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Original Article

FORMULATION AND EVALUATION OF MICROEMULSION CONTAINING NEEM SEED OIL

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ABSTRACT

Objective: The objective of the present study was to formulate Microemulsion containing seed oil. Neem seed (Azadirachtaindica) oil was extracted from its seeds by the soxhlet apparatus. Acetone is used as a solvent. PEG 400 and Carbopol 940p was select as surfactant, co-surfactant and hydrogel thickening agent. Microemulsions were characterized for pH, viscosity, spreadability, *in vitro* drug transport study and *in vivo* antibacterial activity and shows satisfactory results. Antibacterial activity of formulation against E. coli Shows at a concentration of 3%. The neem seed oil microemulsion has the potential for antibacterial activity.

Methods: A ratio of surfactant and cosurfactant i.e, S/CoSchoosen and corresponding mixture was made. The mixture was mixed with oil. Each mixture was mixed thoroughly using magnetic stirrer until homogenous dispersion/solution was obtained. Double distilled water was used in this formulations as to prevent the incorporation of surface active impurities. The mixture was titrated with water and ambient temperature with constant stirring at the endpoint where the mixture become clouded, the quantity of aquous phase added. The percentage of three different pseudo-phases incorporated were calculated.

Results: Solubility studies in various solvents reveals that the oil is insoluble in distilled water and ethanol. Soluble in methanol.

Conclusion: It was observed that the microemulsion having multilamellar nature. Batches with carbopol shows better homogenous distribution. The stability of microemulsion prepared with carbopol 71 was gretter than with xanthan gum. The *in vitro* study of microemulsion was performed and Batch (F7) is optimized batch which shows highest drug release.

Keywords: Neem seed oil, Microemulsion, Topical application, Antibacterial activity

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INTRODUCTION

Microemulsions are thermodynamically stable isotropically clear dispersion of two immiscible liquids, like oil and water, stabilized by an interfacial film of surfactant molecules, with a size range of 5-200 nm and have very low interfacial tension [1]. Microemulsions could be an alternative carrier in topical drug delivery and as it has high Solubilization capability and nanometer size, it is believed that microemulsion will be a better candidate in delivering drug topically. They are composed of surfactant, water, and oil having co-surfactants provide better therapeutic action when compared to the traditional cream and lotions [2].

It is a topical delivery in skin, which makes the drug delivery difficult. This factor is consideration of preparation of micro-emulsions which have low skin irritation, high drug loading capacity, It reduce the diffusion barrier of corneum by dissolving the lipids in the Stratum corneum and enhancing the permeation of drug [3].

Traditionally neem seed oil and leaves used as insect repellent and as pesticides. Neem oil containing Azadirachatin could be used in hair care formulation due to their antiheadche, antidandruff, and antifungal properties [4]. Almost all parts of neem have been used as traditional Ayurvedic, in unani, and siddha medicine in india. Neem oil is used to control various skin infections [5].

The aim of the present study is to formulate topical microemulsion using Neem seed oil, tween 80, PEG 400, xanthan gum, carbopol 940. Tween 80 is a non-ionic, non-toxic surfactant that is not affected by change in pH. The antibacterial efficacy of the formulated microemulsion is additionally investigated.

MATERIALS AND METHODS

Table 1: List of chemicals

S. No.	Chemical	Manufacturer/Provier
1	Neem seed oil	Pradip aggrotech pvt. ltd
2	Tween 80	Lab grade
3	Propylene Glycol 400	Lab grade
4	Carbopol 71	Lab grade
5	Xanthan gum	Lab grade
6	Ethanol	Lab grade

Extraction of neem seed oil [6]

Neem seeds were washed three times thoroughly till no dust or other impurities left. Neem seeds were heated in a temp of 50 °C for one hour so they would dry out so kernels can be separated from wooden parts of seeds. The kernels were grounded using grinder. Soxhlet apparatus used for the extraction of neem seed oil. The kernel powder was placed into the soxhlet apparatus. Acetone is used as solvent in the ratio of 1:5 (W/V). The solvent was hated at 50 °C for eight hours so no oil left in the neem kernel. Then the

mixture of oil and solvent was kept in room temperature for 48 h so the solvent evaporates completely and eventually oil is left.

Analysis and characterisation of extracted oil [7]

Determination of specific gravity

The specific gravity of the oil was determined using specific gravity bottle.

Determination of refractive index

This is physical attribute of triglycerine measures by the angle through which a beam of light is bent when passing through a thin film of melted fat few drops of oil were placed on one face of glass prism of a refractometer and it was gently spread, closed and tightened. Ample time is allowed for the prism and oil to achieve steady temperature. The refractive index was then read from demarcation line.

Determination of pH

The pH of oil was determined by using digital pH meter.

Preparation of standard calibration curve of neem oil [8]

Different concentrations of Neem oil were prepared with different solvents and scanned in the UV range. The wavelength 220 nm was selected for neem oil, where it show maximum absorbance. Lower concentrations does not show any absorbance, hence concentration (above 10 mcg) were taken for calibration. The calibration curves were linear over the concentration range of 30-70 ug/ml of neem oil. Absorbance versus concentration plotted

Formulation of microemulsion [6]

A ratio of surfactant and cosurfactanti. e,S/CoSchoosen and corresponding mixture was made. The mixture was mixed with oil. Each mixture was mixed thoroughly using magnetic stirrer until homogenous dispersion/solution was obtained. Double distilled water was used in this formulations as to prevent the incorporation of surface active impurities. The mixture was titrated with water and ambient temperature with constant stirring. At the end point where the mixture become clouded, the quantity of aquous phase added. The percentage of three different pseudo-phases incorporated were calculated. Same procedure was followed for all S/Co ratios. Phase diagrams were prepared after calculating the percentage of each phase required to form microemulsions. After preparing the pseudo ternary phse diagram the medicated microemulsions were formulated.

Evaluation of microemulsion [9]

Physical appearance

Prepared microemuisions were observed for color, homogeneity, consistency, texture, and pH.

Spreadability measurement

The spreadability of the microemulsion was determined by placing 0.5 g micoemulsion within a pre-marked circle of 1 cm diameter on a glass plate over which a second glass plate was placed. A weight of 5 g was allowed to rest on the upper glass plate for five min. The increase in the diameter due to spreading of the microemulsion was noted, and the mean diameter was taken.

Globule size determination

The globule size of the microemulsion was determined by optical microscope.

Viscosity

It was determined at 25±1 °C by means of Brookfield viscometer.

***In vitro* skin permeation [10]**

In vitro skin permeation study was performed by using Franz diffusion cells with an effective diffusion area of 2.8 cm^2. The excised skin samples of rat were clamped in between donar and receptor compartment of Franz diffusion cell.1g of sample was added to the donar compartment. The receptor compartment was filled with PBS pH 7.4 and maintained at 37 °c with stirring at 100 rpm. At predetermined time of interval 3 ml of sample was withdraw and fresh same quantity of sample was added. Samples were filtered through whatman filter paper and analysed by UV spectrophotometer at 220 nm. The amount of drug permeted per unit surface area (µg/cm^2) was plotted versus time (h) and the flux (µg/cm^2) was calculated from slope of the line.

Antimicrobial study [11]

Antimicrobial test was performed on previously prepared agar plates. On which *E. coli* saline solution was spread with the help of glass spreader. Wells were prepared with the help of borer, samples were withdrawn with the help of micropipette (100ug/ml) and poured into the prepaped wells. Then plates were incubated at 37 °c for 24h. And zone of inhibition was measured with the help of scale after 24h.

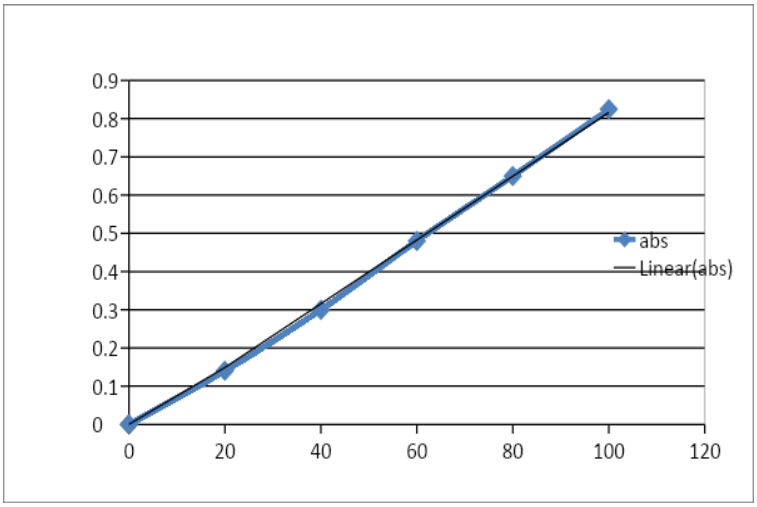
RESULTS AND DISCUSSION

Determination of solubility study

Solubility studies in various solvents reveals that the oil is insoluble in distilled water and ethanol. Soluble in methanol.

Calibration curve for neem oil

Calibration curve for Neem seed oil by UV spectrophotometer. The equation found was Y=mx+c and R^2 value was 0.9985. The concentration of drug was in the range of 30-70µg/ml.



FTIR of neem seed oil

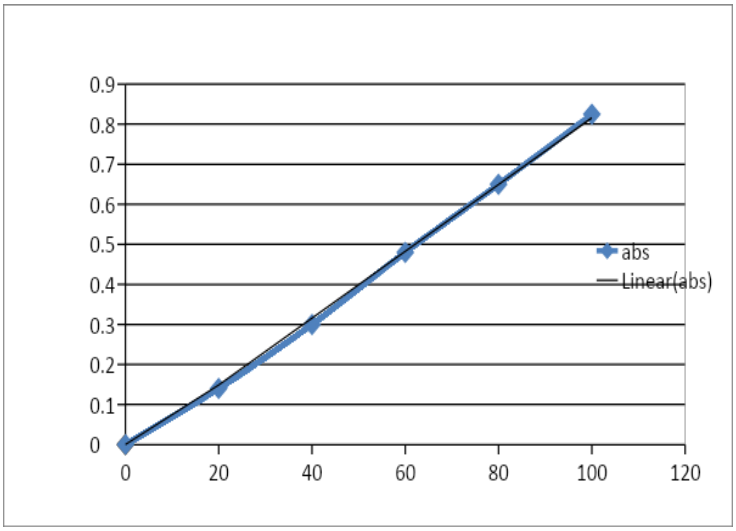


Fig. 1: FTIR of neem seed oil

Table 2: Neem seed oil evaluation

Property	Experimental value	Unit
Refractive index	1.2564	ml/g
pH	5.4	
Specific gravity	0.931	

Table 3: Formulation of neem seed oil microemulsion

Batches	Stirring time (1000 rpm)	Oil	Tween 80	PEG 400	Xanthan gum	Carbopol 71	Water upto 100 ml
F1	30 min	10 %	20%	5%	0.3%	-	100
F2	40 min	10 %	20%	5%	0.3%	-	100
F3	50 min	10 %	30%	5%	0.7%	-	100
F4	60 min	10 %	30%	5%	0.9%	-	100
F5	30 min	10 %	40%	5%	-	0.3%	100
F6	40 min	10 %	50%	5%	-	0.3%	100
F7	50 min	10 %	50%	5%	-	0.7%	100
F8	60 min	10 %	60%	5%	-	0.9%	100

Table 4: Apperence and transparency

Batches	Appearance and transparency
F1	Cloudy
F2	Cloudy
F3	Clear
F4	Clear
F8	Clear And Transparent
F6	Clear And Transparent
F7	Clear And Transparent
F8	Clear And Transparent

Table 5: Globule size determination

Characters	Globule size (nm)		
Batches	**F5**	**F6**	**F7**
X_{10}	388.54	416.15	42.29
X_{50}	434.51	476.31	48.33
X_{90}	497.66	574.24	58.05
X_{16}	392.01	424.75	41.09
X_{84}	486.73	554.01	57.52
X_{99}	544.74	654.11	62.94
SMD	438.02	487.21	44.78
VMD	431.15	491.78	43.92
Sv	12.84m^2/cm^2	13.49m^2/cm^2	124. m^2/cm^2
Polydispersity index	44.33	32.21	24.21

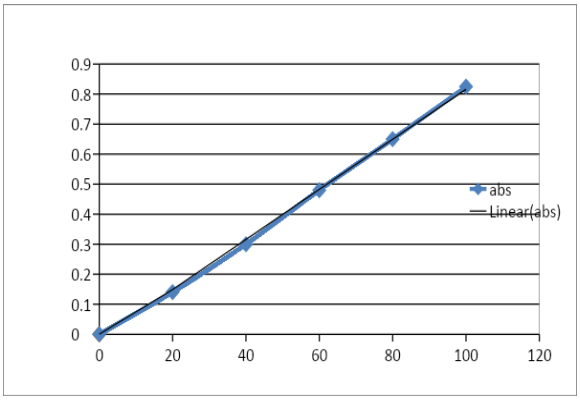


Fig. 2: Globule size determination of batch F5

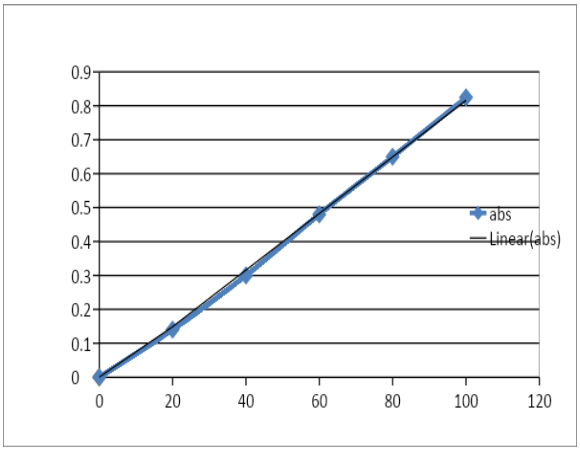


Fig. 3: Globule size determination of batch F6

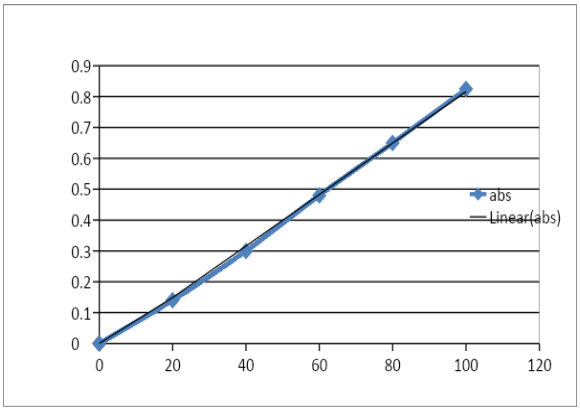


Fig. 4: Globule size determination of batch F7

Table 6: Measurement of pH

Batches	pH
F1	6.3
F2	7.1
F3	7.7
F4	7.3
F8	7
F6	6.8
F7	7.4
F8	7.1

Table 7: Refractive index

Batches	Refractive index
F1	1.4440±0.001
F2	1.4556±0.002
F3	1.4215±0.003
F4	1.4362±0.007
F8	1.4960±0.003
F6	1.4645±0.004
F7	1.4645±0.008
F8	1.4916±0.001

Table 8: Viscosity

Batches	Viscosity cp (spindle speed 0.3) RPM spindle no 63
F1	9897
F2	10119
F3	11325
F4	11444
F8	11550
F6	11230
F7	12690
F8	11856

Table 9: Spredability

Batches	Spredability gm. cm/sec
F1	2.8
F2	2.4
F3	2.9
F4	3.1
F8	3.8
F6	3.41
F7	3.9
F8	3.2

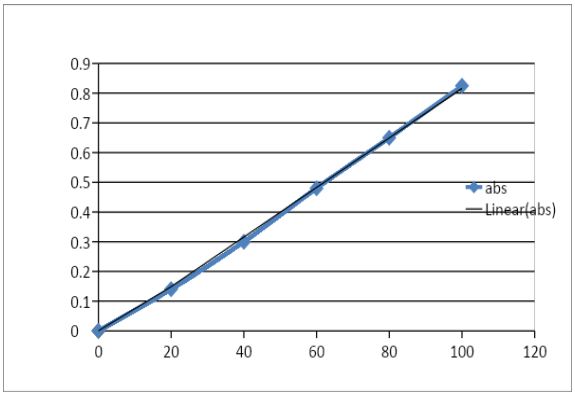


Fig. 5: Antimicrobial activity of neem seed oil 1. Marketed formulation, 2. Neem seed oil microemulsiom, 3. Placebo

Cumulative percent drug release of batch F1-F8

Antimicrobial activity of microemulsion

Antimicrobial activity of oil was performed on *E. coli.* The inhibitory action was showed at concentration of 3%. The seed oil at concentration of 0.3% on agar plate was found to be active against *Staphylococcus Aureus* and at 0.4% was active against *Salmonella typhosa*. The Neem seed oil was found to be inactive against *Pseudomonas aeruginosa*.

The bacteria (*E. coli*) was tested against marketed formulation, as a placebo and with oil.

CONCLUSION

Neem seed oil microemulsion was prepared in order to enhance skin penetration. Microemulsion were prepared by phase titration method using tween 80 surfactant or PEG 400 as cosurfactant and thickening agent. Neem seed oil as antibacterial agent. It was observed that the microemulsion having multilamellar nature. Batches with carbopol shows better homogenous distribution. It is also depends on stirring time. Batches with carbopol 71 having better viscosity than batches with xantham gum. The stability of microemulsion prepared with carbopol 71 was gretter than with xanthan gum. The *in vitro* study of microemulsion was performed and Batch (F7) is optimized batch which shows highest drug release. The formulation tested against bacteria (E. coli) and have antimicrobial activity. The bacteria tested against marketed formulation, neem seed oil microemulsion, and as a placebo. Highest zone of inhibition was observed by Neem seed oil microemulsion.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declare none

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