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Formulation and Characterization of Invasomes Gel of Bacitracin for Effective Treatment of Topical Disease

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Abstract

Topical drug administration is a localized drug delivery system anywhere in the body through Optimized invasomal formulation was sealed in 10ml glass vial and stored at refrigeration temperature (4 - 8°C) and room temperature for one month. Entrapment efficiency, physical appearance was determined at regular intervals ophthalmic, rectal, vaginal and skin as topical routes. Skin is one of the most readily accessible organs on human body for topical administration and is main route of topical drug delivery system. Invasomes are considered an inventive drug delivery system for the transdermal route. It improves the permeability of drugs across the skin layers which limits the absorption of poorly permeated drugs. It is also used for enhancing the efficacy and duration of action for drugs that had first-pass metabolism in the liver requires multiple daily doses. Invasomes contain unique components (Phospholipids, terpenes and ethanol) that act as safe and effective drug permeation enhancers across skin layer. Bacitracin is a cyclic polypeptide antibiotic used to prevent wound infections, treat pneumonia and empyema in infants, and to treat skin and eye infections. Bacitracin (200mg) was loaded in to invasomes by mechanical dispersion technique using Phospholipon 90H, terpene (Limonene) and ethanol, The optimized Bacitracin -loaded invasomes was incorporated into carbopol 934p (0.5 to 2%) solution to get a hydrogel for improving convenience in superficial application. FT-IR studies revealed no interaction between the drug and excipients. The formulated hydrogel formulation was evaluated with parameter pH, viscosity, gel strength, drug content, spreadability, *in-vitro* release test, washability, extrudability study and stability studies. The formulation IG-2 showed a drug content of 98.74% and drug release of 99.85% in 12hrs, which contains carbopol 934p concentration 2%w/w. The present work also focuses on making the formulation more pharmaceutically acceptable.

Keywords: Topical drug administration Bacitracin, Invasomes, Carbopol, Phospholipon 90H.

INTRODUCTION

The transdermal route is an essential route that overcomes many challenges in drug delivery because the drug must permeate the outer hard stratum comeum layer of the skin which is considered a limiting step in the permeation of drugs [1]. Drugs used for transdermal delivery should have several requirements to be a candidate for this route. Drugs should be lipophilic (log Ko/w=1-4), have a low melting point (<200oC, low molecular weight (<500 Daltons), short half-life (<10 h.), and low dose (<20 mg/day) [2]. vesicles discovered by Dr. Alfred Fahr in 2016. These vesicles are considered modified liposomes, ethosomes, and transfersomes in structure composition. Invasomes consist of phospholipids (e.g., egg lecithin, soybean lecithin) as the building block for the structure of the vesicles and a low percentage of ethanol when compared to ethosomes which avoid or minimize the local irritation effect caused by a large percentage of ethanol. Invasomes are unique from other vesicles in the presence of terpenes. These are natural components in plants and are present either as monoterpenes like cineol, fenchone, D-Limonene, and α-Pinene or as diterpenes. These terpenes consist of hydrocarbons and have different lipophilic-hydrophilic properties. Previous researches showed that terpenes facilitate the transdermal permeation of pharmaceuticals in dermal formulations and have a synergistic effect with ethanol and phospholipids [3]. Bacitracin (BC) is a peptide antibiotic

produced by Bacillus subtilis and B. licheniformis that exhibits activity against Gram-positive organism. BCis a complex of many similar compounds and bacitracin A (BC-A) is a main component with the most potent activity among the components. BC-F is a degradation product of BC-Athat shows no antibiotic activity and is nephrotoxic. The structures of the above two components were elucidated and their biological properties are well known. However, in spite of some trials for characterization, the structures and biological properties of other minor components have remained unresolved for over 40 years [4]. To terminate these problems, we have been carrying out a series of studies concerning the separation and characterization of BC minor components. Sciences the discovery of BC in 1943, many researchers have tried to isolate and characterize its components. In 1948, Barry *et al*., [2]) reported that BCis separable into 3 or more components by countercurrent distribution (CCD) and the main component yields 9 amino acids by acid hydrolysis. A few years later, Craig *et al*., ^ and Newton *et al*., established new CCD conditions and separated BC into 5 and 10 components, respectively, and they named the components BCs-A, -B, -C, -D, -E, -F and -G based on the separation. The two groups competitively characterized the BC components and published many papers. Craig *et al*., [10]) established the absolute configurations of constituent amino acids of BC-A and proposed a cyclic structure for these peptides. Newton *et al*., [1] suggested the presence of a thiazoline ring in the structure of BC-A. Following this, many workers reported additional structural information such as sequence data by partial hydrolysis studies of BC-A. Based on such information, several structures were proposed for BC-A one after another between 1953 and 1957 but they were all unsatisfactory. The final and well accepted structure of BC-A was proposed [8]) by Ressler and Kashelikar in 1966, and the structure was partially confirmed by synthesis. The suggestion that BC-F is a degradation product of BC-Awas made by Craig *et al*., who explained the conversion from BC-Ato -F by oxidation of the TV-terminal amino thiazoline moiety to a keto-thiazole. The structure of BC-F has been completely confirmed by synthesis. A few partial structures of other minor componentshave also been reported. Craig *et al*., [6]) suggested that the structure of BC-B is the same as that of BC-A except that one isoleucine residue was replaced by valine, however the exact location of this substitution was unknown. They also suggested that BC-B is transformable to a BC-F type component. On the other hand, Newton *et al*., [5]) reported that BCs-B, -D and -E yield valines by hydrolysis and these components show the same UV absorption spectra as does BC-A, suggesting structural similarity among them. In addition, they reported that two groups of components, BCs-C and -G and BCs-F1? -F2 and -F3 show the same UV spectra as each other. BCs-B, -C, -D and -E were reported to be antimicrobially active but to be less potent than BC-A4'5'40~42) [5].

MATERIALS AND METHODS

Materials

Bacitracin was obtained as a gift sample from Bioplus life science, Bangalore (India). disodium hydrogen phosphate, di potassium hydrogen orthophosphate, methanol, carbopol 934p, methyl paraben, propyl paraben, propylene glycol obtained from. D. Fine Chern. Ltd., Mumbai India. Chloroform, Methanol was purchased from Central Drug House Pvt Ltd, Mumbai, India. All other reagents and chemicals used were of analytical grade. vasomes can also be prepared by the conventional film method. Phospholipids in ethanol are dissolved in methanol: Chloroform (2:1, v/v). This mixture is dried to a thin film by slowly reducing the pressure from 500 to 1 mbar at 50°C using the rotary flash evaporator. The film is kept under vaccum (1 mbar) for 2 h at room temperature and subsequently flushed with nitrogen. Then, the film deposited is either hydrated for 30 min at lipid phase transition with a mixture of phosphate buffer (pH: 7.4; PBS) containing ethanol and terpenes or it is hydrated using PBS (pH: 7.4) and after cooling to room temperature. Drug and terpene or mixtures of terpenes are dissolved in ethanolic phospholipid solution. The mixture is vortexed for 5 min and then sonicated for 5 min in order to obtain a clear solution. Phosphate buffer saline '. (PBS) (pH: 7.4) is added to the solution by a syringe under constant vortexing. The vortexing is continued for an additional 5 min to obtain final invasomal preparation. Optimized invasomal formulation was sealed in 10ml glass vial and stored at refrigeration temperature (4 - 8°C) and room temperature for one month. Entrapment efficiency, physical appearance was determined at regular intervals.

RESULTS AND DISCUSSION

Results of Pre formulation study

Results of physical evaluation

The taste of bacitracin is notably bitter, a sensory characteristic that aligns with many antibiotic compounds. This bitterness can influence patient compliance, especially in oral formulations, where a palatable taste is often desirable. Overall, the sensory profde of bacitracin characterized by its appearance, minimal odor, and bitter taste plays an important role in its formulation and application, ensuring that it can be effectively utilized without compromising user experience.

Table 1: List of sensory characters of Bacitracin

S. No	Sensory characters	Result
1.	Colour	White to off-white powder
2.	Odor	Odorless or faintly odoriferous
3.	Taste	Bitter

Solubility

Solubility of the drug was determined by taking some quantity of drug (about 1-2 mg) in the test tube separately and added the 5 ml of the solvent (Water, ethanol, methanol, 0.IN HC1, and 7.4 pH phosphate buffer) Shake vigorously and kept for some time. Note the solubility of the drug in various solvents (at room temperature).

Table 2: Solubility of Bacitracin

Solvent used	Results of Solubility
Distilled Water	Slightly soluble
0.1 N Hydrochloric acid	Soluble
0.1 N NaOH	Soluble
Ethanol	Slightly soluble
Methanol	Soluble
Chloroform	Insoluble
Phosphate buffer pH 7.4	Soluble

Melting point

The melting point of Lincomycin was found 250-252℃ °C.

Loss on drying

The percentage of loss on drying of Lincomycin was found 0.109±0.005%.

Table 3: *In vitro* drug release study of gel formulation

S. No	Time (hr)	% Cumulative Drug Release*		
		1G-1	1G-2	IG-3
1	0.5	23.32	20.32	18.85
2	1	36.65	33.12	29.98
3	2	49.98	45.65	39.98
4	4	68.85	62.23	46.65
5	6	86.65	74.45	59.98
6	8	97.74	83.32	68.85
7	10	98.85	94.45	78.85
8	12	99.45	98.85	89.98

The in vitro drug release study of gel formulations provides valuable information on the release profile of the active pharmaceutical ingredient (API) over time, aiding in the selection of the optimized formulation for further development and testing, in this study, the cumulative drug release from three different gel formulations, namely 1G- 1, IG-2, and IG-3, was monitored at various time points (0.5, 1, 2, 4, 6, 8, 10, and 12 hours). The percentage cumulative drug release increased progressively over time for all formulations, indicating sustained drug release characteristics. Among the formulations tested, IG-2 demonstrated the most favorable drug release profile, with higher cumulative drug release percentages compared to IG-1 and IG-3 at each time point. Notably, at the final time point of 12 hours, IG-2 exhibited the highest cumulative drug release percentage, indicating superior drug release kinetics and sustained release properties. Based on these results, formulation IG-2 was identified as the optimized formulation for further development. Its enhanced drug release profile suggests that it may offer improved therapeutic efficacy and patient compliance compared to the other formulations tested. Further studies, including stability testing and in vivo evaluation, can validate the suitability of IG-2 for clinical use and provide valuable insights into its pharmacokinetic and pharmacodynamic properties.

Table 4: *In vitro* drug release study of optimized gel formulation IG-2

S. No.	Time (hr)	% Cumulative Drug Release*
1	0.5	20.32
2	1	33.12
3	2	45.65
4	4	62.23
5	6	74.45
6	8	83.32
7	10	94.45
8	12	98.85

The in vitro drug release study of the optimized gel formulation IG-2 provides crucial insights into its release profile, which is essential for evaluating its efficacy and suitability for clinical use. Over the course of the study, the percentage cumulative drug release from IG-2 increased steadily with time, indicating sustained release characteristics. At each time point, from 0.5 to 12 hours, IG-2 exhibited a progressive release of the drug, demonstrating its ability to provide a controlled and sustained release of the active pharmaceutical ingredient (API). Notably, at the final time point of 12 hours, IG-2 showed a high cumulative drug release percentage of 98.85%. This suggests that IG-2 has achieved a significant release of the drug over the specified duration, meeting the requirements for sustained drug delivery. The release profile of IG-2 aligns with the desired pharmacokinetic profile for the intended therapeutic application, ensuring prolonged and controlled drug release, which is crucial for maintaining effective drug concentrations in the body over an extended period. He in vitro drug release study confirms that formulation IG-2 is an optimized gel formulation with favorable drug release kinetics, making it a promising candidate for further preclinical and clinical evaluation. Additional studies, including stability testing and in vivo studies, will provide valuable insights into its performance and therapeutic efficacy.

Release kinetics of Invasomes encapsulated formulation IG-2

Table 5: *In-vitro* drug release data for optimized formulation IG-2

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative* % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	20.32	1.308	79.68	1.901
1	1	0	33.12	1.520	66.88	1.825
2	1.414	0.301	45.65	1.659	54.35	1.735
4	2	0.602	62.23	1.794	37.77	1.577
6	2.449	0.778	74.45	1.872	25.55	1.407
8	2.828	0.903	83.32	1.921	16.68	1.222
10	3.162	1	94.45	1.975	5.55	0.744
12	3.464	1.079	98.85	1.995	1.15	0.061

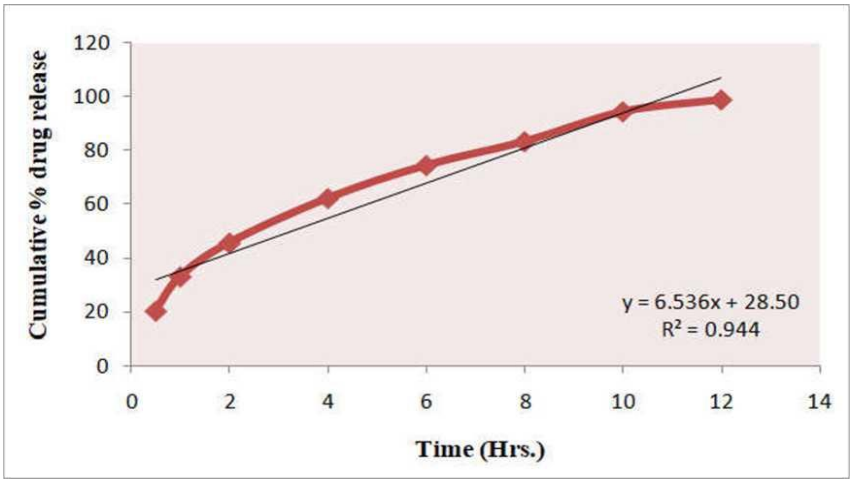

Figure 1: Cumulative % drug released Vs Time (Zero Order Kinetics)

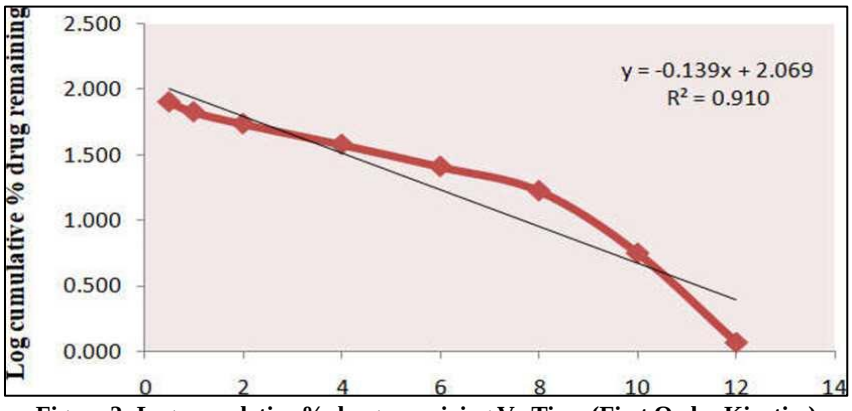

Figure 2: Log cumulative % drug remaining Vs Time (First Order Kinetics)

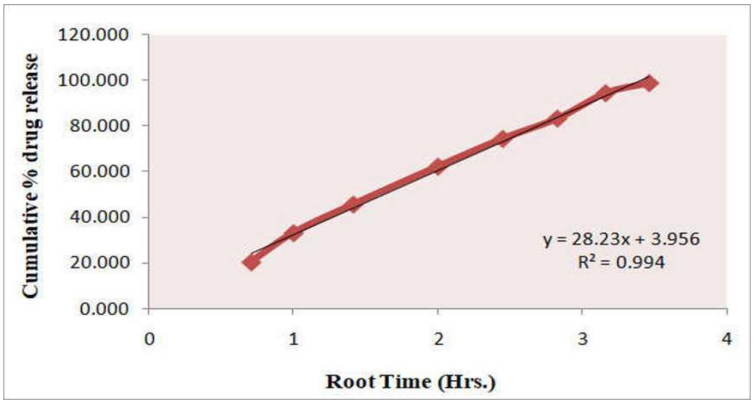

Figure 3: Cumulative % drug release Vs Root time (Higuchi Release Kinetics)

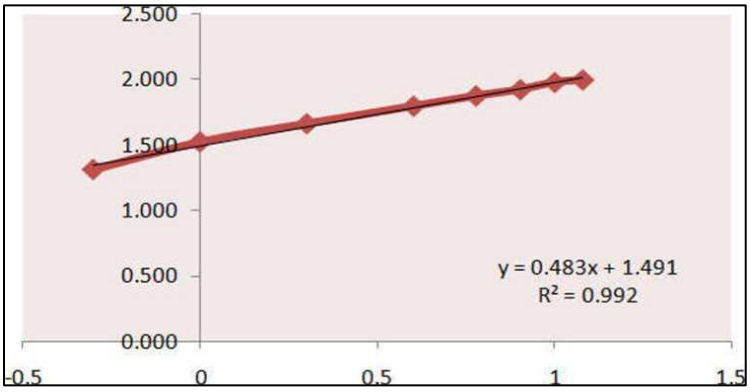

Figure 4: Log Cumulative % drug release Vs Log time (Korsmeyer Peppas Model)

Table 6: Regression analysis data of optimized gel formulation IG-2

Batch	Zero Order	First Order	Higuchi	Korsmeyer Peppas
	R^2	R^2	R^2	R^2
IG-2	0.944	0.910	0.994	0.992

The regression analysis data of the optimized gel formulation IG-2 provides valuable information regarding the kinetics of drug release from the formulation. By analyzing the coefficient of determination (R^2) values for different mathematical models, we can gain insights into the mechanism of drug release and the suitability of the formulation for sustained release applications. These R^2 values indicate the goodness of fit of the experimental data to the respective kinetic models. A higher R^2 value indicates a better fit of the data to the model. The R^2 value for the Higuchi model is notably high (0.994), suggesting that the drug release from IG-2 follows a diffusion-controlled mechanism, as proposed by the Higuchi model. This indicates that the drug release is primarily governed by the dissolution of the drug from the gel matrix and subsequent diffusion through the gel. Additionally, the R^2 values for the Zero Order and Korsmeyer Peppas models are also relatively high, indicating that these models provide a good fit to the drug release data. The First Order model, although exhibiting a slightly lower R^2 value compared to the others, still demonstrates a reasonable fit to the data. The regression analysis suggests that the drug release from the optimized gel formulation IG- 2 is predominantly diffusion-controlled, as indicated by the high R^2 value for the Higuchi model. This information is valuable for understanding the release kinetics of IG-2 and optimizing its formulation for sustained release applications.

Results of Stability

Table 7: Stability of optimized formulation of invasomes gel IG2

Characteristic	Time (Month)					
	1 Month		2 Month		3 Month	
Temp.	**4.0±0.2°C**	**25-28±2°C**	**4.0 ±0.2°C**	**25-28±2°C**	**4.0 ±0.2°C**	**25-28±2°C**
Viscosity (cps)	**3465**	**3352**	**3455**	**3265**	**3445**	**3145**
Drug Content (%)	98.95	98.45	98.15	97.65	98.10	96.44
Physical Appearance	Normal	Turbid	Normal	High turbid	Normal	High turbid

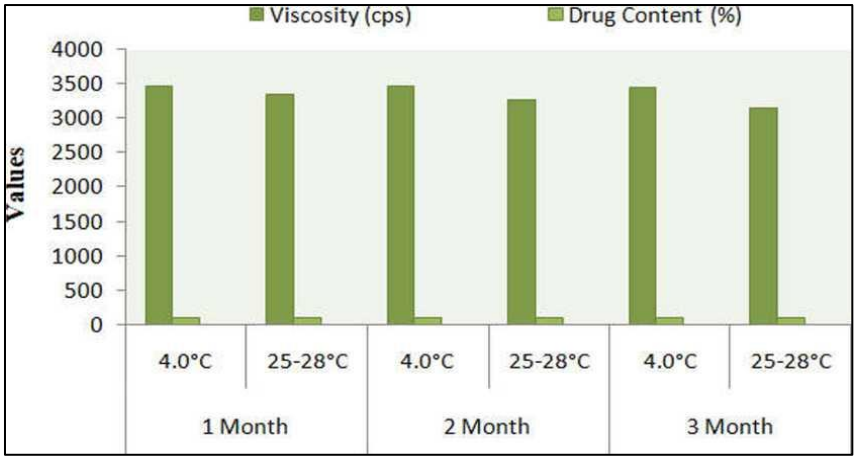

Figure 5: Graph of stability study of Invasomes gel formulation IG2

Temperature variations were observed at two different conditions: 4.0 ± 0.2°C and 25-28 ± 2°C. At both temperature settings, the viscosity of the gel was monitored over the three- month period. The results indicate that there were slight fluctuations in viscosity over time, but they remained within an acceptable range. However, it's noteworthy that at the 25-28 ± 2°C condition, a gradual decrease in viscosity was observed, which may indicate some degree of instability under ambient temperatures. Similarly, the drug content of the gel was assessed over the three-month period. The data reveal minimal changes in drug content, with slight decreases observed over time. However, the drug content remained above 96% throughout the study period, indicating good chemical stability of the formulation. Physical appearance was also evaluated as part of the stability assessment. At the beginning of the study and after one month, the gel maintained a normal appearance. However, after two and three months, there were instances of turbidity observed, particularly at the higher storage temperature (25-28 ± 2°C). This turbidity suggests the possibility of phase separation or aggregation of components within the gel, indicating a potential loss of physical stability under elevated temperatures. The stability study of the optimized invasomes gel formulation IG2 demonstrates satisfactory physical and chemical stability under refrigerated conditions (4.0 ± 0.2°C) for up to three months. However, some concerns arise regarding its stability under ambient temperatures (25-28 ± 2°C), where slight changes in viscosity and turbidity were observed. Further investigation may be warranted to optimize the formulation for enhanced stability under varying storage conditions.

SUMMARY AND CONCLUSION

The characterization of bacitracin provides a comprehensive overview of its physical and chemical properties, essential for understanding its efficacy as a pharmaceutical agent. Bacitracin appears as a white to off-white powder, is generally odorless or faintly odoriferous, and has a bitter taste. In terms of solubility, it shows variability depending on the solvent: it is slightly soluble in distilled water and ethanol, but readily soluble in 0.1 N hydrochloric acid, 0.1 N sodium hydroxide, methanol, and phosphate buffer at pH 7.4. Notably, it is insoluble in chloroform. The melting point analysis indicates a standard melting point of 252°C, with the sample melting point ranging from 250-252°C, suggesting high purity. The FT-IR spectral analysis of bacitracin identifies critical functional groups, with peaks indicative of N-H stretching at 3340.72 cm^{-1}, C=O stretching at 1631.19 cm^{-1}, C-H stretching (aliphatic) at 2985.16 cm^{-1}, and C-H bending at 1362.03 cm'. These spectral features reinforce the molecular characteristics of bacitracin, confirming its structure as a cyclic peptide. Moisture content determination reveals a moisture level of 0.109% for bacitracin, indicating its stability and suitability for formulation. The calibration curve analysis shows a strong linear relationship between concentration and absorbance, with a slope of 0.050, an intercept of 0.006, and an impressive correlation coefficient (r^2) of 0.999, validating the reliability of the analytical method for quantifying bacitracin. The evaluation of bacitracin-loaded invasomes indicates promising results in terms of drug delivery efficiency. The entrapment efficiency ranges from 65.56% to 76.65%, with formulation F5 exhibiting the highest efficiency The optimized formulation IG-2 exhibits a consistent release profile, suggesting effective drug delivery over an extended period. The regression analysis indicates that IG-2 follows a Higuchi release model (R^2 = 0.994) and Korsmeyer- Peppas model (R^2 = 0.992), confirming a diffusion-controlled release mechanism. The impressive release profiles, particularly for the optimized formulation IG-2, suggest a reliable, sustained drug release mechanism that aligns with therapeutic needs. Stability studies indicate that IG-2 maintains its properties over time, although care must be taken to manage temperature fluctuations to preserve clarity and performance. These findings provide a solid foundation for the development and optimization of bacitracin formulations, paving the way for further research and potential clinical applications in the treatment of various infections. The combination of high drug retention, effective release mechanisms, and

favorable physical characteristics positions these formulations as promising candidates in the pharmaceutical landscape.

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