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Nanoemulgel using Neomycin: Nanoemulgel Formulation and Evaluation

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ABSTRACT: Based on the research, it was determined that a stable neomycin nanoemulsion formulation could be created when co-surfactants, surfactants, and oils were optimized. Twelve distinct formulations were made.
The spontaneous emulsification method, which uses the concentrations of oil (5% neem oil), surfactant (36% tween 80), and co-surfactant (9% ethanol), produced the best results based on the various concentrations of all the components of co-surfactants, surfactants, and oils.

Keywords— Nanoemulgel, Neomycin, Emulsification, Surfactants, Co-surfactants.

I. INTRODUCTION

The efficacy of topical therapies in eradicating fungal infection, however, is more limited because the use of the stratum corneum serves as the skin shield resulting in longer treatment time and poor compliance of the patient when used as a typical form of gel and emulsion [1,2].

Nanoemulsion is one of the most effective droplet size distributed nano-systems with a submicron size spectrum. The Nanoemulsion device, characterized by high stability, is usually transparent or semi-transparent. The scale of submicron droplets and the high concentration of surfactants make it an important medium for tropical distribution. Study results suggest that nanoemulsion is much more powerful than other systems in terms of drug delivery [3-5].

Thermodynamically safe clear nanoemulsions / ultrafine emulsions / sub-micron emulsions stabilized by an interfacial surface of surfactants & surfactants molecules with droplet size smaller than a hundred nm [6].

Its durability and clarity are usually described as classified as multiphase. Tiny grains or raindrops in the dispersion method, very small oil surface tension is commonly used. Nanoemulsion is readily and frequently formed naturally usually without either high input because the droplet is smaller than a quarter of a millimeter in diameter. In the same circumstances, a co-surfactant or co-solvents is used in addition to the surfactants, oil process, & water volume. Based on the makeup three types of nanoemulsions are most likely to form [7-9]. Nanoemulsions of water oil in which oil droplets are scattered during the continuous aqueous phase. An acceptable mixture of co-surfactants and surfactants stabilizes the interface. The primary difference between emulsifiers and Nanoemulsions is that the former are thermodynamically unstable and ultimately exhibit phase separation, while they can show excellent kinetic stability. Their color is another important distinction; emulsifiers are nanoemulsions that are transparent or translucent [10-12].

The nanoemulsion formulations method is divided into two strategies: highly energetic & low energy. Devices like high-pressure homogenizer, microfluidizer, and sonicator are examples of high-energy formulation, while low-energy formulations relies on the dispersion of the bioactive substance to add a tiny droplet of nanoemulsion highly effective. The formation of nanoemulsions by low-energy means is required for the spontaneous generation of emulsifiers relying on the phase's action of particular surfactant, oil, and water infrastructure [13-14].

There's really increased attention in utilizing relatively low strategies in the emulsified forming procedure due to economic advantages and growing quantities of research into the efficacy of various low-energy methods. Self-emulsifying systems are an approach for improving the solubility of compounds (drugs and oils) that are not easily dissolved in water [15]. The goal of our research was to come up with a thermally robust nanoemulsion.

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II. METHODOLOGY

A. Material Required

Neomycin (Hi media), neem oil, , Indonesia) Tween 80, Tween 20, ethanol, butyl hydroxy toluene (Merk Germany), methanol, isopropyl myristate (IPM).

B. Preformulation studies

Preformlation is a phase of the design phase in which researchers characterise the chemical, physical, and mechanical characteristics of the drug material in order to create a dosage form that is effective, durable, and secure. As a result, pre-formulation experiments are required to describe the medicine so that the delivery of drugs can be properly designed [16].

1. Description:

The drug's organoleptic properties were evaluated and documented utilizing illustrative terminology [17].

2. Melting point:

Volumetric flask of one end closed is filled with enough dry powder to produce a 0.25cm long to 0.35cm column at the end of the hole, that is tightly compacted as tightly as feasible by gentle hammering on a solid surface. The device is operated in accordance with industry best practices. The block is warmed until it reaches a temperature that is approximately 30 degrees Celsius below the projected melting point. The capillary is put into the hot plate, and the temp is raised at a rate of around 1 to 2 degrees Celsius per minute till the melting is finished [18]. The start of melt is classified according to the temp at that the sensor signal initially leaves its initial value, as well as the end of melt, or the melting point, is described as the temp at that the sensor signal achieves its final value. Both of these temperatures are inside the melting range.

3. Solubility Studies

An excess volume of medication (± 250 mg) in 5 ml of selected neem oils, co surfactant (polyethylene glycol 400, tween 20, glycerin, tween 80and ethanol), and surfactants, fixed by the vortex mixer. The combination is blended for 24 hours in a shaker. From the saturated oil procedure, a specimen of Neomycin was obtained and centrifuged for 10 min minutes @ 3000 rpm. The centrifuges sample effluent sheet acts as a solvent of the medications in oils, surfactants, and co-surfactants. 1 ml of the residual layer was reduced to 10 ml in methanol. The quantity of Neomycin then was determined utilizing a UVspectrophotometer at 304 nm [19].

4. UV – Spectroscopy

Sample were subjected to a UV spectroscopic test to determine their authenticity. Using the dilution procedure, it was dissolved in ethanol in a 10 ml volumetric flask and diluted up to 10 times. UV spectroscopy was used to establish the maximum concentration and the standard curve in ethanol was also displayed [20].

C. Optimization of co-surfactants, oils, and surfactants

Optimization of the concentration of surfactants, oils, and co-surfactants was carried out by using the various types of ratios. The ratio of co-surfactant mixture surfactant (smix) varies from 1:4, 1:3, 1:2, and 1:1.

The ratio of the smix and neem concentration is 5:1, 4:2, 3:3, 2:4, and 1:5. Aqua distilled water is added by performing the titration and then stirred by using a magnetic stirrer till it converts to translucent and has no phase separation [21].

D. Nanoemulsion formulation

Formulation of nanoemulsion was carried out by using the method known as spontaneous emulsification method. The Neomycin was added to the oil phase consisting of standardized butylated hydroxytoluene, further the smix solution was added to it, which is a mixture of co-surfactant and surfactant, then allowed for stirring with the magnetic stirrer until homogeneous mixture prepared. Then the Aqua dart (aqua distilled water) is added by using the mode of titration and then left for stirring condition until the nanoemulsion will be formed which is noticeable by the development of a translucent solution [22].

E. Nanoemulsion evaluation

The evaluation of the nanoemulsion after formation was carried out by following the various criteria [23]:

Organoleptic test: Observation was carried out on the basis of the changes that appeared on odor, clarity, color, and phases parathion.

Value of pH: pH of a nanoemulsion is a critical criterion for evaluation. The excipients included in the formulation dictate the pH of the final deployment and thus the administration route. The

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pH of the formulation was determined to use a wireless pH meter.
The measurements were collected three times to reduce the chance of error.
Viscosity: The nanoemulsion viscosity was measured at room temperature (23°C±2°C) using the Brookfield viscometer. Two spindle speeds were used for viscosity tests and studies were performed three time.

F. *Type of Nanoemusion*

The type of emulsions was determined by decomposing the emulsion in methylene blue. Methylene blue granules are located on the surface of the liquid in the oil is a form of preparation.

G. *Size of particle:*

Using the particle size analyzer (PSA) SZ-100, particle size are calculated [24].

H. *FTIR analysis*

The responsive units in a molecule are identified using FTIR. The drug was examined at 4mm/s with a precision of 2cm over a wave number range of 400 to 4000cm^{-1} on a KBr disc. The typical peaks were captured on film [25].

III. RESULT & DISCRIPTION

A. *Pre-formulation studies description*

Mixing is carried out systematically and observed so that no further tests are chosenfor the inconsistent formulations that have blurry and phase separation. The goal of the optimization is to obtain the best formula from each point in the formulation.

The best evaluation of ethanol and tween 80 (Smix) is 1:4, which will be used to create the formula for nanoemulsion. The mixture and oil ratio is 9:1as seen in Table 3, as the neomycin nanoemulsion is formulated. The excipient concentration of the formula nanoemulsions F1, F2, F3, F4, and F5 was unable to stabilize the preparation since it was already cloudy and precipitating after stirring.

Formula F6 and F7 were originally stable and smooth, but the solution became mildly cloudy and precipitated after waiting for some time because the concentration of the excipient did not maintain the nanoemulsion.

B. *Physiochemical properties of drug:*

The melting point of drug is 380 ±0.25℃. The physical form the neomycin at 20℃ -25℃ was whitish in colour and then changed to off white to yellowish powder.

C. *Study solubility of Neomycin:*

Table 4 demonstrates the solubility of neomycin in surfactant, oil, and co- surfactant. The best solubility of neomycin from the findings of Table is Tween 80 (surfactant), neem oil (oil), and ethanol (co-surfactant).

Table 1: Studies in the solubility of neomycin

S No.	Excipients	Solubility (mg / ml)
1.	Tween 80	Dissolve in Moderate Amounts
2.	Tween 20	Soluble
3.	Neem oil	Dissolve in moderate amount
4.	Coconut oil	Soluble
5.	Castor oil	Soluble
6.	Propylene Glycol	Soluble
7.	Ethanol	Dissolve in Moderate Amounts

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D. ***λmax determination of Neomycin:***

In this experiment, the neomycin was scanned in the 300 nm – 700 nm range. The maximum absorbance value was obtained at 420 nm (figure 1). Further the standard curve of neomycin was prepared as shown in figure 2.

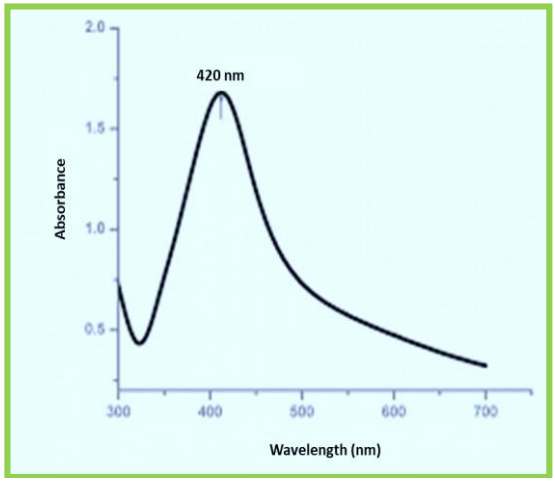

Figure 1: λmax of neomycin.

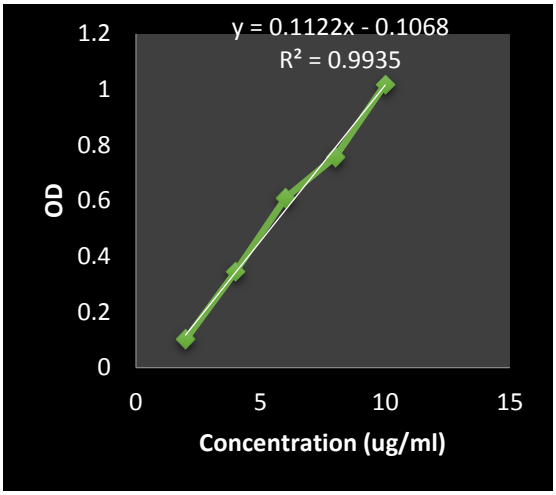

Figure 2: Standard curve of neomycin

E. ***Study of components of neomycin by FTIR analysis:***

The IR spectrum analysis for the neomycin required for the formulation, combined with the report spectrum showing the drug purity.

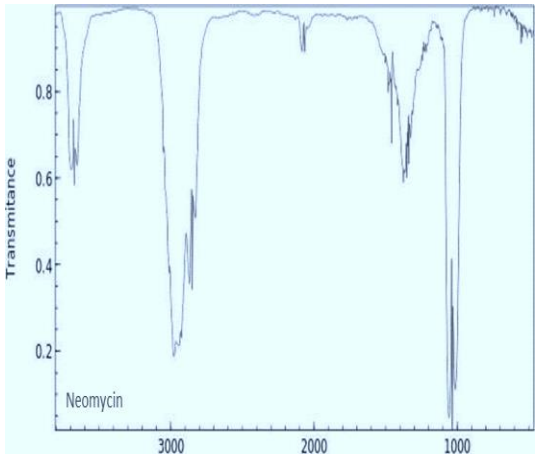

Figure 3: FTIR spectrum of pure drug Neomycin

According to the FTIR spectrum assessment data, neomycin nitrate has its peakscorresponding at 3150.70-3220.11 (O-H), 3678.49 (O-H stretch), 1276.59 - 1240.48 (C-O),2700.81 (C-H), and 954.18 (C-O) (C-Cl).

Individual components including neem oil, methanol, tween, and neomycin with tween have displayed the peaks in Figures 6.1 to 6.9. The absorption peaks of the antifungal medication in conjunction with various polymers include 3422.08 (O- H), 2359.79 (C=C), 2918.12 (C-H), 1654.11 (C=O), 1078.55 (C=O). The absorption band of Neomycin nitrate remains unchanged and indicating that there is no significant chemical reaction between Neomycin nitrate and the polymers utilized in the production of niosomal gels, based on the above findings.

F. ***Evaluation of nanoemulsion:***

The findings of the nanoemulsion evaluation during the first week were shown inTable.

Organoleptic:

Formulas F4, and F5 are pale, less viscous, transparent, in appearance and have no phase distinction. This means that theneem oil (oil), tween 80(surfactant), and ethanol 96 percent (co-surfactant) concentrations used are sufficient. The formulation quickly dissolves and leaves a subtly oily nuance when added to the skin.

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pH:

In the pH spectrum of the skin (4.5 – 6.5) which is the desired pH of the formulation. pH must not be too acidic so that it may irritate the skin because it can cause scaly skin, similarly, it should not be too alkaline [14]. All formulations produced have apH that is still within the skin's pH spectrum.

Viscosity:

A Brookfield viscometer was used to determine the fluidity or viscosity. This emulsion comes in five different viscosity formulas. The content of twenty-eight as surfactants would likewise increase in order to impact its fluidity, Graph depending on the intended consequences of the rise in the oil content in the formulation.

The globule's size will shrink as the quantity of 80 rises. The globule's smaller sizeraises the number of particles, which is proportional to the system's effort to maintain a constant density. The viscosity of globule contact could be improved byraising the number of tiny globules. [15, 16].

Table 2: Observation of combined ethanol and tween 80 (Smix) comparison with neem oil

S no.	Smix :oil	Observations of ethanol and tween 80 mixtures (Smix)			
		1:1	**2:1**	**3:1**	4:1
1.	5:1	++	++	+++	++
2.	4:2	+++	++	+++	++
3.	3:3	++	+	+	+
4.	2:4	+++	+	++	+
5.	**1:5**	+	+	++	+

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Table 3: Optimization of drug-loaded nanoemulsion

Formulation	Composition (%)			
	Neomycin (g)	Neem oil (ml)	Ethanol (ml)	Tween 80 (ml)
F1	2.5	5	5	**2.5**
F2	3	4	5	**5**
F3	25	6	5	**2.5**
F4	1	2	5	**5**
F5	1	7	5	**2.5**
F6	1.5	5	5	**5**
F7	1.5	4	5	**2.5**
F8	2	6	5	**5**
F9	2.5	2	5	**2.5**
F10	1	7	5	**5**
F11	1	5	5	**2.5**
F12	**1**	**4**	**5**	**5**

Table 4: Evaluation of best 5 formulation of nanoemulsion in the 1stweek

Formula	Colour	Odor	Clarity	pH	Viscosity
F1	Soft Pale	Specifics	Less transparent	5.7	318.25
F2	Soft yellow	Specific	Translucent	6.9	326.05
F3	Soft yellow	Specific	Translucent	5.95	365.24
F4	Soft yellow	Specific	Translucent	6.54	369.20
F5	Soft yellow	Specific	Translucent	6.18	389.35

G. ***Nanoemulsion type:***

Both formulations involve oil-type nanoemulsion in water (o / w). This is due to the fact that the majority of the ingredients in the mix are hydrophilic or polar, so the nanoemulsion style stays oil in water (o / w) while there is a hydrophobic component.

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H. ***Particle size analysis:***

In the 1st week, calculations were made using PSA. The findings of the particle calculation demonstrate that each formula has a variable scale, which is due to the complexity of homogenizing two distinct processes, the formula is not homogeneous in addition to some other variables such as length or intensity of stirring.

As most of the 80 is undergoing hydrolysis, the particle size increases from the first week of the eight, thus reducing the efficacy of the nanoemulsion interface film layer of the globule [17]. During storage, temperature modification can induce a decrease in the surfactants efficiency such that oil droplets begin to close together and gradually create larger droplets. The distribution of globule size is an essential aspect of the nanoemulsion system as it can influence the release of the drug and the stabilization of the preparation [18]. The polydispersity index (PDI) is a measurement of the emulsion's particles size variation, with a PDI near zero indicating mono dispersal and a PDI near 1.0 indicating a relatively large size and distribution. Thus, PDI values less than 0.2 imply homogenous communities, whereas a value of 0.3 shows heterogeneity.

The high tolerance of two immiscible materials to remain combined as a single phase emulsion is the attractive characteristic of formulation. Based on the sample form [19], the appropriate PDI value should be < 0.7. The findings of this analysis show the PDI value is > 0.7 for the F* & F9 formula, indicating that the 2 formula have a large range of particle size and have an unstable formulas.

The PDI values of the F10 - F12 formula is less than 0.7, hence it falls into the polydispersion rank while remaining in the secure formula category. The globule size distribution calculation effects of the nanoemulsion formula using PSA are shown in Table.

Table 5: The nanoemulsion formula's particle size & PDI value

S no.	Formulation	Particle size (nm)	Intensity	PDI
	F4	43.09	0.90	**1.15**
		255.50	0.06	
	F5	2.98	0.05	**0.71**
		15.90	0.65	
		59.01	**0.36**	

Thermodynamically safe clear nanoemulsions/ultrafine emulsions / sub-micron emulsions stabilized by an inter - facial surface of surfactants & surfactants molecules with droplet sizes smaller than a hundred nm.

Its durability and clarity are usually described as classified as multiphase. Tiny grains or raindrops In the dispersion method, very small oil surface tension is commonly used. Nanoemulsion is readily and frequently formed naturally usually without either high input because the droplet is smaller thana quarter of a millimeter in diameter

An acceptable mixture of co-surfactants and surfactants stabilizes the interface. The primary difference between emulsifiers and Nanoemulsions is that the former are thermodynamically unstable and ultimately exhibit phase separation, while they can show excellent kinetic stability. Their color is another important distinction; emulsifiers are nanoemulsions that are transparent or translucent. Because emulsions need a high energy input,

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whereas nanoemulsions are described the below table.

IV. CONCLUSION

On th basis of the work, it was concluded that the after optimization of the co-surfactants and surfactants, and oils to prepare a constant neomycin nanoemulsion formulation. We prepared 12 different types of formulation.

On the basis of the different concentrations of all the components of co- surfactants and surfactants, and oils, best results were obtained by using the spontaneous emulsification method which is the concentration of oil 5% (neem oil), 36% surfactant concentrations (tween 80) and co-surfactant (ethanol) 9%.

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