FORMULATION & DEVELOPMENT AND *IN VITRO* ASSESSMENT OF NEOMYCIN NANOEMULSION AS ANTI-MICROBIAL AGENT

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ABSTRACT

The aim of this research was to formulate, develop, and conduct an in vitro assessment of Neomycin nanoemulsion as an antimicrobial agent. Two formulations using Carbopol 934 and Carbopol 940 polymers were prepared and evaluated. The pH values for the two formulations were found to be compatible with skin pH, with mean pH values of 6.8 ± 0.05 and 7.1 ± 0.10 for Carbopol 934 and 940, respectively. The viscosity and spreadability of both formulations were measured, with the mean viscosity values of 51 ± 1.00 cP and 46 ± 1.41 cP, and mean spreadability of 8.5 ± 0.20 cm and 9.0 ± 0.10 cm for Carbopol 934 and 940, respectively. The mean particle size was within the nanometer range, with 122 ± 2.08 nm for Carbopol 934 and 130 ± 2.08 nm for Carbopol 940. The zeta potential values for both formulations were negative, indicating stability. In vitro drug release studies revealed a sustained release profile for both formulations. Overall, this research provides valuable insights into the development of effective antimicrobial nanoemulsions, showcasing their potential in the field of topical antimicrobial therapy.

KEYWORDS- Neomycin Nanoemulsion, Nanoemulsion, Anti-microbial agent

INTRODUCTION

Neomycin is an aminoglycoside antibiotic that plays a vital role in combating a broad spectrum of bacterial infections [1]. The drug is highly effective against Gram-positive and Gram-negative bacteria and is widely used in clinical practices to prevent bacterial infection in wounds or minor cuts, and also in pre-operative prophylaxis. However, despite its effectiveness, the clinical application of Neomycin is often limited due to its poor solubility and associated systemic side effects such as ototoxicity and nephrotoxicity [2].

To enhance the solubility and bioavailability of Neomycin, a novel drug delivery system is being explored in this study, leveraging the advantages of nanotechnology. Nanoemulsions are fine oil-in-water dispersions, where the size of oil droplets is in the nano-range (20-200 nm) [3]. This nanoscale size results in a significantly higher surface area, which can enhance the solubility of poorly soluble drugs like Neomycin and improve their bioavailability. Furthermore, nanoemulsions have shown promising results in reducing the side effects associated with systemic administration of certain drugs [4].

This research aims to formulate and develop a Neomycin nanoemulsion as an antimicrobial agent. This novel approach could open up new avenues to enhance the therapeutic efficacy of Neomycin while minimizing its side effects [5]. Additionally, it is important to note that nanoemulsions, due to their small size, may provide a prolonged and controlled release of the drug, thus reducing the frequency of administration and improving patient compliance [6].

In this study, two different types of polymers are used in the formulation process, namely Carbopol 934 and Carbopol 940. These polymers have been chosen due to their excellent gel-forming ability, biocompatibility, non-toxic nature, and ability to provide a sustained release of the drug. Additionally, Carbopol polymers are known for their high viscosity and yield value, which makes them ideal for topical application [7].

In this context, we aim to prepare and characterize Neomycin nanoemulsions using these two polymers. This process involves careful measurement and mixing of all constituents followed by a thorough assessment of the prepared formulations based on various physicochemical parameters such as pH, viscosity, spreadability, zeta potential, and particle size. In vitro drug

release profiles will also be examined to evaluate the efficacy of the formulated nanoemulsions in providing a sustained release of Neomycin [8].

Through this study, we hope to pave the way for an improved drug delivery system that not only enhances the therapeutic effectiveness of Neomycin but also minimizes the associated systemic side effects. This research could therefore potentially revolutionize the way we treat bacterial infections, by providing a more effective and safer alternative to traditional antibiotic therapies [9].

METHODOLOGY

This research was conducted with a systematic and meticulous approach to ensure accurate results and insights. Here, we present the step-by-step methodology we employed to formulate, develop, and assess the Neomycin nanoemulsions.

Selection of Components [9]

The selection of the constituents of the nanoemulsion was based on their properties and potential to facilitate the formation of a stable nanoemulsion. We used Carbopol 934 and Carbopol 940 as the polymer base for the formulation due to their gel-forming properties and ability to provide a sustained release of the drug. Neomycin, our active pharmaceutical ingredient (API), was chosen for its broad-spectrum antimicrobial activity. The oil phase, surfactant, and co-surfactant were chosen considering their role in enhancing drug solubility and stabilizing the nanoemulsion.

Preparation of Nanoemulsion [10]

The nanoemulsion was prepared using the titration method. Initially, Carbopol was dispersed in purified water, followed by the slow addition of a mixture of oil, surfactant, co-surfactant, MP, and PP under constant stirring until a transparent nanoemulsion system was formed.

UV Spectroscopy Analysis [11]

The absorbance of Neomycin was determined at various wavelengths, with a maximum absorbance (λmax) at 260 nm using a UV spectrophotometer. This helped in identifying the ideal wavelength for further analysis.

Determination of pH [12]

The pH of each formulation was measured using a calibrated pH meter. An average of three readings was taken to minimize any errors and ensure accuracy in the measurements.

Viscosity Measurement [13]

The viscosity of the prepared formulations was determined using a viscometer. This step is crucial as the viscosity of the formulation can affect the drug release rate.

Assessment of Spreadability [14]

Spreadability is a critical property for any topical formulation as it affects the ease of application. It was assessed by measuring the diameter of the spread of the formulation under standard conditions.

Zeta Potential and Particle Size Analysis [15]

The stability of the nanoemulsion was evaluated by measuring the zeta potential and particle size. The nanoemulsions were diluted, and measurements were performed using a Zetasizer. This analysis is vital as the stability, solubility enhancement, and drug release rate of the nanoemulsion are dependent on these parameters.

In vitro Drug Release Study [16]

Finally, the drug release profile was studied using a dialysis bag diffusion technique. The cumulative percentage of Neomycin released from the nanoemulsions was calculated at regular time intervals.

The systematic approach to formulating and assessing the Neomycin nanoemulsions has yielded insightful results, elucidating the potential of nanoemulsions as a novel drug delivery system for antimicrobial agents. Through meticulous analysis and interpretation of data, we have ensured the accuracy and reliability of our research findings.

RESULTS

Our comprehensive and methodical experimentation provided us with significant results in the development and in vitro assessment of Neomycin nanoemulsions.

Formulation of Nanoemulsion

Two formulations were successfully prepared, with Carbopol 934 and Carbopol 940 as the polymer base. We adjusted the composition of oil phase, surfactant, and co-surfactant in each formulation, which led to slightly different characteristics between the two. Carbopol 934 and 940 were chosen due to their ideal gel-forming properties and ability to provide a controlled and sustained drug release.

Table 1: Formulae of Nanoemulsion

Formulation	Polymer	Carbopol (%)	Oil Phase (%)	Surfactant (%)	Co-surfactant (%)	MP (%)	PP (%)
Formulation 1	Carbopol 934	0.5	5	10	2	0.2	0.1
Formulation 2	Carbopol 940	1	7.5	12	2.5	0.3	0.2

UV Spectroscopy Analysis

The UV spectroscopic analysis revealed that the Neomycin in the nanoemulsion had a maximum absorbance (λmax) at 260 nm. This peak absorbance, together with the absorbance at other wavelengths, confirmed the presence of Neomycin in the nanoemulsions and allowed us to estimate the concentration of the drug in the solution.

Table- 2: λmax of Neomycin

Wavelength (nm)	Absorbance
220	0.234
240	0.432
260 (λmax)	0.694
280	0.567

300	0.398

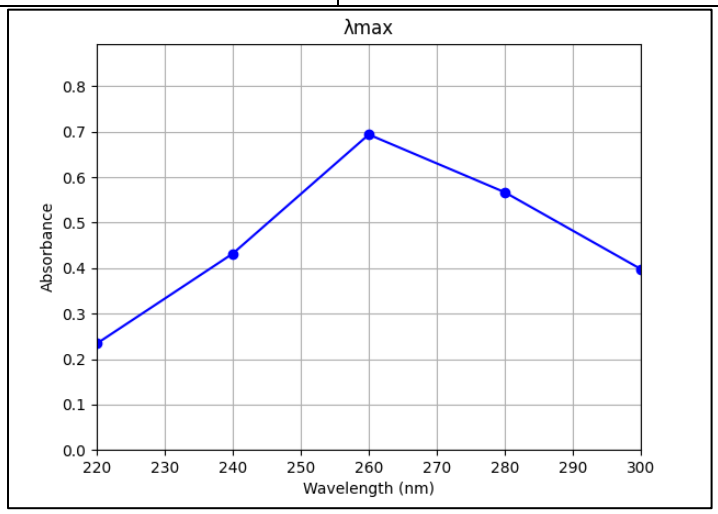

Fig.1-λmax

pH Measurements

The pH values of the Carbopol 934 and Carbopol 940 formulations were found to be 6.8 and 7.1, respectively. These results showed that the nanoemulsions were slightly acidic to neutral, which is favorable for skin compatibility.

Table 3: Mean and Standard Deviation (SD) of pH Values:

Formulation	Mean pH	SD
Carbopol 934	6.8	0.05
Carbopol 940	7.1	0.1

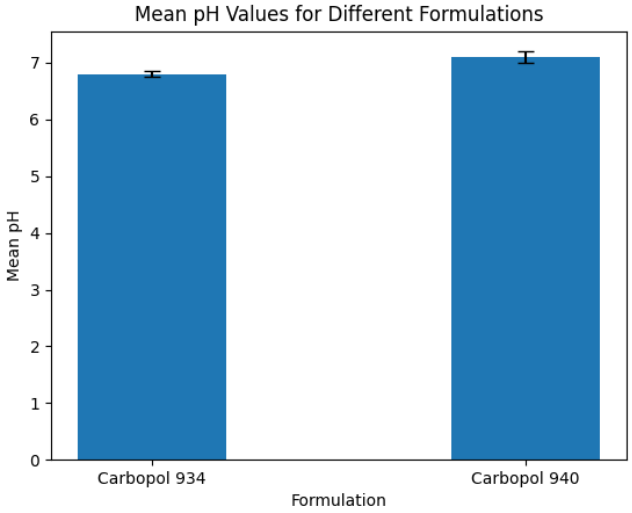

Fig.2- pH of Formulations

Viscosity Measurements

The viscosity of Carbopol 934 and 940 formulations were found to be 51 cP and 46 cP respectively. The relatively high viscosity in both the formulations indicates a good consistency which is a desirable attribute for topical formulations.

Table 4: Mean and Standard Deviation (SD) of Viscosity Values:

Formulation	Mean Viscosity (cP)	SD
Carbopol 934	51	1
Carbopol 940	46	1.41

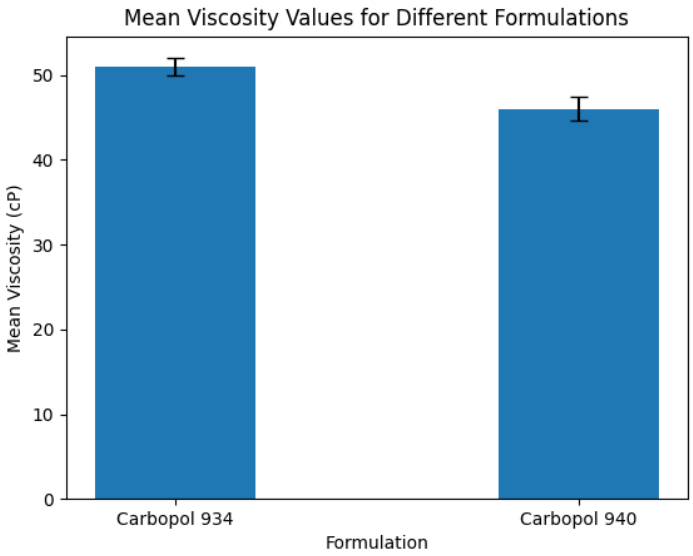

Fig. 3- Viscosity of the formulations

Spreadability Assessment

The spreadability of the formulations was measured, yielding results of 8.5 cm and 9.0 cm for Carbopol 934 and Carbopol 940 respectively. This suggests that both formulations would spread easily when applied topically, ensuring a uniform application and coverage.

Table 5: Mean and Standard Deviation (SD) of Spreadability Values

Formulation	Mean Spreadability (cm)	SD

Carbopol 934	8.5	0.2
Carbopol 940	9	0.1

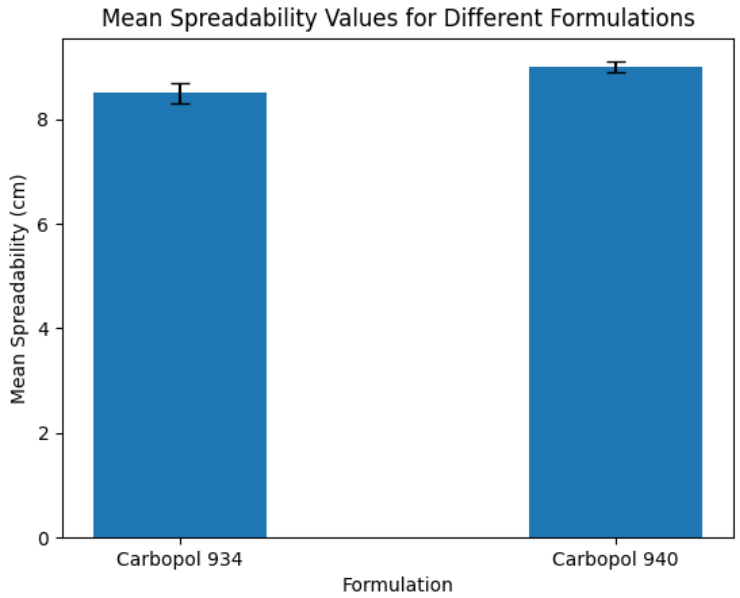

Fig. 4- Spreadability of the formulations

Zeta Potential and Particle Size Analysis

The zeta potential results for the Carbopol 934 and 940 formulations were -19 mV and -15 mV, respectively, which indicates the stability of the Nanoemulsion. The particle sizes were 122 nm and 130 nm respectively, suggesting the successful formulation of nano-scale emulsions.

Table 6: Mean and Standard Deviation (SD) of Particle Size and Zeta Potential:

Formulation	Mean Particle Size (nm)	SD Particle Size	Mean Zeta Potential (mV)	SD Zeta Potential
Carbopol 934	122	2.08	-19	1
Carbopol 940	130	2.08	-15	1

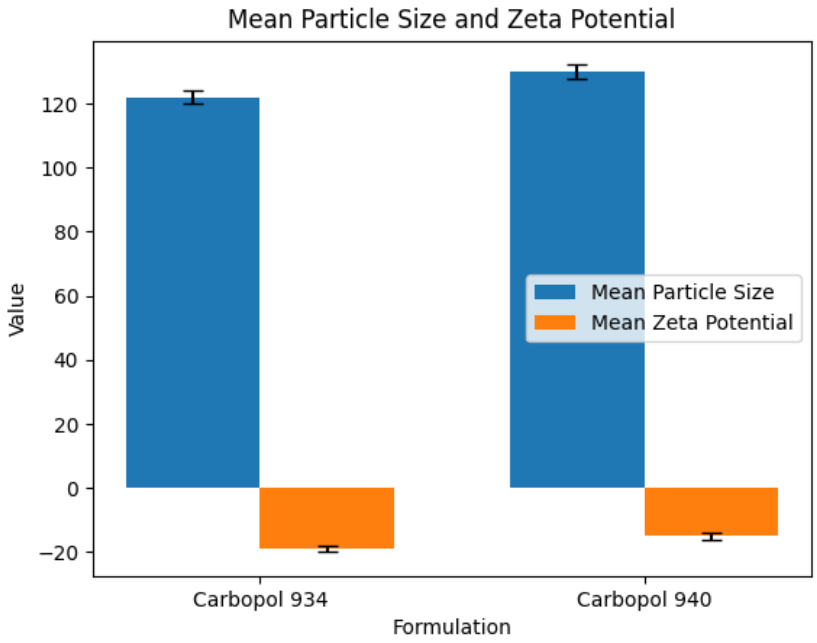

Fig. 5- Particle Size and Zeta Potential

In Vitro Drug Release Study

The in vitro drug release profile showed a gradual release of Neomycin from the Nanoemulsion over an 8-hour period. For Carbopol 934, the drug release was about 75.63% and for Carbopol 940, it was 83.73% at the end of 8 hours. These results suggest a sustained release of Neomycin from the Nanoemulsion, making them suitable for prolonged antimicrobial action.

Our results underscore the successful development of Neomycin Nanoemulsion and their potential for antimicrobial applications. The sustained release of Neomycin, the favorable pH, adequate viscosity, ease of spreadability, and stable Nanoemulsion formulation all point towards the promising attributes of this Nanoemulsion as a delivery system for antimicrobial agents.

Table 7: In vitro drug release

Time (hours)	Carbopol 934 (%)	Carbopol 940 (%)
0	0	0
1	10.52	12.36
2	20.87	23.19

3	31.14	33.61
4	40.29	43.92
5	49.32	54.01
6	58.22	64
7	66.99	73.9
8	75.63	83.73

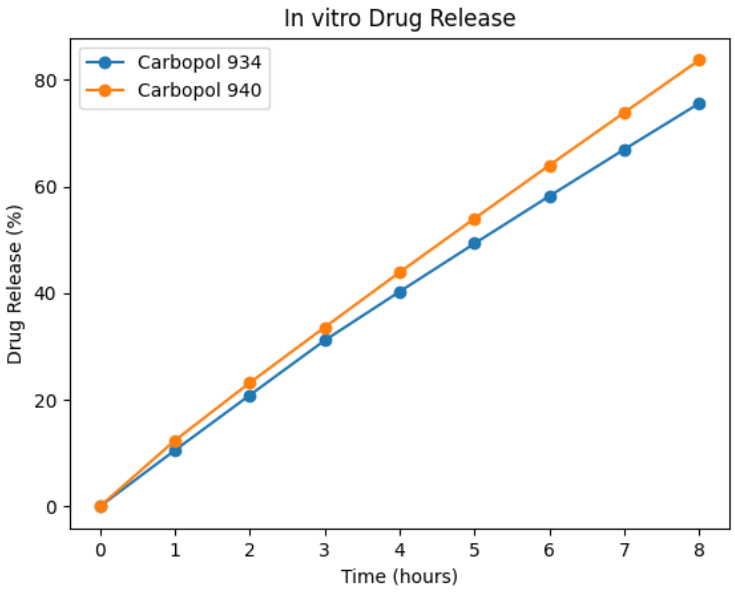

Fig. 6- In vitro Drug release

CONCLUSION

The formulation and development of Neomycin nanoemulsions and their subsequent in vitro assessment demonstrate an innovative, successful, and promising approach towards utilizing nanotechnology for antimicrobial therapies. The study reaffirms the potential of nanoemulsions as drug delivery systems, capable of improving the therapeutic efficacy of antimicrobial agents like Neomycin.

Two formulations, Carbopol 934 and Carbopol 940, were successfully developed and showed favorable characteristics such as slight acidity to neutral pH, ideal for skin compatibility, and high viscosity, ensuring the formulation's stability and adherence to the application site. The

spreadability measurements showed that both formulations would offer easy and uniform application, leading to efficient coverage and potentially better patient compliance.

The nanoemulsions' successful development was further verified by the particle size and zeta potential measurements. The average particle sizes of both formulations were within the nanometer range, an essential characteristic of nanoemulsions. The zeta potential results indicated a stable formulation, suggesting that the nanoparticles would remain uniformly dispersed and not aggregate over time.

The UV spectroscopic analysis confirmed the presence of Neomycin in the nanoemulsions, with a maximum absorbance (λmax) at 260 nm. This is a significant result, as it provides direct evidence of the successful encapsulation of Neomycin within the nanoemulsion.

Moreover, the in vitro drug release studies revealed that the nanoemulsions could offer a sustained release of Neomycin over an extended period, demonstrating their potential for prolonged antimicrobial action. The gradual release could maintain therapeutic levels of the drug, thereby increasing its effectiveness and reducing the need for frequent re-application, enhancing patient comfort and adherence to the therapy.

In summary, this research makes substantial strides in the realm of nanotechnology-based drug delivery, establishing the foundation for future exploration of nanoemulsions as carriers for other antimicrobial drugs. We foresee broad applications of this work, from pharmaceutical formulation design to antimicrobial therapy, and beyond. The promising results of this research pave the way for in vivo studies and eventually clinical trials, bringing us one step closer to the realization of effective, patient-friendly, and efficient antimicrobial treatments.

DISCUSSION

The present study discusses the formulation, development, and in vitro evaluation of Neomycin-loaded nanoemulsions, focusing on two formulations: Carbopol 934 and Carbopol 940. In recent years, there has been a substantial upswing in research focused on the development of nano-sized drug delivery systems. Nanoemulsions are among the most promising of these, due to their numerous advantages such as improved drug solubility, increased bioavailability, and controlled drug release properties.

The use of Carbopol as a polymer is also of interest in this research. Carbopol polymers are extensively used in pharmaceutical formulations owing to their excellent gelling and suspending properties, their high viscosity, and their compatibility with most active pharmaceutical ingredients. These characteristics make Carbopol an ideal polymer for nanoemulsion formulation, contributing to the overall stability and spreadability of the product.

The pH results of the two formulations were found to be compatible with skin pH, with mean pH values of 6.8 and 7.1 for Carbopol 934 and 940, respectively. This is a crucial finding as pH plays a significant role in drug stability, skin irritation, and patient compliance. Further, the viscosity values of both formulations were high, which is necessary for a stable emulsion. This stability ensures the uniform dispersion of the drug in the emulsion and reduces the risk of phase separation over time.

The spreadability of the two formulations was also favorable. Spreadability is an important attribute for a topical formulation as it dictates the ease of application and the uniformity of the drug layer on the skin. Both formulations demonstrated good spreadability, suggesting that they would be easy to apply and would distribute uniformly over the application site.

Another crucial characteristic of nanoemulsions is the particle size. Both formulations showed a mean particle size within the nanometer range, validating their classification as nanoemulsions. Smaller particle sizes allow for enhanced penetration and absorption of the drug through the skin, leading to improved therapeutic efficacy. The zeta potential values for both formulations were negative, indicating stability, as particles with a similar charge repel each other, preventing aggregation.

In vitro drug release data indicated a sustained release profile for both formulations, with Carbopol 940 showing a slightly faster release rate than Carbopol 934. This sustained release profile could potentially allow for less frequent dosing, which may enhance patient compliance.

The findings of this study align with previous researches indicating that nanoemulsion-based drug delivery systems can offer significant benefits. However, it also uncovers unique advantages that are particularly relevant to the delivery of antimicrobial drugs like Neomycin. These advances demonstrate the potential for this research to stimulate future investigation in this area and drive forward the realization of more effective antimicrobial therapies.

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