FORMULATION AND EVALUATION OF ETHOSOMAL GEL FOR ANTI-AGING

Gupta Rajat Kumar Rajesh[1]*, Dr. Arun Patel[2], Dr. Shailendra Patel[3] Dr. Naveen Shivavedi[4]

[1*2,3,4]Shri Ram Group of Institutions, Department of Pharmacy,Jabalpur-482002 M.P.,India. Rajiv Gandhi Proudyogiki Vishwavidyalaya,Bhopal-462033, Madhya Pradesh.

***Corresponding Author -** Gupta Rajat Kumar Rajesh.

***Gmail:** guptarajat1214@gmail.com **Mobile No:**8174865558.

Abstract

Ethosomes are soft, malleable, tiny bubble like lipid vesicles composed mainly of phospholipids, ethanol and water. The objective is problem of appearance of wrinkle before age and to prevent chronic effect of signs. The neem plant extract (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered and estimated total alkaloids contents. Ethosomes were prepared by solvent dispersion method. The phospholipids (2 - 3 mg) and neem extract (1 mg) was dissolved in ethanol (30 to 40ml) and heated to 30ºC ± 1ºC on water bath. We preparated ethosomes suspension containing ethanolic extract with eight ethosomal formulations. We evaluated physical evaluation, measurement of pH, spreadibility, consistency, homogeneity, viscosity, drug content, compatibility studies. We found that formulation FE8 had good values of spreadability, viscosity, pH, drug content and during the accelerated stability studies the appearance was clear and no significant variation in spreadability, pH and drug content was observed. • The formulated gels were subjected to stability studies. The pH of all formulations remained unchanged and was found to be within the range of 6.8-7.3. The viscosity and spreadability of all gels were also unaltered and found to be within the range. The drug content was found to be in the limit 70.21% to 77.52 %, for all gel formulation at all temperature conditions. All the studies performed provides a that novel topical ethosomal gel containing neem extract that can be used as an alternative remedy for management and treatment of anti aging related disorder and disease.

Key words: *Ethosomal gel, Anti-aging, Cosmaceuticals, Topical drug delivery systems, Drug release kinetic modelling, Spreadibility.*

Introduction

Cosmaceuticals has been used to describe the products that yield benefits traditionally, and active constituents thought to be cosmetic in nature, such as moisturization, as well as product that make marketing claims approaching those of drug products, such as reducing wrinkles, regenerates skin, firms, heal and penetrates into skin. Consumers seek "anti-wrinkle" cosmetic products that treat or delay the visible signs of actual aging and weathered skin, such as wrinkles, lines, sagging, and hyper pigmentation and age spots. The present consumers are highly specific about choice of product; consumers prefer mainly products free of synthetic active constituents, synthetic preservatives and base free of animal derivatives. Unnatural, chemically- synthesized products may be perceived as being environmentally or personally unsafe[1,2]. In contrast, natural products are perceived as pure, mild, and superior to chemically synthesized products. Natural based products extracted from plants or herbs are believed to contain antioxidant/free-radical scavenging agents that can neutralize the effects of free-radical damage[3,4]. Additionally, they contain agents that stimulate the synthesis and restoration of damaged connective tissue structures in the dermis and barrier function in the epidermis[5,6]. In spite of the various anti- aging cosmetic products on the market for the treatment of skin, there remains a need for effective topically applied cosmetic compositions that provide anti-aging or rejuvenating benefits to the skin, using natural ingredients as active components. They also expect the latest technology advances to be incorporated into innovative formulations[7].

Organs of all the creatures including human grow from birth, and gradually decline with age, then, functions thereof are deactivated. When the deactivated part exceeds a certain extent, the creature dies. The process that the functions thereof are gradually declining is called aging. Skin is directly affected by surroundings and has important functions to maintain circumstance of the inner part of living bodies. Intrinsic aging, also known as the natural aging process, is a continuous process that normally begins in our mid-20s. Within the skin, collagen production slows, and elastin, the substance that enables skin to snap back into place, has a bit less spring. Dead skin cells do not shed as quickly and turnover of new skin cells may decreases slightly. While these changes usually begin in our 20s, the signs of intrinsic aging are typically not visible for decades[8,9].

In the early 1980s, Mezei and his group described liposomes as the first topical lipid vesicular system for enhanced drug delivery to the skin. Since then, many works have shown that lipid vesicular systems are able to increase the accumulation of various molecules in the SC or other upper skin layers. Ethosomes are soft, malleable, tiny bubble like lipid vesicles composed mainly of phospholipids, ethanol (relatively high

concentration) and water. These "soft vesicles" represents smart vesicular carrier for enhanced delivery to/through skin. All components of the Ethosomal systems are considered as being safe for pharmaceutical and cosmetic use[10,11].

Up till now work has been done over ethosomes to deliver one of herbal derivative that is ammonium salt of glycyrrhizic acid (antinflammatory agent) of Glycyrrhiza glabra. *In-vitro* and *in-vivo* experiments were carried out; Ethosome suspension showed very good skin tolerability in human volunteers, increases of in vitro percutaneous permeation and significantly enhanced anti-inflammatory activity of glycyrrhizic acid. Ethosomes even reported to deliver many synthetic drug for example Salbutamol Sulfates, Minoxidil, Cannabidol, Acyclovir, Anti-HIV Agents, zidovudine, Lamivudine, Trihexiphenidyl hydrochloride Insulin, Azelaic acid, Erythromycin, Bacitracin, DNA, Diclofenac, peptides[12,13].

The main objective behind choosing this work is to treat world's today major problem that is appearance of wrinkle before age and to prevent chronic effect of signs which are results of various environmental factors like pollution, UV rays, toxic chemicals through effective and safe treatment (by complete natural product) within less period of time. Rationale behind choosing ethosomes as delivery system to fulfill objective of topical delivery system that is enhanced delivery of active agents at deeper level of skin, packed less tightly than conventional vesicles but has equivalent stability, allowing a more malleable structure and improves distribution in *stratum corneum* lipids, phospholipids present in ethosomes rejuvenate cell membrane and ethanol present in formulation act as preservative. Ethosomes containing extract via lotion are incorporated into cream. Even the cream is formulated by taking all natural components and no preservative was added in cream to make it safe. Cream is full of natural oils which nourishes and moisturizes skin effectively and are rich in omega oils which is highly required for collagen production[14,15].

Materials and Methods

Collection of plant material: Neem leaves were collected locally from Bhopal, a tree in college campus, required whole leaves were collected from same tree. Collected Neem leaves were washed by distilled water two times and dried under shade for one month.

Preparation of plant powder: The plant was dried under shade and then powdered coarsely with a mechanical grinder. The powder was passed through sieve No. 40 and stored in an airtight container for further use.

Preparation of extracts: The dried powder of plant leave was extracted with

Ethanol using Soxhlet apparatus. Accurate 250 gm of dried powder leave of plant was subjected to soxhlation. It was first defatted with petroleum ether then exhaustively extracted with solvent in a Soxhlet apparatus for 36 hours. The temperature was maintained at 40-50 degree centigrade.

Table No. 1: Formulations of Gel containing ethanolic extract of *neem* leaves

Ingredient	F1	F2	F3	F4	F5	F6	F7	F8
Carbopol 934 (gm)	1	1	1	1	1	1	1	1
Sodium CMC (gm)	1	1	1	1	1	1	1	1
Ethosomes of *neem* (1% w/w)	1	1	1	1	1	1	1	1
Methyl Paraben (0.2%)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Triethanolamine (ml)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Distilled water (100ml)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Characterization and Evaluation of Gel Formulation

Physical Evaluation: Physical parameters such as color and appearance of the herbal gel were observed manually.

Measurement of pH: The pH of various gel formulations was determined by using digital pH meter. One gram of gel was dissolved in 100 ml distilled water and stored for two hours. The measurement of pH of each formulation was done in triplicate and average value was calculated.

Spreadibility: Spreadibility was determined by the apparatus which consists of a wooden block, provided with pulley at one end. By this method spreadibility was measured on the basis of slip and drag characteristics of gels.

$$S = M \times L / T$$

Where, S = Spreadibility, M = Weight in the pan (tied to the upper slide), L = Length moved by the glass slide and T = Time (in sec.) taken to separate the slide completely each other.

Homogeneity: All developed gels were tested for homogeneity by visual inspection after setting gels in the container. They were observed for their appearance and presence of any aggregates.

Viscosity: Viscosity of gel was measured by using Brookfield viscometer with spindle No. 7 at 50 rpm at room temperature.

Drug content: 1 g of the prepared gel was mixed with 100 ml of suitable solvent.

Compatibility studies: Fourier transformed infrared (FTIR) spectra technique has been used to study the physical and chemical interaction.

Drug release kinetic modelling: The kinetics of ethosomal gel FE release was determined using the release kinetics method of drug release into various kinetic equations: zero order release kinetics, first order release kinetics and Higuchi model.

Accelerated Stability Studies: The optimized formulations were subjected to a stability testing for six months as per ICH norms at a temperature and RH of 40°C ± 2°C/75% RH ± 5% RH respectively.

RESULT AND DISCUSSION

Preliminary Study

Morphological Characterization of *Neem* leaves

Table No. 2: Morphology of *Neem* leaves

S. No.	Character	Observation
1	Color	Green
2	Odor	Bitter
3	Taste	Bitter
4	Size	Length – 1.5-3cm,Width -1-1.5cm
5	Shape	Ovate
6	Texture	Rough outer periphery

Physiochemical analysis of *Neem* leaves powder

Table No.3: Physiochemical analysis of powder of *Neem* leaves

S. No.	Parameters	Observation (%)
1	Total ash value	9
2	Loss on drying	1.2
3	Acid insoluble ash value	2.9
4	Water soluble ash value	1.6
5	Foaming index	6 (ml)

Extract of *Neem* Leaves

Table No 4: Extractive values of extracts of *Neem* Leaves

S. No.	Solvents	% Yield (w/w)	Color	Consistency
1	Pet. Ether	2.91 %	Dark green	Greasy
2	Methanol	9.34 %	Dark green	Semi Solid

Total alkaloid content estimation (TAC)

Total alkaloid content was calculated as atropine equivalent mg/100mg using the equation based on the calibration curve: $y = 0.008x + 0.010$, $R^2 = 0.999$, where X is the Atropine equivalent (AE)and Y is the absorbance.

Table No. 5: Preparation of calibration curve of Atropine

S. No.	Concentration (µg/ml)	Mean Absorbance
1	0	0
2	40	0.352
3	60	0.514
4	80	0.679
5	100	0.845
6	120	0.997

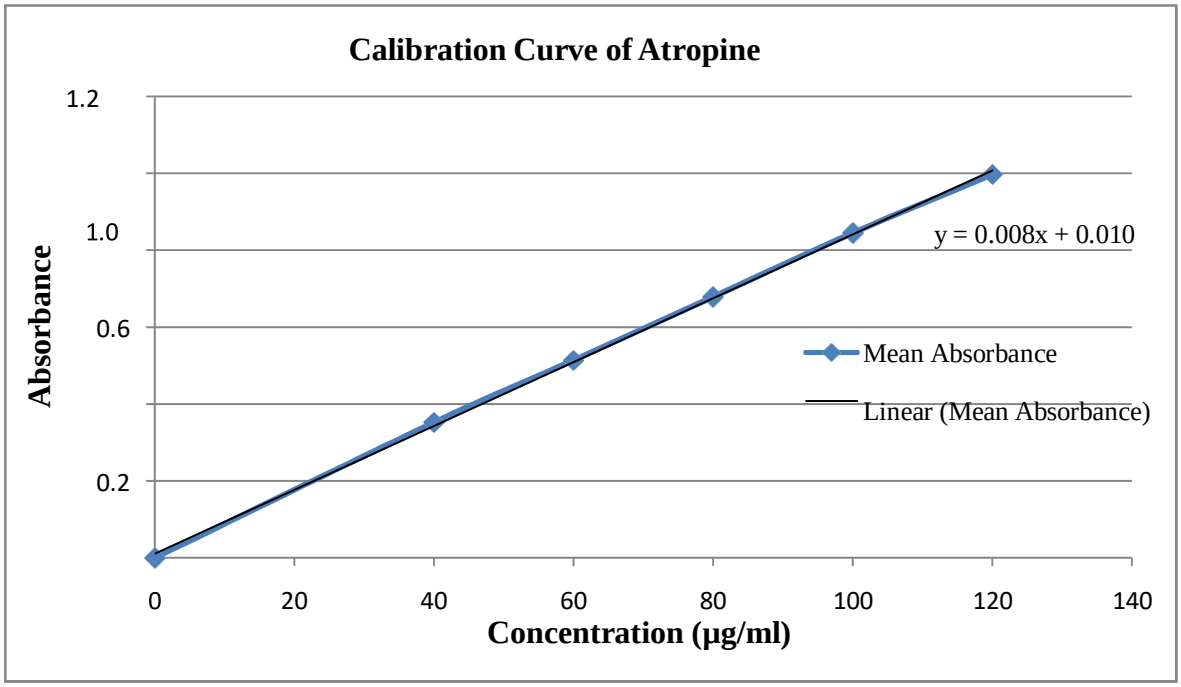

Figure 1: Graph of Calibration Curve of Atropine

Table 6: Estimation of total alkaloid content *Neem Leaves* extract

S. No.	Extract	Total alkaloid content (mg/ 100 mg of dried extract)
1.	Methanol extract	0.632

Formulation of Ethosomes

Table 7: Preparation of Ethosomes Suspension Containing Ethanolic Extract

Ingredient	FE1	FE2	FE3	FE4	FE5	FE6	FE7	FE8
Ethanol extract of Neem (mg)	1	1	1	1	1	1	1	1
Phospholipid (mg)	2	2	2	2	3	3	3	3
Ethanol (ml)	30	40	30	40	30	40	30	40
Tween 20 (ml)	-	-	2	2	-	-	2	2
SLS (mg)	-	-	500	500	-	-	500	500
Distilled Water (upto 100ml)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Characterization of Neem Extract Loaded Ethosome Suspension

Image analysis of ethosomes by optical microscope: For the initial vesicle characterization of ethosome suspension were examined by compound microscope. The result revealed that formulation without added SLS and Tween 20 shown aggregation process among the

structure. Formulation in which SLS and Tween 20 was added shown spherical shaped vesicle structure without aggregation. Hence, Formulation containing SLS and Tween 20 in ehanolic extract (FE3, FE4, FE7 and FE8) were emphasized in further study.

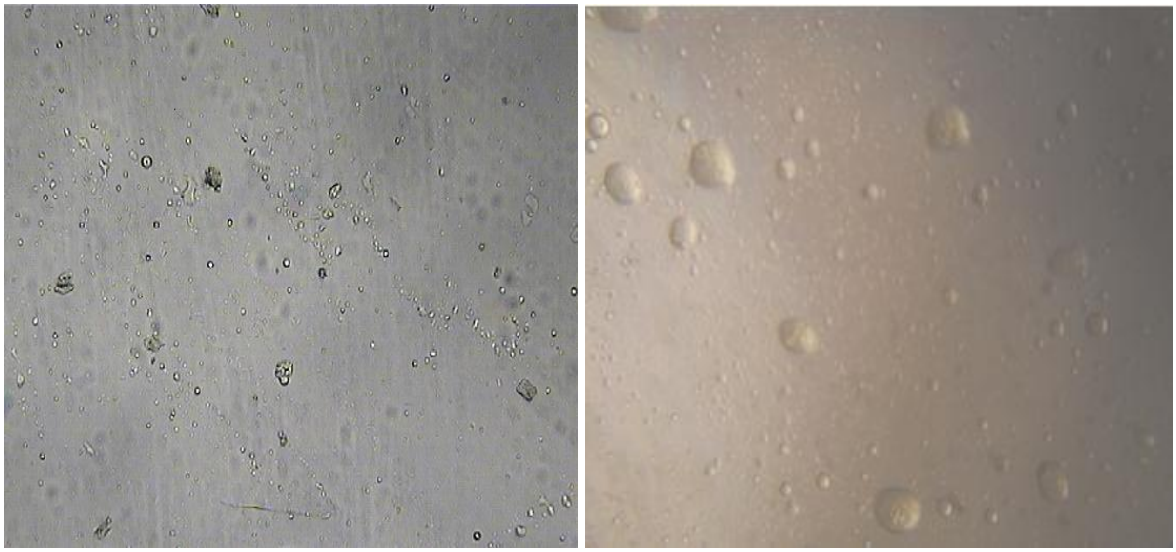

Figure 2: Microphotographs of Ethosome suspension with SLS and Tween (left) withoutSLS and Tween (right)

Vesicular shape and surface morphology by TEM: The vesicular shape and surface morphology of ethosomes of formulation FE8 examined by Transmission Electron Microscope (TEM). The TEM image showed that ethosomes were spherical shaped.

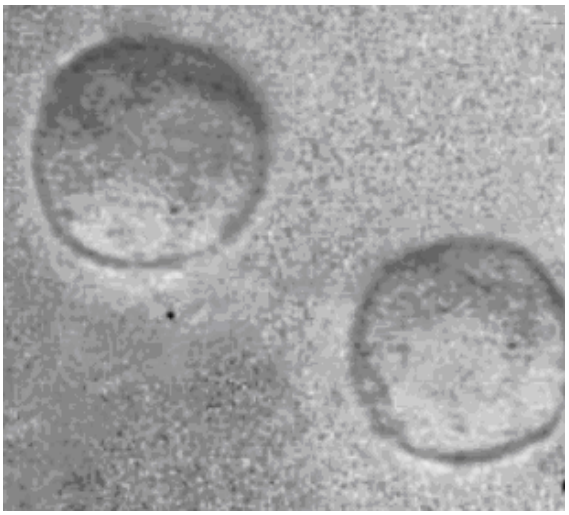

Figure 3: Visualization of ethosomes by TEM

Table No. 8: Characterization of Neem Extract Phytosome

Batch Code	Yield (%)	Drug Content (%)	Mean Particle Size (nm)	Encapsulation Efficiency (%)
EF1	89.12 ± 0.05	70.21 ± 0.21	732 ± 13	78.67 ± 1.52
EF2	88.32 ± 0.08	72.35 ± 0.76	694 ± 29	84.27 ± 0.81
EF3	88.91 ± 0.03	77.52 ± 1.90	637 ± 17	81.34 ± 0.64
EF4	89.45 ± 1.06	71.41 ± 1.63	713 ± 44	82.52 ± 1.30
EF5	83.48 ± 1.95	70.44 ± 1.02	694 ± 21	72.19 ± 1.68
EF6	81.32 ± 0.32	73.32 ± 0.08	765 ± 62	71.62 ± 0.37
EF7	82.91 ± 0.31	72.91 ± 0.03	876 ± 32	90.62 ± 0.11
EF8	87.45 ± 0.06	71.45 ± 1.06	954 ± 27	93.87 ± 0.47

Determination of Entrapment efficiency of ethosomes suspension: The entrapment efficiency of various ethosomes formulations are presented in Table. The entrapment efficiency of formulation FE8 was found to be highest (93.87%), EF7 (90.62%), EF4 (82.52%) while FE3 formulation showed entrapment efficiency (81.34%). However it has been observed the formulation containing phospholipid with ethanol has maximum entrapment efficiency.

In-vitro **drug release study:** Percentage drug release of hydrogel containing ethosomes (FE1- FE8) was observed at 360min 78.31%, 66.31%, 83.23%, 87.83%, 79.22%, 75.63%, 82.33% and 90.53% respectively. It was observed that addition of ethanol in formulation increase the release by increasing permeation properties of ethosomes. The ethosome (formulation FE8) showed maximum drug release as compared to others formulation.

Table No. 9: Cumulative % of drug release from Ethosome

Time (min)	FE1	FE2	FE3	FE4	FE5	FE6	FE7	FE8
15	12.42	11.21	10.33	12.42	12.52	11.22	12.42	12.02
30	19.43	15.92	13.93	18.12	18.21	16.13	18.22	19.43
60	24.53	22.42	17.47	20.32	22.42	24.33	25.32	26.45
90	32.55	36.32	27.32	31.47	30.61	38.85	40.64	46.38
120	44.32	41.31	36.21	41.22	42.53	42.63	50.17	51.53
180	59.46	58.27	54.23	59.64	58.39	59.93	68.34	69.81
240	77.12	62.42	68.31	69.93	74.17	67.24	75.57	78.39
360	78.31	66.31	83.23	87.83	79.22	75.63	82.33	90.53

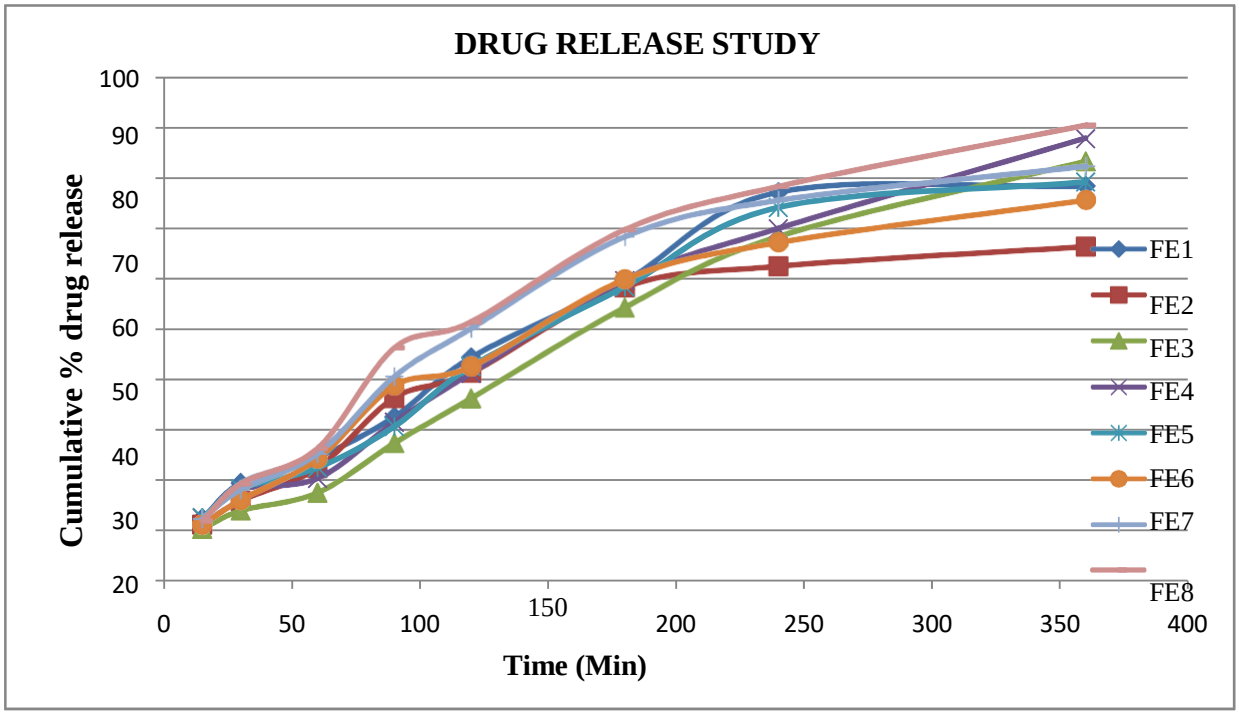

Figure 4: Drug release profile of ethosome suspension containing neem leaves extract

Formulation of topical gel of *Neem* extract loaded ethosome

Table No. 10: Formulations of Gel containing ethanolic extract of *neem* leaves.

Ingredient	F1	F2	F3	F4	F5	F6	F7	F8
Carbopol 934 (gm)	1	1	1	1	1	1	1	1
Sodium CMC (gm)	1	1	1	1	1	1	1	1
Ethosomes of *neem* (1% w/w)	FE1	FE2	FE3	FE4	FE5	FE6	FE7	FE8
Methyl Paraben (0.2%)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Triethanolamine (ml)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Distilled water (100ml)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Evaluation of Neem Extract Loaded ethosomal gels

Table No. 11: Physical Evaluation of Neem Extract Loaded ethosome gels

Formulation code	Clarity	Odor	Phase Separation	Wash ability	Homogeneity	Grittiness
F-1	Clear	No	No	Washable	Homogeneous	No
F-2	Clear	No	No	Washable	Homogeneous	No
F-3	Clear	No	No	Washable	Homogeneous	No
F-4	Clear	No	No	Washable	Homogeneous	No
F-5	Clear	No	No	Washable	Homogeneous	No
F-6	Clear	No	No	Washable	Homogeneous	No
F-7	Clear	No	No	Washable	Homogeneous	No
F-8	Clear	No	No	Washable	Homogeneous	No

Table No. 12: Evaluation of Neem Extract Loaded Ethosome gels

Formulation	pH	Spreadability (cm)	Viscosity(cp)	% Permeation

code				
F-1	6.9	5.6 ± 0.3	100 ± 1.8	83.2%
F-2	6.8	6.8 ± 0.2	99 ± 2.0	86.3%
F-3	7.1	7.1 ± 0.6	114 ± 1.2	92.7%
F-4	7.3	7.6 ± 0.2	100 ± 0.8	91.0%
F-5	7.1	7.9 ± 0.6	118 ± 2.6	90.1%
F-6	7.0	6.9 ± 0.3	123 ± 2.6	90.7%
F-7	7.2	7.3 ± 0.1	105 ± 2.6	89.1%
F-8	6.9	7.1 ± 0.7	113 ± 2.6	90.5%

Table No. 13: *In-Vitro* Drug Release Profile of Phytosome gel from FE 8 Formulation

Time (T) (Hr.)	S.R.T.	Log T.	%C.R	Log % C.R	Drug remaining	Log% drug remaining
0	0	0	0	0	100	2
15	3.873	1.176	12.02	1.080	87.02	1.939
30	5.477	1.477	19.43	1.288	80.57	1.906
60	7.746	1.778	26.45	1.778	73.55	1.867
90	9.487	1.954	46.38	1.954	53.38	1.727
120	10.954	2.079	51.53	2.079	48.47	1.685
180	13.416	2.255	69.81	2.255	30.81	1.489
240	15.492	2.380	78.39	2.380	21.39	1.330
360	18.974	2.556	90.53	2.519	09.47	0.976

Drug release kinetic modeling

On comparison of kinetic modeling and release profile data it was evident that ethosome FE-8 was found to release the drug in accordance to Higuchi kinetics, the regression coefficient was not found to be exactly near to 1, which could be due to influence of some other factors.

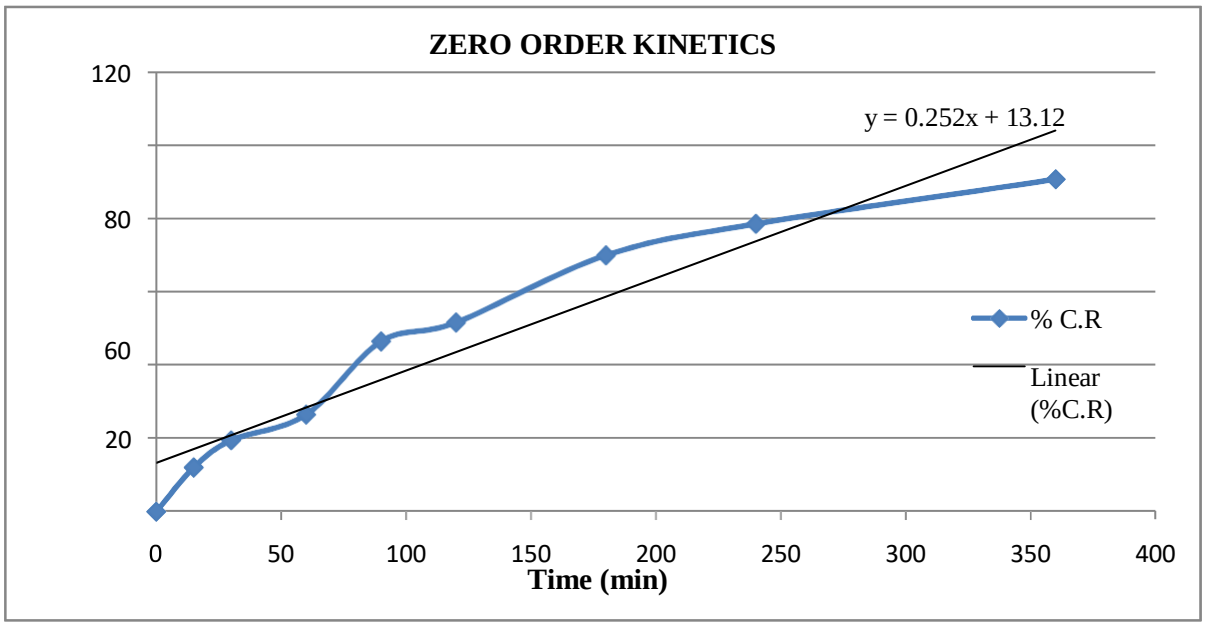

Figure 5: Zero order kinetics of formulation FE8

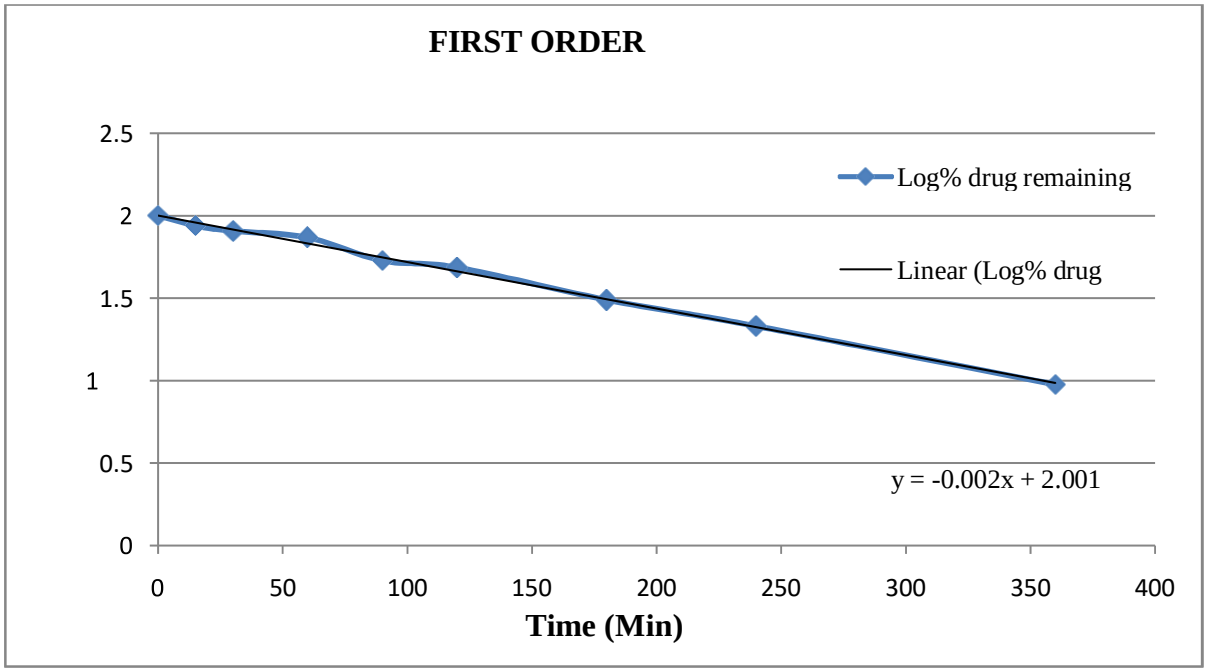

Figure 6: First order kinetics of formulation FE8

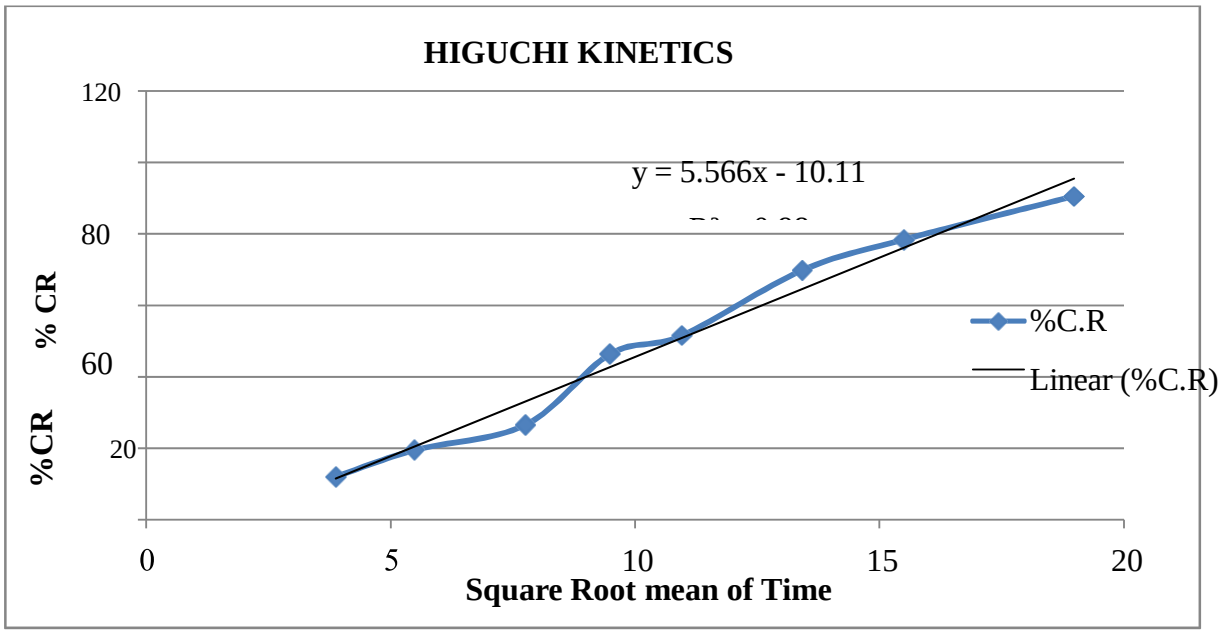

Figure 7: Higuchi kinetic Model of formulation FE8

Stability Study

The formulated gels were subjected to stability studies. No color fading was observed for all prepared gels. The pH of all formulations remained unchanged and was found to be within the range of 6.2-7.2. Viscosity and spreadability of all gels remained unaltered and found to be within the range. Drug content was found to be in the limit 90% -103% for all gel formulation.

Table 14: Accelerated Stability study of formulated gel

Batch	Clarity/ Homogeneity	Phase Separation	Spreadibility (gm.cm/sec)	Viscosity (cps)	pH	Drug Content (%)
F-1	Homogeneous	No	4.9 ± 1.3	142 ± 1.3	7.0	69.01 ± 0.32
F-2	Homogeneous	No	6.1 ± 0.3	134 ± 1.4	7.0	70.45 ± 1.06
F-3	Homogeneous	No	7.0 ± 1.2	119 ± 1.2	7.2	76.12 ± 1.10
F-4	Homogeneous	No	6.8 ± 0.3	123 ± 1.2	7. 1	71.14 ± 1.13
F-5	Homogeneous	No	7.7 ± 0.1	152 ± 1.3	7. 3	72.63 ± 1.12
F-6	Homogeneous	No	7.0 ± 0.4	149 ± 0.4	7.2	72.62 ± 1.03
F-7	Homogeneous	No	7.3 ± 0.5	110 ± 0.3	7.2	70.97 ± 1.05
F-8	Homogeneous	No	7.3 ± 0.2	117 ± 0.5	6.8	70.86 ± 1.71

Discussion

Ethosomes of neem leave extract has been successfully formulated in this procedure firstly Azadirachta indica leaves were collected locally from Bhopal then washed and dried. Neem leaves were morphological Characterized as green in color bitter in taste with ovate shape. Neem leave powder was subjected for various physiochemical analysis like total ash value (9), Loss on Drying (1.2), Acid Insoluble Ash Value (2.9), Water Soluble Ash Value (1.6) and Foaming Index (6 ml) and then extracted out methanolic fraction of neem leaves, yield of the extract which was 9.34%. Estimation of total alkaloid content in extract of neem leaves as Total alkaloid content was atropine equivalent 0.632mg/100mg using the equation based on the calibration curve: y = 0.008x + 0.010, R2= 0.999, where X is the Atropine equivalent (AE) and Y is the absorbance.

Eight different types of Ethosome formulations were formed using fixed amount of ethanol extract of neem leaves, Phospholipid (2-3mg), ethanol (30-40ml) and surfactants e.g. tween20 and SLS. These formulations were characterized under various parameters like yield, drug content, particle size and Encapsulation Efficiency, also morphology like size and shape was observed via TEM. For all formulation yield was 81.32 % to 89.45%, drug content was 70.21% to 77.52 %, Mean Particle Size (nm) 637nm to 954nm and entrapment Efficiency was 71.62% to 93.87%. Percentage drug release of hydrogel containing ethosomes (FE1-FE8) was observed at 360min 78.31%, 66.31%, 83.23%, 87.83%, 79.22%, 75.63%, 82.33% and 90.53% respectively.

It was observed that addition of ethanol in formulation increase the release by increasing permeation properties of ethosomes. The ethosome (formulation FE8) showed maximum drug release as compared to others formulation. All eight Ethosome formulations were incorporated with gel and formed clear, odorless, washable, homogeneous, stable and free from grittiness gel was evaluated under the various parameter, pH of all formulations were observed between 6.8 to 7.3 and Spreadability between 5.6 to 7.9 cm, and viscosity

between 99 to 123 centi poice (cp) and % permeation between 83.2 % to 92.7 %. But on the basis of drug release profile F-8 formulation was very good because its % drug release was 90.53% followed First Order Kinetic Model. Three good formulation's drug releases were F-3 (83.23), F-4 (87.83) and F-7 (82.33). All gel formulations were F-1 to F-8 are stable at 4oC and 40oC showing homogeneity, no significant change in phase separation, Spreadibility, Viscosity, pH and Drug Content (%).

CONCLUSIONS

In the present study, an attempt was made to prepare, characterize and evaluate of topical therapeutic system of anti-aging system from Azadirachta indica herbal plant. Various formulations such as hydrogel, hydroalcohalic gel and ethosomal gel were designed and optimized. From the present study, the following conclusion can be drawn: The extraction of Azadirachta indica was carried out with ethanol by using soxhlet apparatus. The hydrogel and hydroalcohalic gel formulation of ethanolic acetate extract were designed by using varied concentration of carbopol and sodium CMC polymer. During the trial, the excipients concentrations of carbapol and Sodium CMC were gradually increased and then decreased as several problems like homogeneity, spreadibility and viscosity were encountered. These problems occured in some of the batches of polymer based gel containing Azadirachta indica. Hence, these batches were discarded and remaining batches were characterized for various parameter. The result showed that the developed herbal gel was greenish in color, translucent in appearance and showed good homogeneity with absence of lumps. Formulation FE8 had good values of spreadability, viscosity, pH, drug content and during the accelerated stability studies the appearance was clear and no significant variation in spreadability, pH and drug content was observed.

Percentage drug release of hydrogel containing ethosomes (FE1-FE8) was observed at 360 min 78.31%, 66.31%, 83.23%, 87.83%, 79.22%, 75.63%, 82.33% and 90.53% respectively. It was observed that addition of ethanol in formulation increase the release by increasing permeation properties of ethosomes. The ethosome (formulation FE8) showed maximum drug release as compared to others formulation. The formulated gels were subjected to stability studies. No color fading was observed for all prepared gels. The pH of all formulations remained unchanged and was found to be within the range of 6.8-7.3. The viscosity and spreadability of all gels were also unaltered and found to be within the range. The drug content was found to be in the limit 70.21% to 77.52 %, for all gel formulation at all temperature conditions. Stability studies revealed that all optimized formulations containing Azadirachta indica extract are stable and can be explored as a marketed product.

Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Herbal formulations have growing demand in the world market. In the undertaken study an attempt has been made to establish that herbal gel containing Neem extract has promising anti-aging action. All the studies performed provides a that novel topical ethosomal gel containing neem extract that can be used as an alternative remedy for management and treatment of anti aging related disorder and disease.

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