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Formulation and Evaluation of Chlorhexidine loaded Transethsomal gel

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ABSTRACT

The current research emphases on the preparation and analyzing of a transethosomal gel loaded with chlorhexidine to enhance its dermal delivery and antimicrobial efficacy. Chlorhexidine, a broad-spectrum antiseptic, often suffers from poor skin permeability and retention in conventional formulations. To overcome these limitations, transethosomes ultra-deformable vesicle carriers made up of edge activators, phospholipids and ethanol were employed to encapsulate chlorhexidine using the thin-film hydration method. The formulated vesicles were placed into a Carbopol 934P-based gel to improve topical application and sustained release. Preformulation studies confirmed the physicochemical compatibility of chlorhexidine with selected excipients. The optimized transethosomal formulation (TE5) exhibited a particle size of 112 nm, zeta-potential of –26 mV, and high entrapment efficiency (80%). The transethosomal gel (TE5) displayed favorable organoleptic characteristics, pH (6.4±0.123), spreadability, viscosity, and extrudability. *In-vitro* drug release followed a controlled and sustained pattern, showing approximately 88.76 % release over 12 hours, fitting best with the First order model, indicating diffusion-controlled release. Stability studies confirmed the physicochemical stability of the formulation over two months. Overall, the chlorhexidine-loaded transethosomal gel demonstrated enhanced drug penetration, prolonged retention, and improved antimicrobial potential, offering a best strategy for skin delivery in dermal and wound care.

Keywords: *Chlorhexidine, Transethosomes, Thin-film hydration, Entrapment efficiency.*

1. INTRODUCTION

Chlorhexidine is a widely used antiseptic agent with broad-spectrum antimicrobial activity, frequently employed in the treatment of skin infections, wound care [1,2]. Despite its proven efficacy, the therapeutic effectiveness of conventional chlorhexidine formulations is often limited by inadequate skin penetration and retention [3,4].

Transethosomes ultra-deformable lipid vesicles composed of phospholipids, ethanol, and edge activators [5,6]. Their high deformability enables them to overcoming the barrier posed by the stratum corneum [7]. This makes transethosomes particularly advantageous for the delivery of antimicrobial agents like chlorhexidine [8].

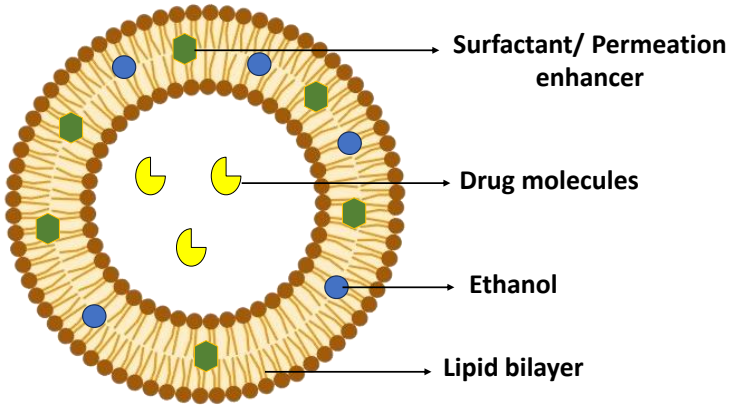

Fig. 1: Structure of transethosomes. The figure was developed using Biorender (www. biorender.com) (Accessed on 19 June 2025).

This study aims to develop and optimize a transethosomal gel loaded with chlorhexidine for improved skin delivery. The formulation is expected to enhance drug penetration, sustain release, and increase antimicrobial efficacy while minimizing systemic exposure. The prepared formulations were evaluated for entrapment efficiency, and *in-vitro* drug release. The ultimate goal is to create a patient-friendly, efficacious topical formulation

2. MATERIAL AND METHODS

2.1 Materials

Chlorhexidine was procured from Molychem Pvt. Ltd. and used as the model antimicrobial agent in the formulation. Phospholipid 90 G (PL90), serving as the primary lipid component in the vesicle formation, along with sodium deoxycholate, which functioned as the edge activator to enhance vesicular flexibility, were also obtained from Molychem Pvt. Ltd. Ethanol, used as a penetration enhancer and solvent, was supplied by SRL Chem Pvt. Ltd. For gel formulation, Carbopol 934P, a commonly used gelling agent providing appropriate viscosity and consistency, was sourced from SRL Chem Pvt. Ltd. Triethanolamine, employed for pH adjustment and gel neutralization, was obtained from Molychem Pvt. Ltd. All reagents and chemicals used were of analytical grade and used without further purification.

Preformulation may be defined as a phase of the development process whereby the drug's physical, chemical, and mechanical characteristics are characterized by the researchers in order to create a dosage form that is safe, stable, and effective.

2.2 Methods

2.2.1 Preformulation study

2.2.1.1 Organoleptic properties

The drug's sensory attributes were assessed and documented.

2.2.1.2 Melting point

The melting point of chlorhexidine was measured using a digital instrument and capillary flow method.

2.2.2 Ultraviolet/Visible spectrophotometric method development

The calibration curve of chlorhexidine was prepared buffer solution of phosphate (PBS) PH 7.4, to analyze and quantify the chlorhexidine during various stages of formulation development.

2.2.2.1 Absorption maxima (λ_{max}) of chlorhexidine

UV-Visible spectrophotometer (Labindia UV 3000^{+}) was used to confirm the purity and absorption maxima of the drug. UV-Visible spectroscopy was used for the quantitative analysis of chlorhexidine. Stock solutions (1 mg/mL) of the drug were prepared in PBS (PH 7.4) and were further diluted to obtain a concentration of 10 µg/mL. Samples were taken in 1 cm standard cuvettes and scanned in a range of 200-800 nm in a UV-Vis spectrophotometer to determine absorption maxima.

2.3 Formulation of chlorhexidine loaded transethosomes

The chlorhexidine-loaded trans-ethosomes were developed using the THF technique, employing different ratios of PL90 and SDC. Briefly, 10 mg of chlorhexidine, along with specified amounts of PL90 and SDC, were mix in a 10 mL mixture of chloroform and methanol (1:2, v/v). This solution was transferred into an RBF. Using a rota evaporator, the organic diluents were gradually detached to form a uniform, dry lipid film on the inner wall of the flask. Once solvent evaporation was complete, the lipid film was hydrated with 10 mL of phosphate-buffered saline containing ethanol. The resulting lipid dispersion was then subjected to probe sonication at 4 °C for 5 minutes (with a 2-minute interval) at an amplitude of 50, yielding the chlorhexidine-transethsomal formulation [9,10]. Finally, the prepared vesicles were stored under refrigeration for further characterization **(Table 1)**.

Table 1: Composition of chlorhexidine loaded transethosomes.

Formulation code	TE1	TE2	TE3	TE4	TE5	TE6	TE7	TE8	TE9
Chlorhexidine	10	10	10	10	10	10	10	10	10
PL90 (%, w/w)	80	80	80	85	85	85	90	90	90
SDC (%, w/w)	20	20	20	15	15	15	10	10	10
Ethanol (%, v/v)	20	30	40	20	30	40	20	30	40

2.4 Characterization of chlorhexidine loaded transethsomoal

2.4.1 % Entrapment efficiency

. A cold centrifuge was used to spin the centrifugation tubes at a speed of 15000 RPM for 30 minutes at 4°C after adding 1 ml of a drug-loaded transethosomes suspension. The unentrapped drug concentration was determined spectrophotometrically at 260 nm [11].

$$\% EE = \frac{Total\ drug\ concentaion - Free\ drug\ concentation}{Total\ drug\ concentration} \times 100$$

2.4.2 Particle size

Particle size of transethsomoal were determined by Particle size Analyser (Litesizer 500).

2.4.3 Zeta potentials

The Zeta potential of the preparation may analyse using a Zeta meter. A value of 25 mV, regardless of its polarity, may serve as the threshold that distinguishes surfaces with low charge from those with high charge.

2.5 Preparation of gel

Carbopol 934P (1.5% w/w) was immersed in a small quantity of water for one hour, and then 10 ml of transethosomes dispersion containing chlorhexidine was added. The mixture was agitated at a speed of 700 revolutions per minute in a sealed container with a constant temperature of 30°C until a uniform was transethosomes gel formed. In order to preserve the neutral pH, triethanolamine was gradually introduced into the stirring mixture.

2.6 Evaluation of chlorhexidine loaded transethsomoal gel

2.6.1 Physical Evaluation

Visual inspection was used to assess the chlorhexidine loaded transethsomoal gel physical features, clarity, occlusiveness, washability, and organoleptic properties.

2.6.2 Determination of pH

Three duplicate readings of the data were taken, and the average value was determined.

2.6.3 Spreadability

The chlorhexidine loaded transethsomoal gel Spreadability was measured by determining the dia of a 1 g gel after 5 minutes between horizontal plates (20 x 20 cm^2). 500g was the typical weight fastened to the top plate.

2.6.4 Extrudability study

The gel formulations were evaluated by filling collapsible tubes, with the formulation measured according to the weight in grams necessary to extrude a 0.8 cm ribbon of gel [12].

2.6.5 Washability

By smearing a little quantity of the formulated gel formulation to the skin washability test was conducted, followed by rinsing with warm water to evaluate its ease of removal [13].

2.6.7 *In-vitro* drug release study

The optimized gel was conducted on *in-vitro* release study encumbered with chlorhexidine to determine the most effective formulation. This investigation used a dialysis membrane using Franz Diffusion Cell with size of 0.4 µm pore. The membrane is absorbed in a 7.4 buffer solution of phosphate for a duration of 24 hours.

2.7 Stability Study

The drug retention capacity of vesicles in a gel formulation was evaluated by subjecting the gel to various temperatures. The gel was stored in airtight vials with a capacity of 10ml at a temperature of 4 ± 2ºC and at room temperature for a duration of 45 days. The drug content was measured at various time periods to ascertain the percentage.

3. RESULT AND DISCUSSION

3.1 Pre-formulation studies

3.1.1 Organoleptic properties

Organoleptic evaluation revealed that the chlorhexidine is a white to light yellow, crystalline substance with a characteristic odor and bitter taste.

3.1.2 Melting point analysis

The melting point of chlorhexidine analyzed by Stuart smp30 digital melting point apparatus. The sample analyzed in triplicate and mean was found to be 132 ℃. The mean was almost the similar as that of the reported melting point i.e., 132–136 ℃ (Drug Bank). The results of melting point are given in **Table 2.**

Table 2: Melting point analysis of chlorhexidine.

Observed Melting Point				**Reported Melting Point**
MP1 ℃	**MP2 ℃**	**MP3 ℃**	**Mean ℃**	
132	132	134	132	132–136 ℃

3.1.3 Solubility study

The solubility of chlorhexidine in dissimilar solvents was studied, namely ethanol, DMSO, Dimethyl formamide and water etc. The solubility of chlorhexidine with ethanol, DMSO, dimethyl foramide and water were found to be 10, 15, 10, and 0.0261 mg/mL..

3.1.4 Preparation of calibration curve and Determination of λ_{max}

The spectrum of chlorhexidine was examined and the wavelength of chlorhexidine was found to be 260 nm which is as according to standard values**.** Then the selected wavelength of 260 nm was used for the further studies. The dilutions were prepared in the concentration range 10, 20, 30, 40, 50 and 50 µg/ml. The result of the linearity curve of chlorhexidine was found to be R^2 0.9992 (**Table 3, Fig. 2**).

Table 3: Calibration curve of chlorhexidine in PBS (pH 7.4) at 260 nm.

Concentration (µg/ml)	**Absorbance**
10	0.1
20	0.24
30	0.378
40	0.52
50	0.687
60	0.834
70	0.987

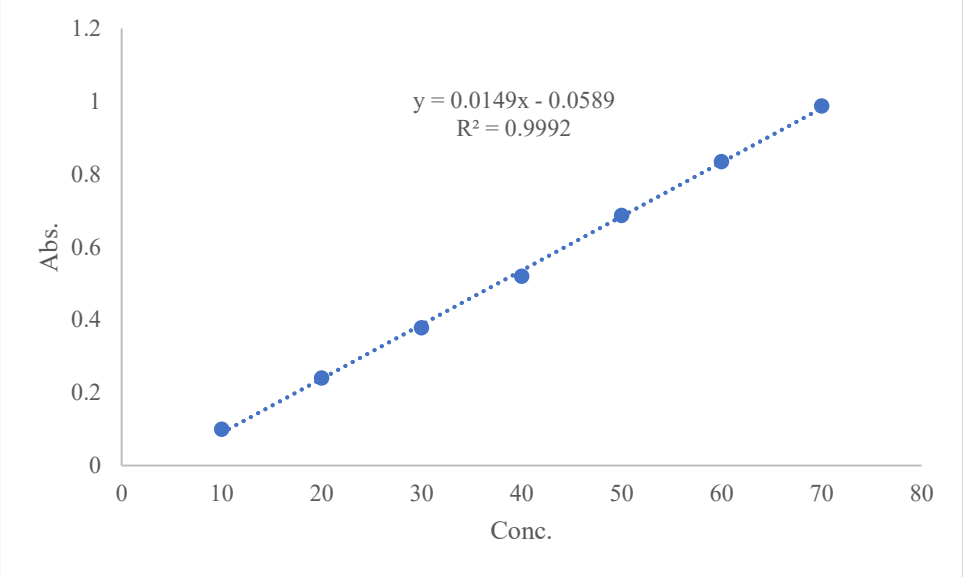

Fig. 2: Linearity curve of chlorhexidine in PBS in PH 7.4 at 260 nm.

3.2 Evaluation of chlorhexidine loaded transethsomoal

3.2.1 Entrapment efficiency (%EE)

The EE of all formulations ranged from 60 % to 80%. Among the formulations, Formulation TE5 exhibited the highest entrapment efficiency at 80 % . The data indicates that the entrapment efficiency of transethsomoal increases as the polymer ratio increases.

3.2.2 Particle size analysis and polydispersity index

An instrument Litesizer 500 used to analyse the particle size of the prepared formulation (TE 1 to TE9) was in the range of 112- 202nm. TH5 is the best particle size and it was found to be 112 nm .This result demonstrates that the prepared transethsomoal possess nano-sized particles, indicating their suitability for penetration through the skin. The Polydispersity Index (PDI) of the transethosomes was range to be 0.18-0.24 **(Table 4),** and TE5 was found to be 0.24 indicating a uniform particle size distribution and narrow dispersion within the formulations. A PDI value equal to or less than 0.24 suggests that the sample is monodispersed.

Table 4: PDI of different formulations of chlorhexidine loaded transethsomoal.

S. No.	Formulation	PDI
1.	TE1	0.18
2.	TE2	0.38
3.	TE3	0.44
4.	TE4	0.32
5.	TE5	0.24
6.	TE6	0.42
7.	TE7	0.36
8.	TE8	0.29
9.	TE9	0.21

3.2.3 Zeta potential

Zeta potential is the widely utilized method generally used for the determination of the stability of colloidal dispersion. The zeta potential of TE1 to TE9 was found to be range of -17.0 to -26.0mV **(Table 5).** An increase in zeta potential leads to enhanced repulsion between charged particles, resulting in improved stability against aggregation. In the case of the improved transethosomes preparation, TE5 formulation of zeta potential was measured to be -26.0 mV. A higher absolute value indicates a stronger electrical charge on the surface of the transethosomes, leading to robust repulsive forces among the particles and preventing their aggregation.

Table 5: Zeta potential of different formulations of chlorhexidine loaded transethsomoal.

S. No.	Formulation	Zeta potential (mV)
1.	TE1	-18.0
2.	TE2	-10.0
3.	TE3	-17.0
4.	TE4	-20.0
5.	TE5	-26.0
6.	TE6	-22.0
7.	TE7	-19.0
8.	TE8	-22.0
9.	TE9	-21.0

3.3 Characterization of chlorhexidine loaded transethsomoal gel

Out of the 9 batches of transethsomoal, the TH5 batch exhibited the best encapsulation efficiency (%EE), optimal polydispersity index (PDI), and zeta potential (ZP). This batch was then used to create gel formulations using carboxyvinyl polymer carbomer [Carbopol 934P (0.5%)].

3.3.1 Physical Evaluation

The physical evaluation of gel was shown in **Table 6.**

3.3.2 pH measurement

The pH gel was measured by using an electrode-based digital pH meter. The obtained pH value of gel was shown in **Table 6.**

3.3.3 Drug content

The drug content study of vesicular gel formulation was characterized by using a UV spectrophotometer to find out the amount chlorhexidine of drug present in the formulation. The obtained results of the drug content of transethsomoal were listed in **Table 6.**

3.3.4 Viscosity of gel

The viscosity of transethosomal gel was measured by Brookfield viscometer with the use of spindle no. 7, speed 100 rpm and the optimum viscosity is shown in **Table 6.**

Table 6: Results of chlorhexidine-loaded transethsomoal gel.

Parameters	TE5 (GEL)
Physical appearance	Turbid yellowish
pH	6.4±0.123
Viscosity (cps)	9020
Drug content (%)	91

3.3.5 *In vitro* drug release of chlorhexidine-loaded transethsomoal gel

The *in-vitro* drug release was performed with the help of a dialysis membrane in the beaker. Drug release of gel formulation was characterized by using a UV spectrophotometer to find out the amount of drug (chlorhexidine) released. The obtained results of % drug release is shown in **Fig.3 and Table 7.** The gel released 88.76% of chlorhexidine in 12 hours by the use of the dialysis membrane .

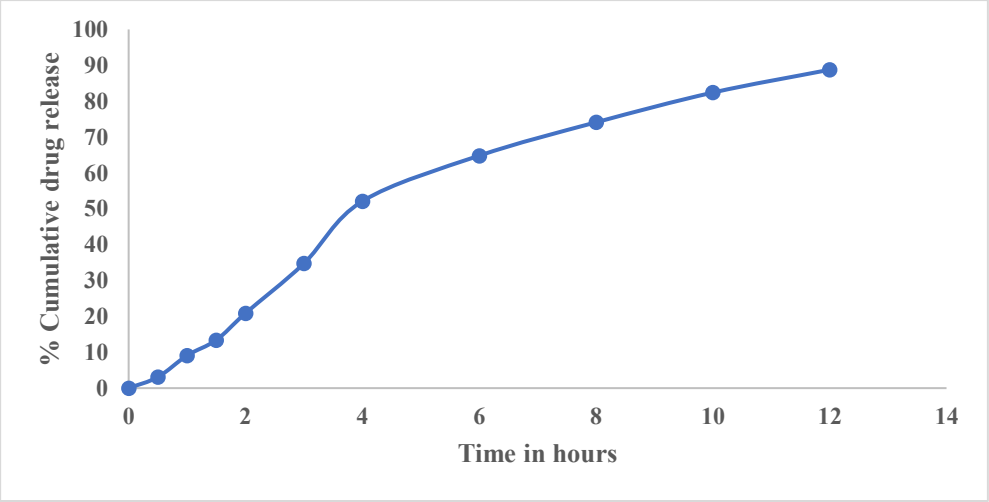

Fig. 3: *In-vitro* cumulative drug release profile of chlorhexidine-loaded transethsomoal gel.

Table 7: *In-vitro* cumulative drug release of chlorhexidine-loaded transethsomoal gel.

Time (hours)	% Cumulative drug release
0	0
0.5	3.08
1	9.11
1.5	13.4
2	20.88
3	34.76
4	52.1
6	64.8
8	74.1
10	82.4
12	88.76

3.3.9 *In-vitro* drug release kinetics studies

The delivery rate was established by computing the gradient of the relevant graphs, and the coefficient of determination (R^2) was also assessed. The model fitting data for the release kinetics of chlorhexidine loaded transethsomoal gel is presented in **Fig. 4, 5, 6, and 7**. Among the different models, the first model exhibited the highest R^2 value, indicating the best fit for the data. This observation was further confirmed by plotting the percentage cumulative drug release against the time (hr), where the R^2 value ranged between 0.9947.

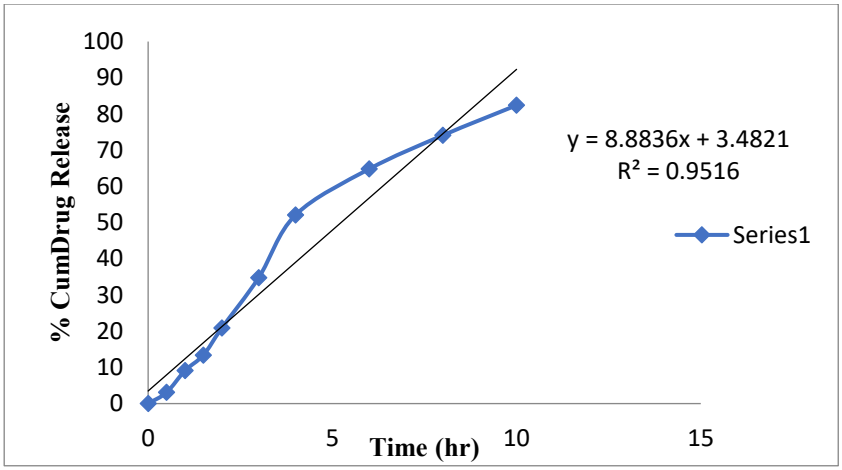

Fig. 4: Zero order plot for release kinetics of chlorhexidine-loaded transethsomoal gel.

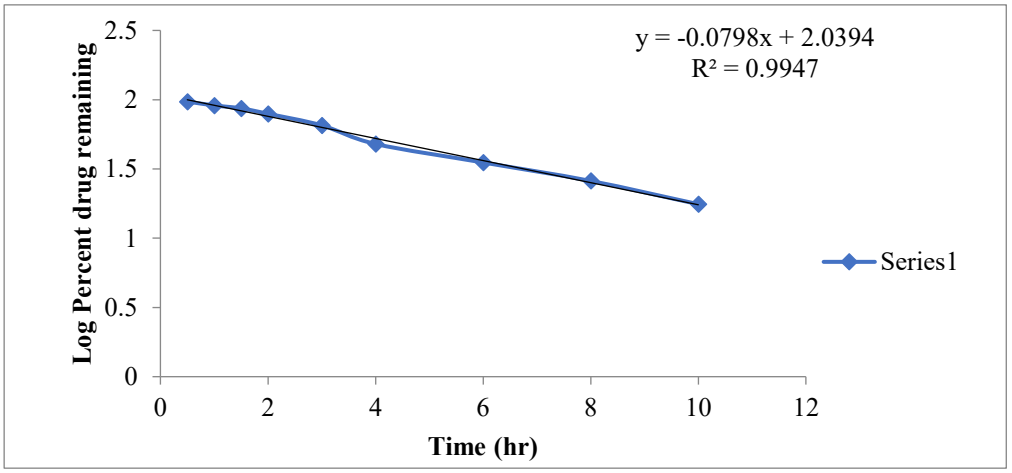

Fig. 5: First order plot for release kinetics of chlorhexidine-loaded transethsomoal gel.

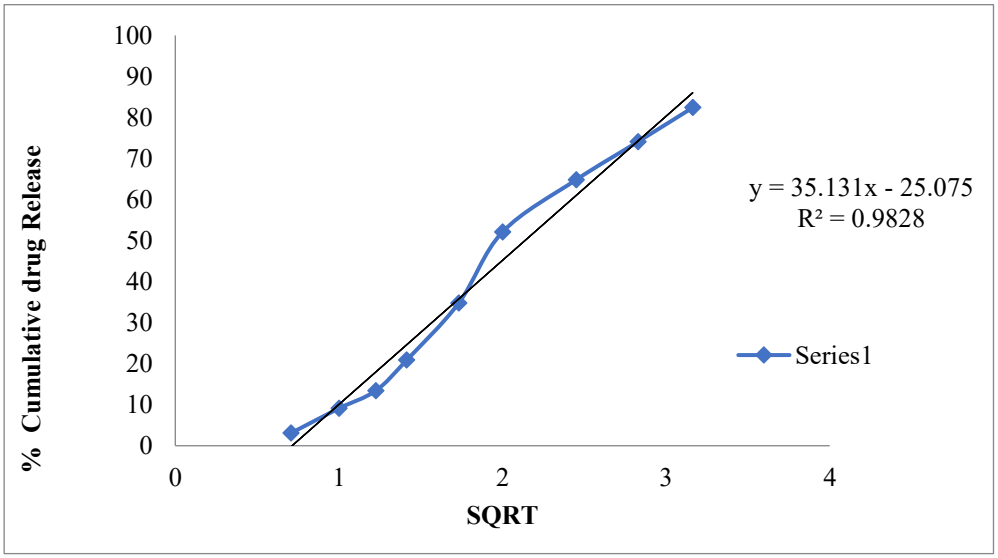

Fig. 6: Higuchi plot for release kinetics of chlorhexidine-loaded transethsomoal gel.

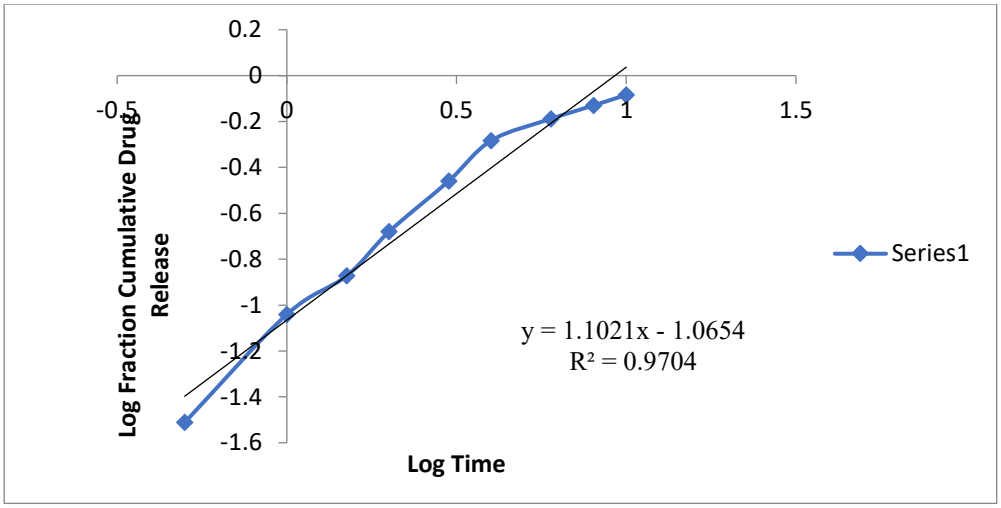

Fig. 7: Peppa's plot for release kinetics of chlorhexidine-loaded transethsomoal gel.

3.4 Stability study

The physical stability studies of formulated gels of chlorhexidine loaded transethosome gel were conducted for 2 months by storing them at different temperature conditions such as 4±2ºC and 25±2ºC/60±5 % RH. **(Table 8)**.

Table 8: Results of accelerated stability studies after two months.

Evaluation parameters	Initial	1st month	2nd month
Colour	Turbid yellowish	Turbid yellowish	Turbid yellowish
Ph	6.4 ±0.004	6.5±0.001	6.4±0.058

4. CONCLUSION

The present study successfully developed and evaluated a chlorhexidine-loaded transethosomal gel with the objective of enhancing dermal drug delivery and antimicrobial efficacy. The optimized formulation (TE5) demonstrated favorable physicochemical properties, including small vesicle size (112 nm), high entrapment efficiency (80%), and good zeta potential (–26 mV), ensuring formulation stability. Incorporation of the transethosomes into a Carbopol 934P-based gel further improved the topical application profile, with desirable spreadability, viscosity, pH, and drug content. In vitro release studies confirmed a sustained and controlled drug release pattern, with 88.76% cumulative release over 12 hours, fitting best to the first-order kinetic model. The formulation also showed excellent stability over a two-month period without significant changes in physical or chemical attributes. Overall, the developed chlorhexidine-loaded transethosomal gel represents a promising and effective strategy for the topical treatment of skin infections and wound care, providing improved drug penetration, prolonged retention, and better patient compliance

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