20)

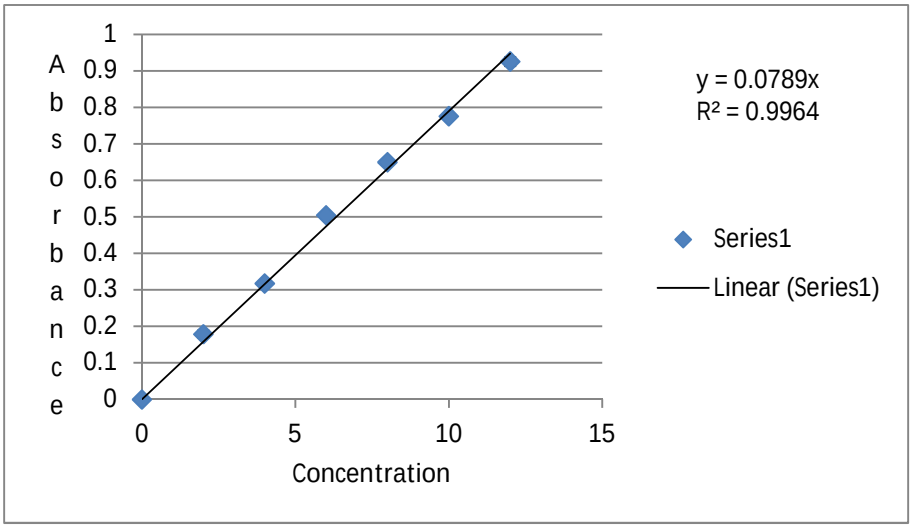


Figure 3: - Calibration curve of adapalene in IPA and Phosphate buffer 6.8 (80:20)

Regression co-efficient was found to be 0.997 indicating linearity in the range of 2 to 12 µg.

***In-Vitro* drug release of preliminary batches** Diffusion of preliminary batches of Adapalene topical sprays were performed by Franz diffusion cell method**.** Polymer eudragit E100 was selected due to greater release profile. Further optimization of batches was done by trial and error method as mentioned in table 2. In optimization process drug concentration was kept constant. Different concentration of ethanol, PG, IPA and eudragit E100 was selected for batch optimization by Trial and Error method and further evaluated for release profile.

Diffusion release Profiles of Optimize batches from F1-F18

Diffusion release of Optimize batches were performed by using Franz diffusion cell. The results are shown in table 10 & 11.

Table 10: - Diffusion release Profiles of Optimize batches from F1-F9.

Time (hr)	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	8.72	9.33	9.43	9.7	**10.34**	**11.12**	6.93	7.56	8.03
2	12.64	13.53	13.69	13.13	**15.23**	**16.66**	9.53	11.67	12.03
3	20.53	21.55	22.69	23.45	**25.01**	**26.67**	15.34	17.78	18.89
4	32.23	32.45	33.52	34.82	**36.67**	**38.56**	25.36	29.45	30.85
5	41.54	42.34	43.63	44.92	**46.26**	**48.13**	35.23	38.43	39.34
6	50.31	50.85	51.67	52.83	**54.75**	**56.81**	44.31	47.21	48.87
7	55.97	56.44	57.42	58.53	**60.23**	**62.74**	49.22	52.81	53.65
8	64.78	65.02	65.23	66.57	**68.12**	**69.57**	57.55	59.42	62.76
24	69.34	71.45	69.98	73.66	**74.55**	**75.12**	65.2	68.71	68.99

Table 11: - Diffusion release Profiles of Optimize batches from F10-F18.

Time (hr)	F10	F11	F12	F13	F14	F15	F16	F17	F18
1	8.23	**9.95**	**10.92**	2.5	2.76	3.02	3.56	4.78	5.85
2	12.43	**14.83**	**15.63**	4.23	4.78	5.67	6.67	7.97	8.67
3	19.73	**24.75**	**25.66**	9.34	9.57	10.34	12.89	13.77	14.43
4	31.22	**35.63**	**37.86**	18.44	19.67	20.78	22.67	23.88	24.56
5	40.43	**45.2**	**47.53**	28.55	29.34	30.67	32.56	33.65	34.76
6	49.28	**53.52**	**55.75**	36.44	37.32	38.54	40.89	42.66	43.76
7	54.91	**59.69**	**61.85**	41.76	43.32	44.56	46.78	47.34	48.77
8	63.77	**67.87**	**68.75**	49.42	50.22	52.21	54.77	55.78	56.43
24	69.23	**73.98**	**74.22**	57.27	59.28	60.02	62.67	63.89	64.98

Formulations F6, F5, F12 and F11 had greater release profile from F1 to F18 batches. When concentration of IPA and PG is increased then release of formulation was also increased. Formulations F6, F5, F12 and F11 had maximum concentration of PG and IPA. Higher concentration of eudragit E100 was responsible for less release of drug from the formulations.

Filling of aerosol container: - Selected batches F6, F5, F12 and F11 were filled in aluminium canister by pressure filling method at room temperature. Following evaluations were performed on selected batches.

Evaluation of Adapalene Topical Spray

Evaluation of batches was based on good results. Optimized batches were selected based on release profile. Further eavaluation was done in following manner.

Physical compatibility of aerosol concentrates with propellant.

Physical compatibility of aerosol concentrates with propellant (LPG) was performed for 20 days in glass container. Containers were checked for physical change.

No physical incompatibility was found after 20 day period so, it was confirmed that product concentrate is compatible with propellant (LPG).

Delivery rate

Four aerosol containers were selected. Each valve was actuated for 5 seconds at a temperature of 25^0C. Each container was weighted accurately. The results are shown in table 12.

Table 12: - Delivery rate data for F6, F5, F12 and F11 batches.

Batch code	Delivery Rate (g/sec) *
F6	1.15 ± 0.03
F5	1.17 ± 0.02
F12	1.14 ± 0.06
F1 1	1.19 ± 0.07

*** Mean ± SD; n=3**

Delivery rate was found similar for all the formulation. The delivery rate of Adapalene topical spray was affected by vapour pressure. At low vapor pressure delivery rate was decreased.

Delivery amount: Four aerosol containers were selected. The valves were pressed continuously for 5 seconds each time until no more spray emerged. Sufficient time was allowed between each actuation to avoid significant canister cooling. Results of delivery amount are shown in table 13.

Table 13: Delivery amount data for F6, F5, F12 and F11 batches.

Batch code	Delivery Amount (%)*
F6	94.47 ± 1.55
F5	94.29 ± 1.34
F12	94.57 ± 1.67
F11	94.04 ± 1.10

*** Mean ± SD; n=3**

Delivery amount was found similar for all the formulation. Delivery amount gives information about material to be dispensed from net content after complete actuation.

Minimum fill: Ten filled containers were selected and weighed individually. The contents were removed from each container. After removal of contents was dismantle the container and rinsed

with few portion of methanol. Parts of containers were dried at 100°C for 5 minutes and weighted to know net content. The results are shown in table 14.

Table 14: -Minimum fill data for F6, F5, F12 and F11 batches.

Batch code	Minimum Fill (%)*
F6	100.55 ± 0. 34
F5	100.16 ± 0.67
F12	100.72 ± 0.89
F11	100.33 ± 0.25

*** Mean ± SD; n=3**

The product passed the minimum fill test if the net weight of the contents is not less than the labeled amount. All formula had a minimum fill of more than 100 %, which mean that the net weight of the contents was not less than the labeled amount and met the requirement.

Pressure test: Pressure was measured by pressure gauge at room temperature**.** The results are shown in table 15. (1bar=14.51 psig)

Table 15: - Pressure test data for F6, F5, F12 and F11 batches.

Batch code	Vapor Pressure (Bar) *
F6	7.45 ± 0. 05
F5	4.33 ± 0.04
F12	5.77± 0.03
F11	6.55 ± 0.04

*** Mean ± SD; n=3**

The delivery rate of Adapalene topical spray was affected by vapour pressure. Propellants that produced high vapour pressure gave higher delivery rate.

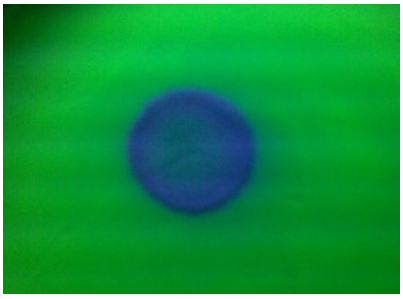
Flammability of the formulated Adapalene Topical spray: The formulation was sprayed for 4 second into a flame. Depending on the nature of the formulation flame was extended and the exact length was measured with the help of a ruler. The results of flammability test are shown in Table 16.

Table 16: - Flammability test results of selected batches.

Batches	Flame Extension (inch)
F6	22
F5	20
F12	24
F11	19

All Topical sprays formulated with different propellant were flammable due to the presence of ethanol used as solvent and LPG as a propellant in the formulation. Flammability test indicated the effect of an aerosol formulation on the extension of an open flame.

Spray pattern: Spray pattern was performed by using silica-gel glass plate method. Contents were sprayed on glass plate containing silica gel. The glass plates were analyzed under UV light to check spray pattern. The results of spray pattern of Adapalene Topical spray are shown in figure 4 and 5.



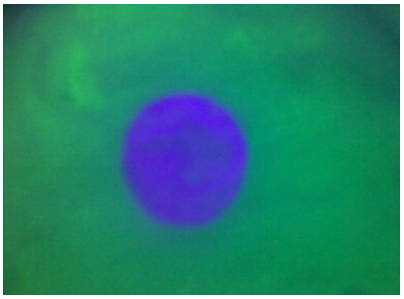


Figure 4:- Spray pattern of F6 and F5 batches.

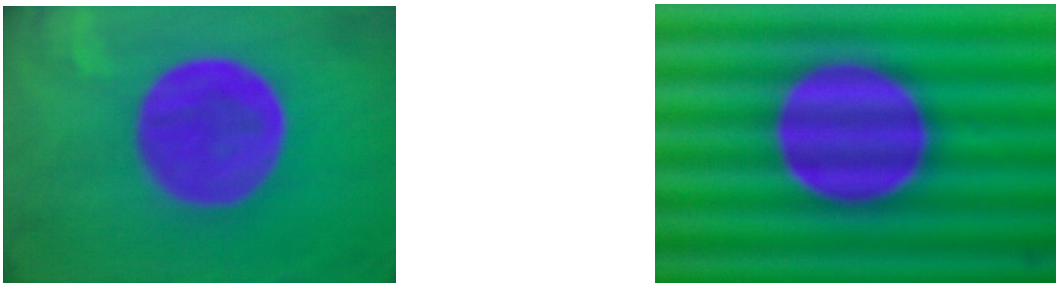


Figure 5:- Spray pattern of F12 and F11 batches.

Table 17 :- Diameter of batches F6, F5, F12 and F11 batches.

Batches	Diameter (mm) *
F6	20 ±2
F5	18 ±3
F12	19±2
F11	20±2

*** Mean ± SD; n=3**

The patterns and size were almost the same since all formula used the same type of valve and container. It means that spray patterns were not significantly affected by type of propellants and vapor pressure. The type of valve used might affect the spray pattern.

Leakage test: Leakage of canisters was confirmed by passing the canisters in water bath at 55°C temperature. Test was performed on selected batches. All batches were passed this test. No change in crimping dimension was found.

Table 18:- Leakage test results of selected batches.

Batches	Results
F6	No leakage
F5	No leakage
F12	No leakage
F11	No leakage

No leakages were found for canisters. All canisters were passed leakage test

Drug content: After removal of spray solution from canister 1ml of sample was analyzed for drug content by UV spectrophotometer. The results of drug content are shown in table 19.

Table 19: - Drug content data for F6, F5, F12 and F11 batches.

Batch code	Drug content * (%)
F6	99.63 ± 0. 05
F5	100.23 ± 0.04
F12	101.11 ± 0.03
F11	98.57 ± 0.04

*** Mean ± SD; n=3**

Drug content was found to be almost same for all formulation. Each ml of spray solution contains 1mg of Adapalene.

Particle size of Topical spray: Particle size was measured by optical microscopic method. The results of particle size of Adapalene Topical spray are shown in table 20.

Table 20: - Particle size data for F6, F5, F12 and F11 batches.

Batch No.	Particle size* (µm)
F6	8.1 ± 2.22
F5	8.4 ± 4.46
F12	11.28 ± 2.78
F11	11.69± 3.65

*** Mean ± SD; n=3**

***Ex vivo* skin permeation study**

Ex vivo skin permeation study was performed by using Franz diffusion cells with an effective diffusion area of 2 cm^2. The excised skin samples (dorsal side) of Albino wistar rat was used as diffusion surface ($2cm^2$). Samples were analyzed by UV spectrophotometer at 268 nm.

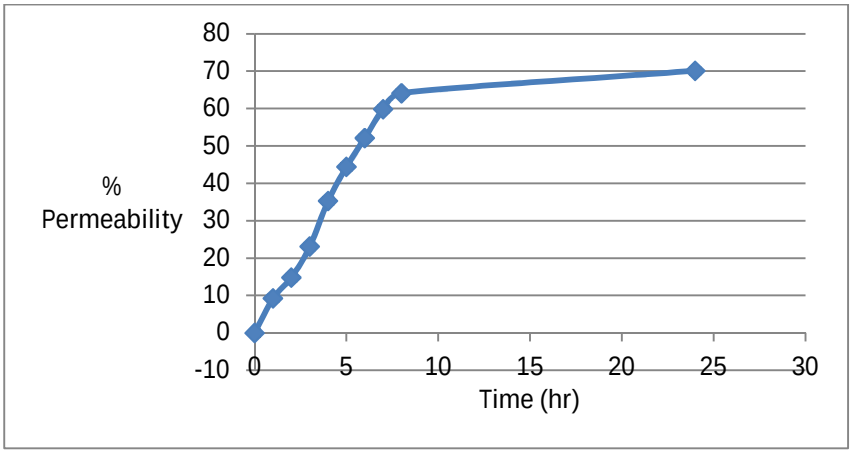


Figure 6:- Diffusion profile of F6 batch.

After 24 hour 70.11% drug release was found for Adapalene topical spray by using rat abdominal skin as semipermeable membrane.

Statistical Data Analysis for Batch F6

The correlation coefficient (R^2) of the kormeyer-peppas model was found to be 0.861 as mentioned in table 21, slightly higher when compared to the other plot. Hence the release of drug from the F6 batch followed kormeyer-peppas (non fickian) model for diffusion.

Table 21: - Diffusion kinetic data for F6 batch.

Diffusion Kinetic	R^2
Zero order plot	0.558
First order plot	0.421
Korsmeyer & peppas plot	**0.861**
Hisxon-crowell plot	0.469
Higuchi plot	0.782

Estimation of skin irritation: Skin irritation test was performed on rabbit back side. Control (without adapalene) and Test formulation (with Adapalene) were observed for 24 hour after application. The total scores for irritation test in each formulation were calculated using the following equation and shown in Table 22.

$$\text{Primary irritation index} = \frac{(\text{Erythema reaction scores} + \text{Edema reaction scores})}{\text{Time interval (h)}}$$

The scores for erythema and edema were calculated on the basis of severity of affected part as shown in Table 22.

Table 22: - Table indicating score of Erythema and Edema formation.

Erythema and Edema formation	Score
No	0
Very slight	1
Well defined	2
Moderate to severe	3
Severe	4

Table 23: - Average response scores of skin irritation for single application.

Groups	Primary Irritation Index	
	8h	24h
Topical spray (control)	0	0
Adapalene topical spray (Test)	0	0

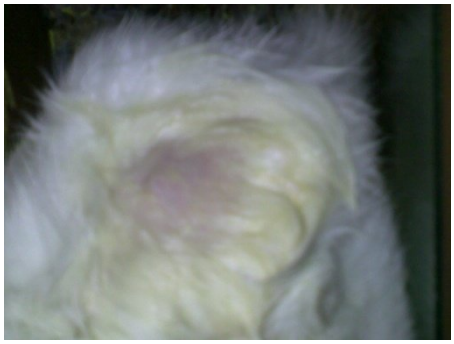


Figure 7:- Control after 24h.

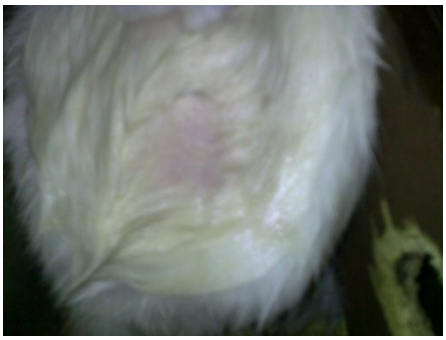


Figure 8:- Test after 24h.

The primary irritation index of Adapalene topical spray was calculated to be 0.00. No erythema or edema were found in the primary skin irritation studies of the optimized formulations on the rabbits hence found to be safe and non irritant for topical application. The formulation developed was more efficient for a period of 24 h after application.

Conclusions

Adapalene topical spray is applied with the push of button at the site of application without contaminating the remaining material. Topical spray of anti-acne agent is expected to form a clear transparent thin film at the site of application having property to adhere to the skin, thus effectively delivering the drug at the site of action, without pain or irritation.

Various polymers were chosen from solubility and preliminary batches study. From preliminary batches study eudragit E 100 polymer was selected on the basis of greater *in-vitro* release. Further optimization was done by altering the concentration of IPA, PG and eudragit E100. Optimized batch containing higher concentration of PG and IPA with lower concentration of eudragit E100 (batch F6) was found to give higher release of drug from formulation.

Adapalene topical spray of optimized batch was evaluated for continuous spray evaluation as per USP, drug content, spray pattern, ex vivo release study, skin irritation study, stability study etc.

Compatibility of aerosol was evaluated using glass container. Product concentrate and propellant (LPG) was found to be compatible. Particles size of Adapalene topical spray was determined by using microscopic method. Particles size of topical aerosol are usually less than 100 μm. Particles size is influenced by many factors. Among them are vapour pressure, the type and amount of solvents present in the formula, the type and amount of the propellant used, and the design of the valve system. Particle size also gives the information about stability of topical spray.

Delivery rate and delivery amount was found to be same and affected by vapour pressure. Net content of topical spray was within labeled amount. No leakage was detected when containers were passed through filled water bath at 55°C. pH of topical spray indicated that Adapalene topical spray was suitable for topical application without producing any irritation.

Adapalene topical spray was found to be flammable due to present of solvent and LPG (propellant). Spray pattern of selected formulation was revealed the similarity with almost same diameter for all formulation.

Skin irritation study was performed by using rabbit (New Zealand white strain). No skin irritation was produced by optimized formulation. Hence, topical spray of Adapalene would have to be a better alternative as a topical drug delivery system for treatment of acne.

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