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DOI: https://doi.org/10.64388/IREV10I3-1723461

Development of Invasomal Encapsulated Antimicrobial Peptide Delivery of Bacitracin for Topical Application in Wound Healing

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Abstract- ***Wound infections are a major clinical concern that can delay the normal healing process and increase the risk of complications. Bacitracin is a peptide antibiotic widely used for topical management of superficial bacterial infections; however, its therapeutic effectiveness at the wound site may be limited by poor penetration into deeper skin layers, instability, and rapid removal from the application site. The present research was aimed at developing an invasomal delivery system for enhanced topical delivery of bacitracin for wound healing. Invasomes are flexible vesicular nanocarriers composed of phospholipids, ethanol, and selected penetration-enhancing terpenes, which provide improved deformability and facilitate penetration across the skin barrier. Bacitracin was encapsulated into invasomal vesicles using an appropriate vesicle preparation technique and the developed formulations were evaluated for vesicle characteristics, particle size, polydispersity index, zeta potential, entrapment efficiency, morphology, deformability, pH, drug content, and in vitro drug-release characteristics. The optimized invasomal formulation was further incorporated into a suitable topical gel base to improve skin residence time and ease of application. The formulation was evaluated for its physicochemical characteristics, drug release, skin permeation, antimicrobial activity, and wound-healing potential. The invasomal system is expected to enhance the deposition and penetration of bacitracin into the skin while providing controlled and prolonged drug release at the wound site. Thus, bacitrans-loaded invasomal gel may represent a promising topical delivery approach for improving the antimicrobial effectiveness of bacitracin and supporting efficient wound healing.***

I. INTRODUCTION

Wound healing is a complex and highly coordinated biological process involving a sequence of overlapping events, including haemostasis, inflammation, proliferation, and tissue remodelling. Successful healing depends on the restoration of tissue integrity and the prevention or control of microbial infection. When a wound becomes contaminated or infected by pathogenic microorganisms, the inflammatory response may become prolonged, resulting in delayed tissue repair, increased tissue damage, and an increased risk of systemic complications. Therefore, effective management of wound infection is an important component of modern wound care.

Topical antimicrobial therapy is frequently used for the prevention and treatment of bacterial infection in superficial wounds, burns, abrasions, and other skin lesions. Local administration offers several advantages over systemic therapy, including direct delivery of the antimicrobial agent to the affected area, reduced systemic exposure, and the possibility of maintaining an effective drug concentration at the site of infection. However, conventional topical formulations may have limitations such as inadequate penetration through the stratum corneum, short residence time, poor drug retention at the wound site, and repeated application requirements.

Bacitracin is a cyclic polypeptide antimicrobial agent belonging to the antimicrobial peptide class. It exhibits antibacterial activity predominantly against Gram-positive microorganisms by interfering with bacterial cell-wall synthesis. Bacitracin is commonly employed as a topical antimicrobial because its systemic use is associated with safety limitations. The development of an appropriate carrier system capable of improving topical deposition and controlled release of bacitracin may enhance its therapeutic performance.

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The skin provides an effective protective barrier against the penetration of foreign substances. The stratum corneum, consisting primarily of corneocytes embedded within a lipid matrix, represents the principal barrier to transdermal and topical drug transport. Although this barrier protects the body from external substances, it also limits the penetration of many therapeutic agents. Conventional formulations generally rely on passive diffusion and may therefore provide inadequate delivery of drugs with unfavourable physicochemical properties. Advanced vesicular and nanocarrier-based delivery systems have consequently received considerable attention for improving topical and transdermal drug delivery.

Vesicular drug-delivery systems can encapsulate therapeutic agents within or around lipid-based structures and may modify their release and interaction with the skin. The incorporation of bacitracin into invasomal vesicles may provide several potential advantages over conventional topical delivery. Encapsulation may protect the peptide from environmental degradation and provide a more controlled release profile.

The deformable nature of invasomes may improve interaction with the skin surface and facilitate penetration into superficial skin layers. Furthermore, incorporation of the optimized invasomal dispersion into a suitable gel can improve formulation viscosity, spreadability, residence time, and patient convenience. Such a combined vesicular-gel system may therefore provide sustained exposure of bacitracin at the wound site while reducing the frequency of application.

The development of an invasomal formulation may therefore provide a rational approach for improving the topical delivery of bacitracin. By combining the antimicrobial activity of bacitracin with the penetration-enhancing and deformable properties of invasomes.

II. MATERIAL & METHOD

2.1 Material

Table 2.1 List of Materials

S. No.	Chemical/Reagent	Source of Procurement
1	Bacitracin (>98% purity)	Yarrow Pharmaceuticals, Mumbai
2	Soya Lecithin	HiMEdia
3	Ethanol	CDH, New Delhi
4	Citral	Yucca Enterprises, Mumbai
5	Eucalyptus oil	Veda oils, New Delhi
6	Sodium dihydrogen orthophosphate	Oxford Fine Chemicals, Mumbai
7	Disodium hydrogen phosphate	Oxford Fine Chemicals, Mumbai
8	Sodium hydroxide	Oxford Fine Chemicals, Mumbai
9	Methanol	Oxford Fine Chemicals, Mumbai
10	Chloroform	Oxford Fine Chemicals, Mumbai
11	Water	Fresh, distilled

2.2 Method

2.2.1 Preformulation Studies

Preformulation testing is the first step in the systematic development of dosage forms. It is defined as an investigation of physical and chemical properties of a drug substance.

2.2.1.1 Organoleptic properties

A small quantity of pure bacitracin powder was taken in a butter paper and viewed in well illuminated place to observe its color; the odor were observed by smelling the drug.

2.2.1.2 Solubility analysis

Solubility of bacitracin was determined in methanol, ethanol, dimethyl formamide, water and phosphate buffer. Solubility studies were performed by shaking small amount of DC in test tubes containing the solvent and observing for undissolved particles (if any).

2.2.1.3 Melting point

The melting point of bacitracin was determined by open capillary method. The pure drug was filled in a capillary tube sealed at one end and placed in the melting point apparatus to observe the temperature at which melting occurs.

2.2.1.4. Loss on drying

The loss on drying was determined by drying the pure drug in an oven at 100°C to 105°C for 3 h. The percent loss of moisture was calculated by the difference between the initial and final weight of the drug.

2.2.1.5 Partition Coefficient

The partition coefficient of the drug was performed by using octanol as oil phase (10 mL) and water as aqueous phase (10 mL). Both the phases were mixed by shaking vigorously in a separating funnel and then 5 mg of drug was added. The drug was allowed to dissolve in both the phases by shaking and allowing for equilibration. Both phases were taken in a conical flask and then analyzed against their respective blank solution and the partition coefficient was calculated.

2.2.2 Standard Curve of bacitracin

The calibration curve of bacitracin in UV was prepared by derivatization of the drug with dabsyl chloride to obtain a orange-red colored solution having absorption maximum at 474 nm. The calibration curve was obtained using different concentrations (2-20 µg/mL) of the drug at the above wave length.

2.2.3 Formulation of Invasomes

Different bacitracin-loaded invasomes were prepared using the conventional thin layer hydration technique. Accurately weighted amount of soy lecithin (200 mg) was dissolved in 10-mL ethanol and to it various concentration of terpene (eucalyptus oil and citral) were added and stirred until a clear solution was obtained. Bacitracin (1% w/v) was dissolved in a 1% v/v hydro-ethanolic solution. Both the solutions were separately sonicated at 60°C for 30 min to assure homogeneity. The organic solvent (ethanol) from the oil phase was evaporated by rotatory evaporator at 120 rpm at 60°C for 15 min to obtain a clear film on the walls of the flask.

The deposited lipid film was then hydrated with aqueous phase by rotation at 120 rpm for 1 h at 60°C, followed by hand shaking for 5 min. The mixture was finally sonicated at 60°C for 30 min to obtain the invasome formulation.

Table 2.2 Formulation design for invasomes

S.No.	Ingredient	F1	F2	F3	F4	F5	F6
1	Bacitracin (%)	1.0	1.0	1.0	1.0	1.0	1.0
2	Soy Lecithin (mg)	200	200	200	200	200	200
3	Methanol-chloroform (2:1 v/v) (mL)	10	10	10	10	10	10
4	Eucalyptus oil (% w/v)	0.5	1.0	1.5	-	-	-
5	Citral (% w/v)	-	-	-	0.5	1.0	1.5

2.2.4. Evaluation of bacitracin-loaded invasomes

2.2.4.1 Entrapment Efficiency

To determine the amount of drug entrapped in the invasomes, 1-mL of bacitracin-loaded invasome dispersion was centrifuged at 13,000 rpm at for 60 min. The clear supernatant was siphoned off carefully to separate the unentrapped bacitracin and the supernatant was analyzed by UV spectrophotometer as per derivatization method. Sediment was treated with 1 ml of 0.1% Triton X 100 to lyse the vesicles and then

diluted to 100 ml with methanol and bacitracin was analyzed by UV spectrophotometry. Amount of bacitracin in supernatant and sediment gave a total amount of bacitracin in 1 ml dispersion. The percent entrapment was calculated using the formula:

% entrapment= amount of bacitracin in sediment/amount of bacitracin added ×100

2.2.4.2 Particle Size and Zeta Potential

The particle size of the prepared invasomes was measured using the dynamic light-scattering by Malvern Zetasizer at temperature of 25 ± 2°C. The zeta potential of a selected bacitracin-loaded invasome was also performed using 90° scattering angle at temperature of 25 ± 2°C.

2.2.4.3 In vitro release study

The in vitro release of bacitracin from different bacitracin-loaded invasomes was evaluated using the dialysis bag diffusion technique.37 Amount of bacitracin-loaded invasomes (equivalent to 2 mg bacitracin) was separated by centrifugation at 13,000 rpm for 1 h, then re-dispersed in 1-mL 3% (v/v) ethanolic aqueous solution and placed in a cellulose membrane bag. The dialysis bag was tightly closed at both ends and immersed in a stoppered bottle containing 100-mL phosphate buffer saline (PBS; pH = 7.4) representing the receptor compartment. The bottle was placed in a water bath shaker, stirred at 100 rpm, and maintained at 37 ± 0.5°C. At pre-determined time intervals, 3-mL sample of the receptor compartment was withdrawn and replaced with an equal volume of fresh medium to keep constant volume. The samples were properly derivatized with dabsyl chloride and analyzed for the amount of drug released was determined by UV spectrophotometry.

III. RESULT AND DISCUSSION

3.1 Preformulation studies

The preformulation studies were carried out using the previously reported methods and the results are presented underneath.

Table 6.1 Physicochemical properties of bacitracin

Test	Observation
Color	White
Odor	Odorless
Melting Point	225-227°C

The partition coefficient of bacitracin was calculated using octanol and water and the Log P value was found to be 2.1.

The percent loss on drying of bacitracin was found to be 0.19%.

Table 6.2 Solubility of bacitracin

Solvent	Observation
Water	Soluble
Ethanol	Soluble
Chloroform	Slightly Soluble
Methanol	Soluble

3.2 Calibration Curve of bacitracin

The UV spectrum of bacitracin upon derivatization with dabsyl chloride showed absorption maximum at 474.0 nm (Figure 6.1). Calibration curve of bacitracin was plotted as absorbance versus concentration (µg/ml) at 474.0 nm.

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DOI: https://doi.org/10.64388/IREV10I3-1723461

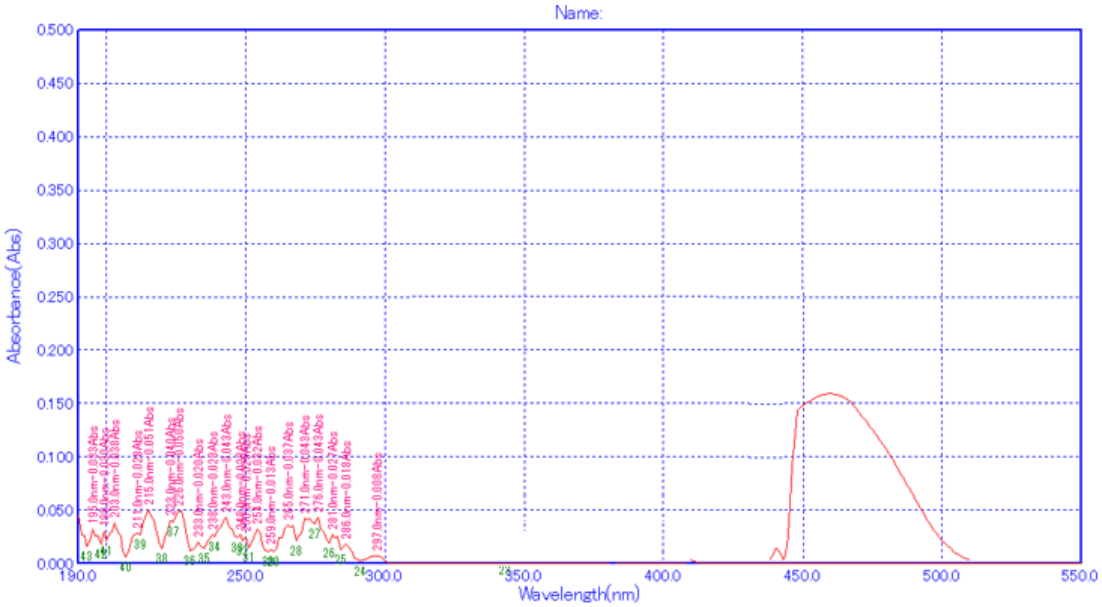

Figure 6.1 UV spectrum of bacitracin

3.3 Particle size, zeta potential and entrapment efficiency of invasomes

Formulation	Particle size	Zeta Potential	% EE
F1	263.4 nm	-19.7 mV	53.44
F2	227.8 nm	-21.5 mV	64.25
F3	167.4 nm	-24.6 mV	69.72
F4	331.5 nm	-23.9 mV	58.46
F5	270.2 nm	-24.7 mV	64.35
F6	226.6 nm	-27.3 mV	79.72

3.4 In vitro drug release

The release of bacitracin from invasomes was studied for a period of 24 hours and the invasomes were able to sustain the release of bacitracin for duration of 12 hours. The results of release study are presented in table 6.5.

Table 6.5 In vitro release of bacitracin from invasomes

Time (h)	% Cumulative Release					
	F1	F2	F3	F4	F5	F6
0	0.00	0.00	0.00	0.00	0.00	0.00
1	5.80	4.10	6.10	7.10	9.1	3.10
2	17.80	14.10	14.20	15.10	16.1	12.10
3	26.20	24.30	25.10	27.20	33.1	20.50
4	31.30	37.20	40.30	41.50	43.7	27.30

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5	41.40	43.70	45.30	48.80	47.3	35.10
6	52.3	49.8	54.3	61.40	64.1	48.3
7	65.1	61.2	70.8	74.6	71.7	52.2
8	72.4	76.1	78.2	79.5	77.1	66.5
9	88.4	86.8	82.1	86.5	82.2	76.8
10	93.7	96.1	85.1	93.2	87.6	85.9
11	99.2	99.3	91.3	98.5	93.9	90.8
12	102.5	102.1	99.8	100.1	100.2	100.1
24	102.5	102.1	99.8	100.1	100.2	100.1

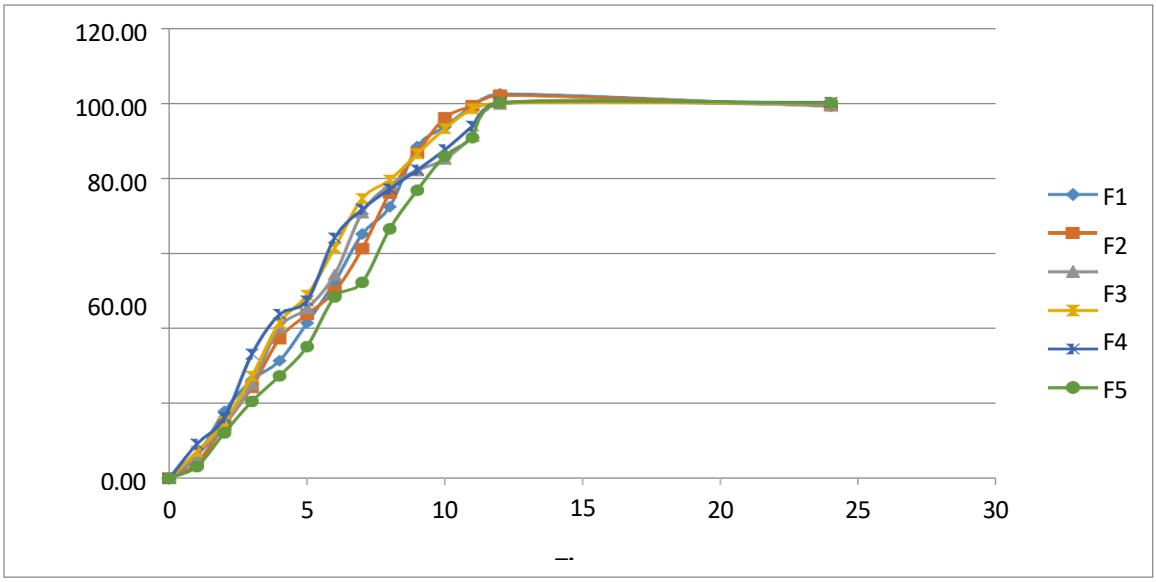

Figure 6.7 In vitro release of bacitracin from invasomes

The in vitro release profile of bacitracin from invasomal formulations (F1–F6) demonstrates a biphasic release pattern characterized by an initial diffusion-controlled phase followed by a rapid release phase leading to near-complete drug liberation within 12 hours. Such behavior is typical of lipid-based vesicular systems where drug molecules are initially retained within the bilayer structure and subsequently released upon vesicle destabilization. During the first three hours, all formulations exhibited a modest release (20–30%), indicating that bacitracin was primarily entrapped within the lipid bilayers. Among the formulations, F5 showed the highest release (33.1% at 3 h), suggesting a relatively less rigid vesicle structure or higher ethanol content, which enhances membrane fluidity and drug diffusion. Between 4–7 hours, a marked acceleration in drug release was observed. Formulations F3–F5 achieved ~70–75% release, while F6 lagged behind (~52%). This phase likely corresponds to vesicle swelling and disruption, allowing bacitracin to diffuse more freely. The differences among formulations highlight the influence of lipid composition, surfactant concentration, and ethanol percentage on vesicle permeability. By 12 hours, most formulations reached near-complete release. F1, F2, F4, F5, and F6 achieved 100% release, while F3 reached 99.8%.

IV. CONCLUSION & SUMMARY

4.1 Summary

The present study focused on the development and evaluation of bacitracin-loaded invasomes for topical wound healing. Six formulations (F1–F6) were prepared by the thin-film hydration method using soy lecithin, ethanol, citral, and eucalyptus oil. The formulations were characterized for particle size, zeta potential, entrapment efficiency, in vitro drug release,

and stability. Particle size ranged from 167.4–331.6 nm, zeta potential from –19.7 to –27.3 mV, and entrapment efficiency from 53.44–79.72%. The formulations showed a biphasic drug-release pattern with nearly complete release within 12 h. The optimized formulation (F3) exhibited good stability with minimal drug degradation. Overall, bacitracin-loaded invasomes demonstrated promising characteristics for enhancing topical drug delivery and providing effective antimicrobial action in wound management.

4.2 Conclusion

The present study successfully developed and evaluated bacitracin-loaded invasomes as a promising nanocarrier for topical wound healing. The formulations exhibited nanoscale particle size, satisfactory zeta potential, good entrapment efficiency, and controlled drug release. Citral-based formulations showed improved drug entrapment, while terpene concentration influenced vesicle characteristics and release behavior. The optimized formulation (F3) demonstrated good stability with minimal drug degradation. Overall, bacitracin-loaded invasomes offer a promising approach for enhancing topical antimicrobial delivery and providing both rapid and sustained drug release for effective wound management.

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