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Research Article

Preparation and Evaluation of Nano-Structured Lipid Carriers of Azelaic Acid in Topical Formulation (Hydrogel)

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ABSTRACT
To prepare a hydrogel nanosystem to deliver Azelaic Acid for topical treatment.A hydrogel system consisting of nanostructured lipid carriers (NLCs) of azelaic acid was prepared by phase separation method. Characterization was done for morphology, particle size, zeta potential, percent encapsulation efficiency and in vitro release rate data. Two formulations gave promising results on the basis of skin permeation profile when compared with marketed formulation

Keywords: nanostructured lipid carriers, azelaic acid, poloxamer, soya lecithin, tween 80

INTRODUCTION

Among the approaches for exploiting new developments in medicine, various nanoparticulates offer some unique advantages as pharmaceutical delivery systems and image enhancement agents. Various nanoparticle based drug delivery and drug targeting systems are currently developed or under development .Their aim is to minimize drug degradation upon administration, prevention of undesirable side effects, and enhancement of drug bioavailability and the fraction of the drug accumulated in the pathological area. Pharmaceutical drug carriers, especially the ones for topical administration, are supposed to be easy and cheap to prepare, biodegradable, should have small particle size, possess high loading capacity, demonstrate prolonged circulation, and, ideally accumulate in required pathological sites in the body. Topical and dermatological dosage forms are prepared for protection purposes. The protective aspects of these applications are passive; even they can not be regarded simply in terms of placebo effects. The lipid organization of the stratum corneum is highly responsible for the transport properties of the skin. The polar head groups of the lipid are gathered in layers with the non polar chains pointed in opposite directions forming layers of methyl groups in the plane where the hydrocarbon ends.[1] not all the lipids are positioned with their polar groups localized in the polar layer. Some of them are actually localized in the region between the methyl groups. A layered structure, such as that in the lipid part of the stratum corneum, is not a perfectly organized array of layers parallel to the skin surface but instead a series of dislocations always occurs. Therefore the diffusion coefficient is the gross one for a partially organized lamellar structure.[2, 3] When a drug molecule moves onto intact skin delivered from a vehicle, it first contacts with the hydrolipidic mantle, cellular debris, bacteria and other exogenous materials which covers the skin. In general the molecule may penetrate to the viable tissue below the horny layer via two potential routes of entry to the subepidermal tissue, which are transepidermal route and transappendageal route.[4, 5]
Nanostructured lipid carriers (NLC) have lipid as their back bone. NLC are formed with both liquid and solid lipid as a blend mixture in such a ratio that they are solid at room temperature. Another advantage of NLC is modulation of drug release. When lipid nanoparticles are produced only with highly pure solid lipids (SLN), they produce relatively perfect crystals of lipid and their loading capacity is limited, especially when high loading is required. [6, 7, 8] In case of NLC, mixing especially very different molecules, e.g. long chain acylglycerols of solid lipids with short chain of acylgylcerols of liquid lipids creates crystals with many imperfections. These imperfections in crystals results in additional loading of drug by providing space between fatty acid chains and lipid bilayers.[9, 10] The active ingredient can be incorporated in particle matrix in a molecular dispersed form or it can be arranged as amorphous clusters. The type of selected lipid also decides the shape of crystals to be formed. In case of highly pure lipids such as tristearin or cetyl palmitate, cubic shaped nanoparticles are obtained. When using identical lipid molecules the cubic shape occurs because they build up crystal like a dense brick wall. In case of rather polydispersed mixtures (which are generally preferred for cosmetic preparations) the nanoparticle obtained are of somewhat larger and smaller and simultaneously different spherical shaped structures.[11,12,13] In this work, an attempt is made to prepare NLC using beeswax and oleic acid as solid and liquid lipid respectively. Different concentration of surfactants like tween 80, poloxamer 180 and Soya lecithin are used. The active ingredient is Azelaic Acid which is used as anti acne and hypopigmentation agent.

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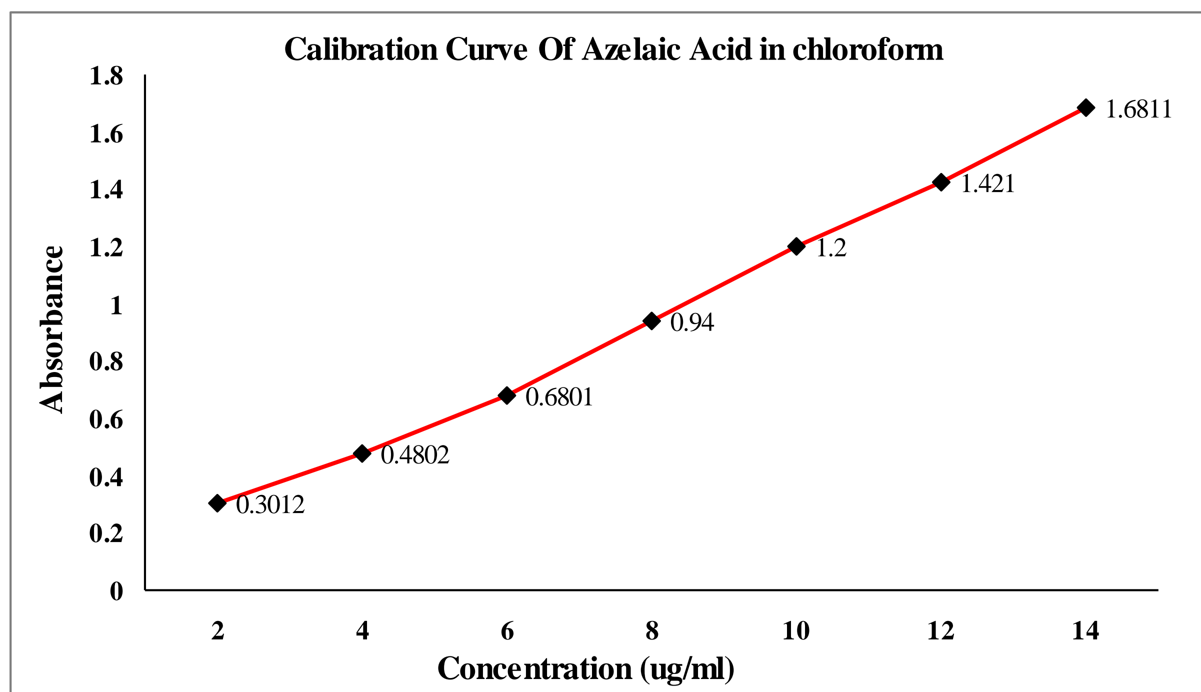

Figure 1: Calibration curve of Azelaic acid in chloroform

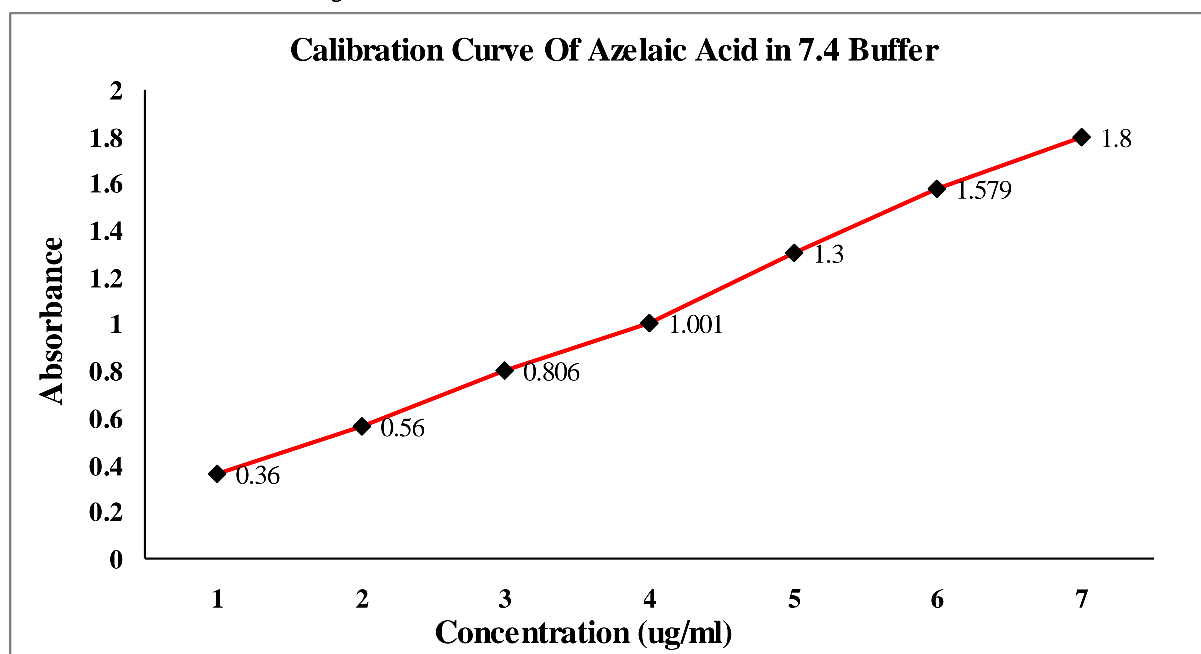

Figure 2: Calibration curve of Azelaic acid in ph 7.4 buffer

MATERIAL AND METHODS

Materials

In preparation of these system three surfactants Tween 80, Poloxamer and Soya Lecithin were used. Beeswax, Oleic acid and cholesterol were used to made lipid phase of emulsion. Azelaic acid was used as active pharmaceutical ingredient. Glycerin, triethanolamine and poly vinly alcohol were used to prepare hydrogel. All chemicals were provided by ALFA AESSAR CHEMICALS. Identification of API and excipients was done with the help of melting point and FTIR analysis. Calibration curve of azelaic acid was prepared in chloroform at max 243.5 nm.

Preparation of NLCs

Oil phase was prepared by dissolving azelaic acid and cholesterol in mixture of beeswax and oleic acid at 60-70˚C. Aqueous phase was prepared by dissolving Tween 80 in water at temperature 60-70˚C. Oil phase was mixed drop wise to aqueous phase and subjected to mechanical stirring for 30-40 minutes at 60-70˚C. Resulting emulsion was cooled below 20˚C and then subjected to ultra sonication for 20 minute. Cooling of emulsion converts it into dispersion and sonication reduces the particle size of

Table 1 : formulation optimization using different surfactants

S. No.	Formulation code	Surfactants
1	T_1	1% tween
2	T_2	2% tween
3	T_3	3% tween
4	T_4	4% tween
5	PC_1	1% lecithin
6	PC_2	2% lecithin
7	PC_3	3% lecithin
8	PC_4	4% lecithin
9	P_1	1% poloxamer
10	P_2	2% poloxamer
11	P_3	3% poloxamer
12	P_4	4% poloxamer

solid lipids and thus produces aqueous dispersion of lipid nanoparticles. This aqueous dispersion was then subjected to centrifugation at 9000 rpm for 30 minutes to remove any large particles escaped from size reduction. Same method was used to prepare nanoparticles using different concentration of other surfactants like poloxamer and lecithin. No residue was found after centrifugation of nano dispersion indicating that all lipid particles are reduced to such a size which remains suspended even after centrifugation at 9000 rpm. All formulations designed contains 6% oleic acid, 4% beeswax, 1% cholesterol and 5% azelaic acid w/w in lipid phase while surfactants and their concentration was variable as shown in table 1 from 1% to 4% w/v . The total volume of each formulation was 100 ml. All formulations produced aqueous dispersions which contain nanoparticles except formulation $\mathbf{T_4}$, $\mathbf{PC_4}$, $\mathbf{P_1}$ and $\mathbf{P_2}$ which showed agglomerates.

Hydrogel preparation

For preparation of hydrogel formulation of NLC of azelaic acid; aqueous dispersion of NLC was used in place of water to prepare hydrogel of PVA as it was not possible to separate NLC from aqueous dispersion in present laboratory conditions. A total of 100 ml of NLC was used to prepare hydrogel with 4gm of PVA and 6 gm of glycerin as softener and triethanolamine as emulsifier.

RESULTS

Characterization of NLCs by particle size and zeta potential determination

The mean diameter, surface charge and size distribution of nano structured lipid carrier for all formulations were done by MALVERN ZETA SIZER MODEL ZS90. Results are recorded in table 2.

Morphological characterization

morphological examination of NLCs was done by transmission electron microscopy.

Table 3: Percent entrapment efficiency of different formulations

S.No.	Formulation code	% Entrapment efficiency in lipid phase	% Concentration of Azelaic acid in aqueous phase
1	T_1	99.1	0.9
2	T_2	99.0	1.0
3	T_3	99.1	0.9
4	PC_1	99.3	0.7
5	PC_2	99.2	0.8
6	PC_3	99.4	0.6
7	P_3	99.1	0.9
8	P_4	98.9	1.1

Fluorescent microscopy

A fluorescent dye RHOHDAMINE 'B' was added to formulation for microscopic analysis of particle distribution.

Percent entrapment efficiency

Azelaic acid in lipid phase

Encapsulation efficiency of NLCs was calculated by dissolving 5 gm of azelaic acid and 1 gm of cholesterol in 6gm of oleic acid and 4gm of beeswax mixture at 60-70˚C. This hot mixture was mixed with a solution of water with surfactant (variable for each formulation) at 60-70˚C, volume made up to 100ml with hot water and mixture stirred mechanically for 30 minutes. The prepared emulsion was cooled to room temperature and lipid phase was allowed to solidify with constant stirring. The resulting dispersion was filtered through Whatman paper no 1 and marc (solid lipid phase) was washed with distilled water twice to displace any aqueous vehicle adhered to lipid particles. The lipid phase was dried in vacuum and approximately but accurately 10 mg was weighed and dissolved in 10 ml of chloroform and 100 µl of this solution was again diluted to 10 ml with chloroform and absorbance was recorded and percent

Table 2: Characterization of nanopaticles

S.No.	Formulation Code	Average Particle size (in nm)	Polydispersitivity Index	Zeta potential
1	T_1	185	0.545	-9.27±3.93
2	T_2	185	0.579	-7.09±4.85
3	T_3	395	0.672	-3.46±15.8
4	PC_1	428	0.357	-24.8±4.68
5	PC_2	525	0.420	-33.3±9.89
6	PC_3	481	0.229	-34.5±7.20
7	P_3	265	0.619	-8.72±3.39
8	P_4	259	0.451	-5.07±3.16

Table 4: percent cumulative drug concentration

S.No	Time (in hours)	Cumulative drug/ml in buffer solution (µg/ml)			Percent cumulative drug concentration in buffer solution		
		PC_2	PC_3	marketed formulation	PC_2	PC_3	Marketed formulation
1	0	0.0	0.0	0.0	0.0	0.0	0.0
2	1	0.72	0.45	1.26	0.40	0.30	0.7
3	2	1.89	0.81	1.98	1.05	0.45	1.1
4	3	2.88	1.35	3.6	1.60	0.75	2.0
5	4	3.42	1.89	3.96	1.9	1.05	2.2
6	5	4.68	2.27	4.86	2.6	1.26	2.7
7	6	5.76	3.06	5.76	3.2	1.71	3.2
8	7	6.48	3.33	7.2	3.6	1.85	4.0
9	8	6.84	3.78	7.92	3.8	2.11	4.4

encapsulation reported in table no 3 for each formulation.

Azelaic acid in aqueous phase

25 ml of filtrate taken after filtering the dispersion (before sonication) through Whatman paper no 1 and it was extracted quantitatively with chloroform three times (each with 10 ml). Extracts were combined and volume was made up to 50 ml. The solution was analyzed for azelaic acid content with UV absorption at 243.5 nm and absorbance recorded for various formulations. Percent concentrations of azelaic acid in aqueous phase of different formulations are tabulated in table no. 3.

Table 5: percent drug remaining after absorption

S.No.	Time after application of formulation	Percent drug remaining in formulation		
		PC_2	PC_3	Marketed
1	0	100	100	100
2	1	83	75	89.08
3	2	63	57.1	76.77
4	3	44	29.90	66.76
5	4	30.99	6.1	59.34
6	5	22	2.02	50.14
7	6	14	undetectable	43.61
8	7	10	undetectable	34.72
9	8	1	undetectable	30.29

FTIR analysis

FTIR analysis was conducted to observe the compatibility between API and other components.

DSC studies

Differential scanning calorimetric analysis was conducted to study the thermal stability of formulation and their individual components.

In-Vitro release studies

Drug that crosses the membrane and reaches the buffer solution

Franz diffusion cell with sample holder opening of 1 cm radius was used in the present study. Fresh goat skin was obtained from slaughter house. It was depilated and kept at pH 7.4 before use. The upper surface of skin was dried which was placed on the sample holder and then one gm of gel was applied on the skin. Franz diffusion cell was used with 25ml of phosphate buffer solution of pH 7.4 and a magnetic bead which allows constant stirring. One ml of aliquots were withdrawn after each hour for total time of 08 hours and volume was compensated by adding one ml of phosphate buffer solution at each withdrawal. Cumulative drug content was calculated on the basis of recorded UV absorption of each aliquot show in table 4. (Figure. 2: calibration curve of azelaic acid in ph 7.4 buffer)

Unabsorbed drug concentration

Approximately 200 mg of hydrogel was applied on isolated depilated goat skin (1cm radius area). The goat skin was marked for circles (total 8) and was tied on the petridish having diameter of 15 cm with modifications (passage was created on petridish base for introducing buffer solution with positive pressure for intimate contact with skin). The petridish was previously filled with buffer

Table 6: *In-vitro* release study

S.No.	Time (in hours)	Percent cumulative drug concentration in buffer solution (a)			Percent drug remaining in formulation (b)			Percent drug concentration in membrane (c) = 100 – (a+b)		
		PC_2	PC_3	Marketed	PC_2	PC_3	marketed	PC_2	PC_3	Marketed
1	0	0	0	0	100	100	100	0	0	0
2	1	0.40	0.30	0.7	83	75	89.08	16.6	24.70	10.22
3	2	1.05	0.45	1.1	63	57.1	76.77	35.95	42.45	22.13
4	3	1.60	0.75	2.0	44	29.90	66.76	54.40	69.35	31.24
5	4	1.9	1.05	2.2	30.99	6.1	59.34	67.11	92.85	38.46
6	5	2.6	1.26	2.7	22	2.02	50.14	75.4	96.72	47.16
7	6	3.2	1.71	3.2	14	0	43.61	82.8	98.29	53.19
8	7	3.6	1.85	4.0	10	0	34.72	86.4	98.15	61.28
9	8	3.8	2.11	4.4	1	0	30.29	95.2	97.89	65.31

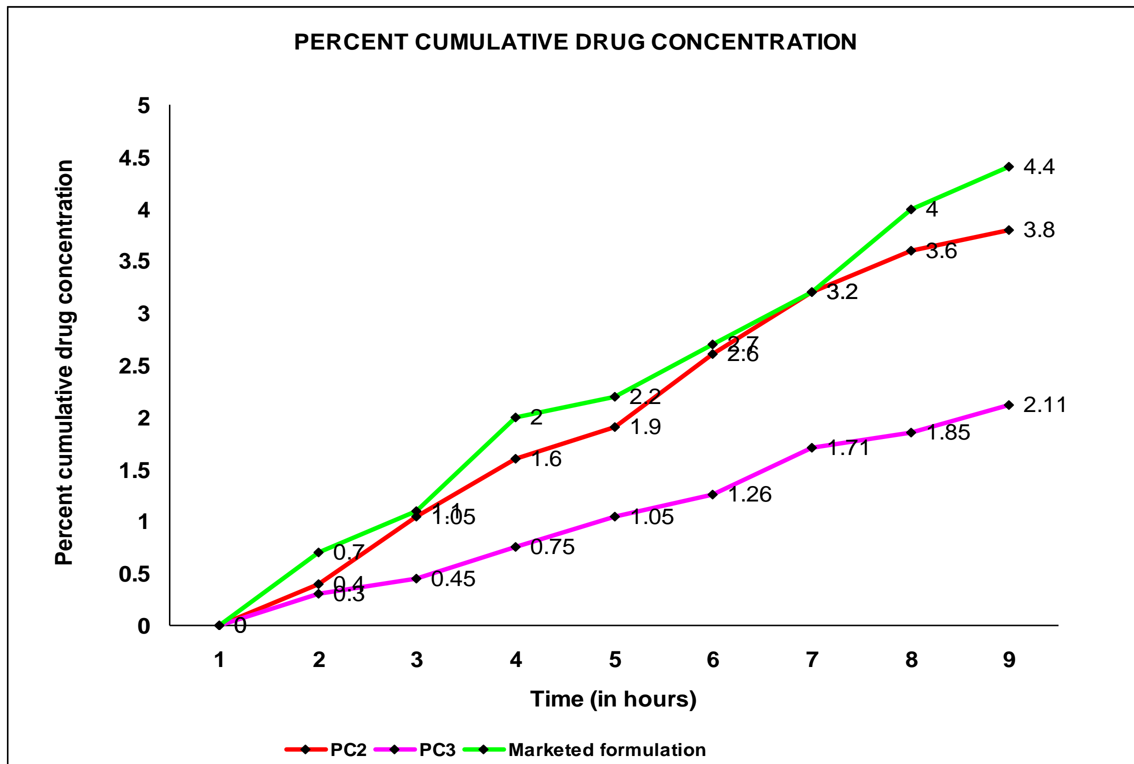

Figure 3: Percent cumulative drug concentration in buffer solution

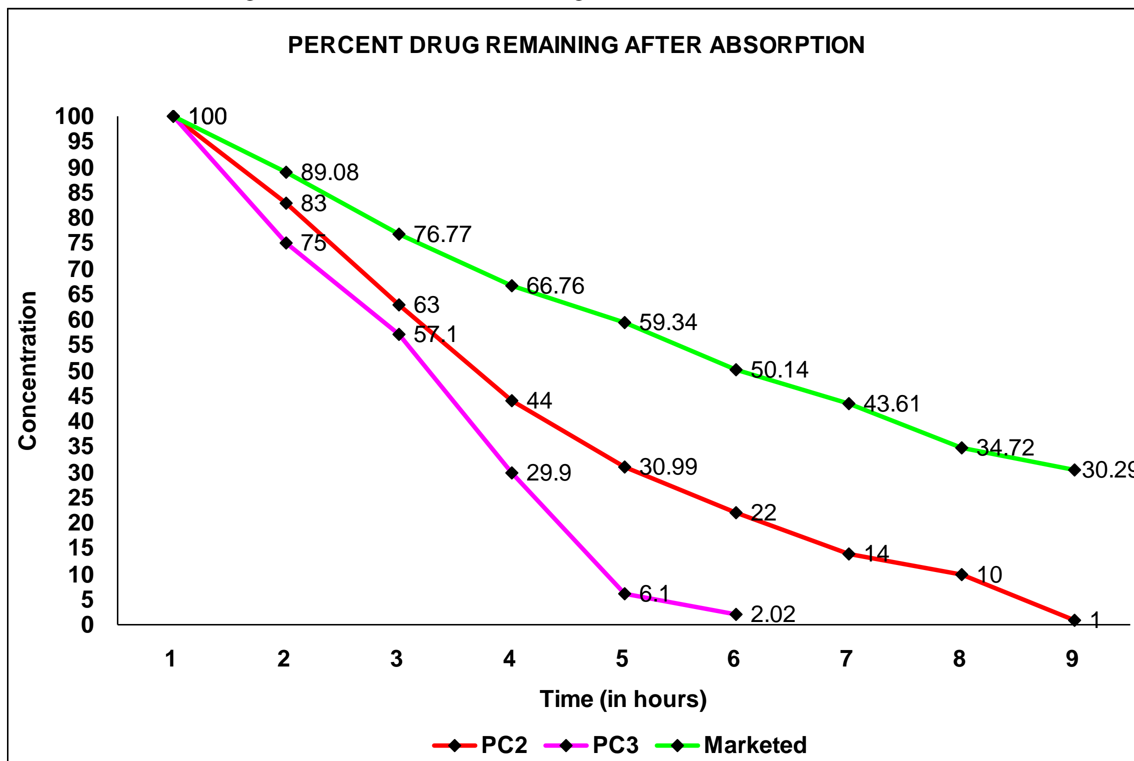

Figure 4: Percent drug remaining after absorption

solution of pH 7.4 and a magnetic bead was placed in for stirring.

After interval of one hour hydrogel applied on circle one was wiped out with help of cotton swab which was previously extracted with chloroform. After wiping, the swab was extracted thrice with chloroform and volume is made up to 25 ml with chloroform. One ml of this solution again diluted to 10 ml with chloroform and absorbance recorded at 243.5 nm. Same method was used for formulation PC_2 and marketed formulation. Samples were wiped out after each hour for up to eight hours from prepared and marketed formulations. With the help of data recorded in Table no. 4 (the percent cumulative drug concentration) percent drug concentration after

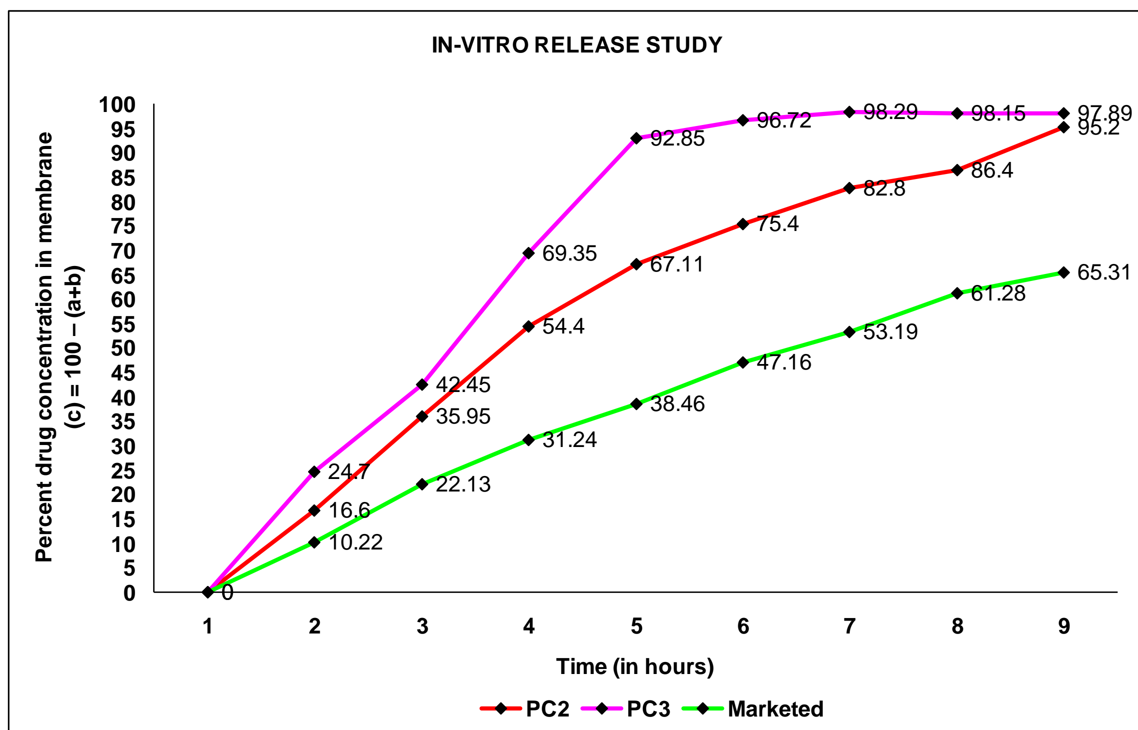

Figure 5: *in-vitro* release study

absorption was recorded in table 5 and in table 6 percent drug concentration that reaches in membrane but does not get systemically absorbed was recorded.

DISCUSSION

Particle size, size distribution and zeta potential of various formulations were studied through MALVERN ZETA SIZER. It was observed that smallest size of 183 nm was present in formulation code T_1 and T_2 and size distribution was better in formulation PC_1. However the zeta potential of formulation code PC_2 and PC_3 was promising. Hydrogel of PVA was selected for incorporation of NLC of azelaic acid as use of any lipid vehicle will dissolve NLC. Hydrogel of PVA 4% was prepared. NLC preparation containing aqueous phase was used to prepare hydrogel instead of using distilled water as it was difficult to separate/filter out NLC from dispersion due to their nano size. Prepared formulations were subjected to release rate studies using Frenz cell and goat skin. Sample from buffer solution were examined periodically and cumulative percent drug concentration was recorded. This gives a picture simulating drug reaching systemic circulation. The same process was used for marketed formulation. However in another study using a petridish base and goat skin marketed formulation and prepared NLC formulations were compared for their drug release to the skin. This was studied by calculating the drug remaining in formulation after each hour. For these calculations formulation and marketed formulation were applied on the goat skin simultaneously and after each hour formulation and sample were wiped out and drug remaining was calculated. After subtracting it from total drug applied on skin, it gives drug penetrating skin. It has been found that prepared formulation penetrated the skin and remained for a longer time.

CONCLUSION

From conducted experimental work it can be concluded that hydrogel formulation of NLC of Azelaic Acid can be used efficiently in treatment of acne while using 3% and 2% lecithin as surfactant providing efficient adherence and penetration in skin.

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