RESEARCH PAPER

Formulation and Evaluation of Withaferin A Extract-Loaded Transethosomal Buccal Film for Enhanced Drug Delivery

Akash Yadav[1] Dr Shankar Lal Soni[2] Dr Vandana Sharma[3] Mo. Firoj Tanwar[4] Ms Aarti Mehta[4]

1. Research Scholar, Department of Pharmaceutics, Arya College of Pharmacy, Kukas, Jaipur, Rajasthan, India.
2. Professor, Arya College of Pharmacy, Kukas, Jaipur, Rajasthan, India.
3. Principal, Arya College of Pharmacy, Kukas, Jaipur, Rajasthan, India.
4. Assistant Professor, Arya College of Pharmacy, Kukas, Jaipur, Rajasthan, India.

Corresponding author: Dr Shankar Lal Soni (drsonikhatu@gmail.com)

Abstract

The present study aimed to formulate and evaluate Withaferin A extract-loaded transethosomal buccal films for controlled and prolonged drug delivery. Withaferin A, a bioactive constituent of *Withania somnifera*, has limited aqueous solubility, which may affect its dissolution and delivery. Transethosomes were developed using soya lecithin and sodium deoxycholate and were incorporated into mucoadhesive buccal films prepared using hydroxypropyl methylcellulose K15M and Carbopol 934. Nine transethosomal formulations (T1–T9) and nine buccal film formulations (F1–F9) were prepared and evaluated. The optimized transethosomal formulation, T5, exhibited a particle size of 142.8 ± 1.74 nm, polydispersity index of 0.198 ± 0.008, zeta potential of −31.6 ± 1.0 mV, entrapment efficiency of 91.6 ± 1.18%, and drug loading of 7.24 ± 0.16%. Among the prepared films, F3 showed the most suitable overall characteristics, including a thickness of 0.134 ± 0.004 mm, folding endurance of 148 ± 3 folds, surface pH of 6.51 ± 0.06, drug content of 98.2 ± 0.8%, and mucoadhesive strength of 29.6 ± 0.7 g. The optimized film released 96.4% of the extract over 12 hours. Release kinetic analysis showed the best fit with the Higuchi model (R^2 = 0.989), while the Korsmeyer–Peppas exponent of approximately 0.67 suggested anomalous or non-Fickian release. Three-month stability studies showed acceptable formulation characteristics under the tested storage conditions. These findings indicate that Withaferin A extract-loaded transethosomal buccal films may provide a promising approach for controlled drug delivery. Further ex-vivo permeation and in-vivo studies are required to establish their absorption and therapeutic performance.

Keywords: Withaferin A, Transethosomes, Buccal Film, Mucoadhesion, Controlled Drug Release.

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Introduction

Withania somnifera (L.) Dunal, commonly known as Ashwagandha, is a medicinal plant widely studied for its bioactive constituents and pharmacological properties. Withaferin A is a naturally occurring steroidal lactone belonging to the withanolide group and has been investigated for its anti-inflammatory, antioxidant, antimicrobial, immunomodulatory, and anticancer activities. Despite its pharmacological potential, the limited aqueous solubility of Withaferin A presents a formulation challenge and may affect its dissolution and delivery. Therefore, the development of an appropriate drug-delivery system is important for improving its incorporation, release, and interaction with biological membranes.

Buccal drug delivery is an alternative approach in which a formulation is placed against the inner lining of the cheek for drug release and absorption through the oral mucosa. This route offers advantages such as ease of administration, avoidance of gastrointestinal degradation, and the potential to reduce hepatic first-pass metabolism. Mucoadhesive buccal films are particularly useful because they can maintain contact with the mucosal surface and provide controlled drug release. However, conventional buccal formulations may be affected by salivary washout, limited mucosal permeability, and insufficient residence time. These limitations have encouraged the investigation of advanced carrier systems that can improve drug incorporation and facilitate transport across biological membranes.

Transethosomes are deformable vesicular carriers composed of phospholipids, ethanol, and an edge activator. The phospholipid component forms the vesicular structure, while ethanol and the edge activator contribute to vesicle flexibility and interaction with biological membranes. These properties make transethosomes a potential approach for delivering compounds with limited aqueous solubility or permeability. Incorporating transethosomes into a mucoadhesive buccal film may combine the advantages of vesicular delivery with prolonged contact at the administration site.

In the present study, Withaferin A extract-loaded transethosomes were prepared using soya lecithin and

sodium deoxycholate and evaluated for vesicle size, polydispersity index, zeta potential, entrapment efficiency, and drug loading. The optimized transethosomal formulation was incorporated into buccal films prepared using hydroxypropyl methylcellulose K15M and Carbopol 934. The prepared films were evaluated for physicochemical characteristics, mechanical properties, mucoadhesive strength, in-vitro drug release, release kinetics, and stability. The study aimed to identify a suitable transethosomal buccal film formulation capable of providing controlled and prolonged release of Withaferin A extract. Further permeation and in-vivo investigations would be required to establish its absorption and therapeutic performance.

Research Gap

The reviewed literature establishes the potential of Withaferin A as a bioactive compound, transethosomes as deformable vesicular carriers, and mucoadhesive films as suitable platforms for buccal drug delivery. However, the sources reviewed here do not establish the formulation performance of Withaferin A extract-loaded transethosomes incorporated into a buccal film prepared using HPMC K15M and Carbopol 934. The present study addresses this formulation gap by preparing and optimizing transethosomes, incorporating the optimized formulation into mucoadhesive films, and evaluating vesicle characteristics, film properties, mucoadhesive strength, in-vitro drug release, release kinetics, and stability. Ex-vivo permeation and in-vivo studies remain necessary to determine whether the formulation improves mucosal transport and therapeutic performance.

Methods

Withaferin A extract was used as the active ingredient for the formulation. Soya lecithin was used as the vesicle-forming material, while sodium deoxycholate acted as an edge activator to improve vesicle flexibility. Ethanol was used as an organic solvent. Hydroxypropyl methylcellulose K15M (HPMC K15M) and Carbopol 934 were used as film-forming and mucoadhesive polymers, respectively. Other excipients, including polyethylene glycol 400, propylene glycol, polyvinyl alcohol, and glycerin, were used as required during formulation development. All materials were of suitable pharmaceutical or analytical grade.

Preformulation Studies

Preformulation studies were performed to assess the basic physicochemical properties of Withaferin A extract. Solubility was examined in selected solvents, including ethanol, methanol, propylene glycol, and polyethylene glycol 400. Ultraviolet spectrophotometry was used to determine the maximum absorption wavelength and establish a calibration curve. Fourier-transform infrared spectroscopy (FTIR) was performed to identify characteristic functional groups and examine possible interactions between the extract and excipients.

Preparation of Transethosomes

Transethosomal formulations were prepared using soya lecithin and sodium deoxycholate in different proportions. Withaferin A extract was incorporated into the vesicular system during formulation preparation. Nine formulations, designated T1–T9, were developed by varying the formulation composition. The prepared dispersions were evaluated to identify the formulation with suitable vesicle characteristics and drug entrapment.

Characterization of Transethosomes

The prepared transethosomes were evaluated for particle size, polydispersity index (PDI), zeta potential, entrapment efficiency, and drug loading. Particle size and PDI were used to assess vesicle size and size distribution, while zeta potential was measured to evaluate surface charge. Entrapment efficiency and drug loading were determined to assess the incorporation of Withaferin A extract into the vesicles. Vesicle morphology was also examined where applicable.

Preparation of Buccal Films

The optimized transethosomal formulation was incorporated into mucoadhesive buccal films using HPMC K15M and Carbopol 934. Different polymer compositions were used to prepare nine film formulations, designated F1–F9. The prepared films were allowed to dry and were evaluated for their physical appearance, mechanical properties, drug content, surface pH, and mucoadhesive characteristics.

Evaluation of Buccal Films

The prepared films were evaluated for thickness, folding endurance, surface pH, drug content, tensile strength, percentage elongation, and mucoadhesive strength. These tests were performed to assess the uniformity, flexibility, mechanical strength, drug distribution, suitability for buccal application, and adhesion of the films to the mucosal surface.

In-vitro Drug-Release Study

The in-vitro release of Withaferin A extract from the optimized buccal film was studied over a period of 12 hours. The percentage of drug released was measured at specified time intervals and compared with the release profile of the pure extract. The release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer–Peppas kinetic models to investigate the drug-release pattern.

Stability Studies

Stability studies of the optimized buccal film were conducted for three months under the selected long-term and accelerated storage conditions. Drug content

and in-vitro drug release were evaluated during the study period to determine whether the formulation maintained its quality and performance during storage.

Results and Discussion

Preformulation Studies

Preformulation studies were performed to establish the basic physicochemical characteristics of Withaferin A extract before formulation development. The extract was evaluated for organoleptic properties, solubility, and melting point. These studies provided preliminary information useful for selecting suitable formulation components and processing conditions for the development of Withaferin A extract-loaded transethosomal buccal film.

Organoleptic Properties

The organoleptic properties of Withaferin A extract were evaluated with respect to colour, odour, appearance, and physical state. The observations obtained are presented in Table 7.1.

Table 1: Organoleptic Properties of Withaferin A Extract

Sr. No.	Parameter	Observation
1	Colour	Yellowish-brown
2	Appearance	Fine, uniform powder
3	Physical state	Solid

The Withaferin A extract was observed as a yellowish-brown, fine and uniform solid powder with a characteristic odour. No visible foreign particles or abnormal physical changes were observed during examination. The observed characteristics were considered satisfactory for further preformulation studies and formulation development.

Solubility Study

The solubility behaviour of Withaferin A extract was investigated in different solvents selected on the basis of their relevance to the formulation and analytical procedures. An accurately weighed quantity of extract was added to 10 mL of each solvent and the samples were examined for dissolution behaviour.

Table 2: Solubility Profile of Withaferin A Extract

Sr. No.	Solvent	Quantity of Extract	Volume of Solvent	Observation
1	Distilled water	10 mg	10 mL	Slightly soluble
2	Ethanol	10 mg	10 mL	Freely soluble
3	Methanol	10 mg	10 mL	Freely soluble
4	Phosphate buffer pH 6.8	10 mg	10 mL	Slightly soluble
5	Propylene glycol	10 mg	10 mL	Soluble
6	Polyethylene glycol 400	10 mg	10 mL	Soluble

The solubility study indicated that Withaferin A extract exhibited limited solubility in aqueous media, whereas better solubility was observed in organic and cosolvent systems. The relatively better solubility in ethanol, methanol, propylene glycol, and polyethylene glycol 400 supported the selection of an appropriate solvent system during formulation development.

Melting Point Determination

The melting behaviour of Withaferin A extract was determined by the capillary tube method. The determination was performed in triplicate, and the onset and end temperatures were recorded.

Table 3: Melting Point Determination of Withaferin A Extract

Sr. No.	Trial	Onset Temperature (°C)	End Temperature (°C)	Melting Range (°C)
1	Trial 1	235.0	239.0	4.0
2	Trial 2	236.0	240.0	4.0
3	Trial 3	235.5	239.5	4.0

The melting behaviour of Withaferin A extract was observed within a narrow temperature range, with an average onset temperature of approximately 235.5°C and an end temperature of approximately 239.5°C. The relatively narrow melting range indicated consistent thermal behaviour of the tested sample. The result provided preliminary information regarding the thermal characteristics of the extract and served as a reference for subsequent compatibility and thermal analysis studies.

UV-Visible Spectrophotometric Analysis

The UV-Visible spectrophotometric analysis of Withaferin A extract was performed to determine its maximum wavelength of absorption (λmax), which was subsequently used for quantitative estimation during the formulation studies.

A suitable solution of Withaferin A extract was prepared and scanned over the wavelength range of 200–800 nm using a UV-Visible spectrophotometer

against the corresponding solvent as blank. The obtained absorption spectrum showed a distinct maximum absorption peak at the characteristic wavelength of the extract.

The λmax was identified from the wavelength corresponding to the maximum absorbance and was used for further spectrophotometric analysis of Withaferin A extract.

Table 4: UV-Visible Spectrophotometric Analysis of Withaferin A Extract

Parameter	Result
Scanning range	200–800 nm
λmax	227 nm
Solvent	Methanol
Method	UV-Visible spectrophotometry

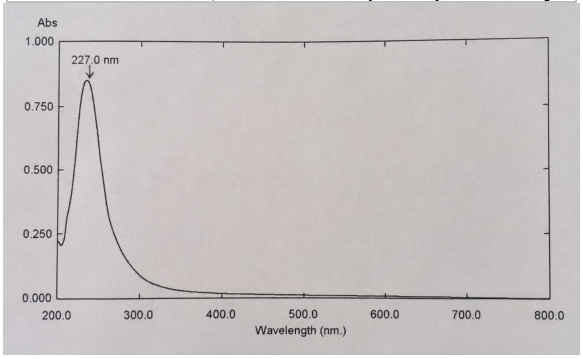


Figure 1: UV-Visible absorption spectrum of Withaferin A extract showing λmax at 227 nm

The UV-Visible spectrum of Withaferin A extract exhibited maximum absorption at 227 nm. The presence of a distinct absorption maximum indicated that the selected wavelength could be used for spectrophotometric estimation of the extract. The identified λmax was subsequently used for preparation of the calibration curve and estimation of drug content in the developed formulations.

Calibration Curve of Withaferin A

The calibration curve of Withaferin A extract was prepared using standard solutions of different concentrations. Standard solutions of 5, 10, 15, 20, 25 and 30 µg/mL were prepared from the stock solution and their absorbance was measured at the predetermined λmax of 227 nm using methanol as the blank.

Each concentration was analysed in triplicate, and the mean absorbance was used for construction of the calibration curve.

Table 5: Calibration Curve Data of Withaferin A Extract at 227 nm

Sr. No.	Concentration (µg/mL)	Absorbance
1	5	0.121
2	10	0.241
3	15	0.361
4	20	0.482
5	25	0.602
6	30	0.721

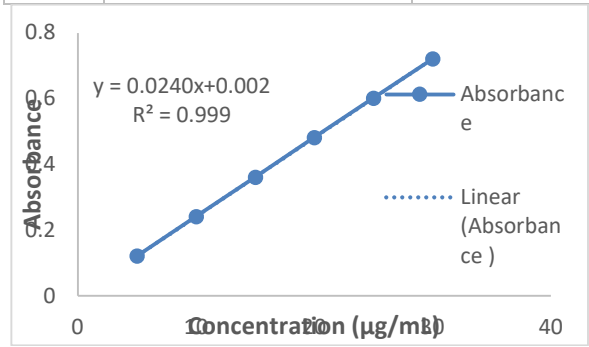


Figure 2: Calibration curve of Withaferin A extract at 227 nm

The calibration curve demonstrated a linear increase in absorbance with increasing concentration of Withaferin A extract over the concentration range of 5–30 µg/mL. The high correlation coefficient (R^2 = 0.9999) indicated good linearity within the selected concentration range. The regression equation obtained from the calibration curve was subsequently used for quantitative estimation of Withaferin A extract during drug-content and formulation studies

FTIR Compatibility Study

The characteristic peaks observed in the spectrum of the pure extract were compared with those of the physical mixture. The major peaks were evaluated for any significant shift, disappearance, broadening, or formation of additional peaks.

Table 6: FTIR Spectral Characteristics of Withaferin A Extract

Sr. No.	Functional Group	Characteristic Wavenumber (cm^{-1})	Observed Wavenumber (cm^{-1})
1	O–H stretching	3200–3500	3420
2	C–H stretching	2850–3000	2930
3	C=O stretching	1650–1750	1705
4	C=C stretching	1550–1650	1618
5	C–O stretching	1000–1300	1235
6	C–O–C stretching	1050–1150	1085

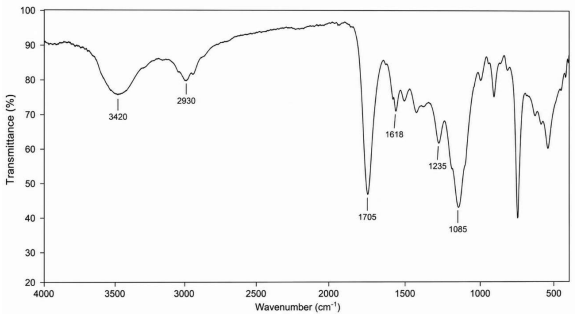


Figure 3: FTIR spectrum of pure Withaferin A extract

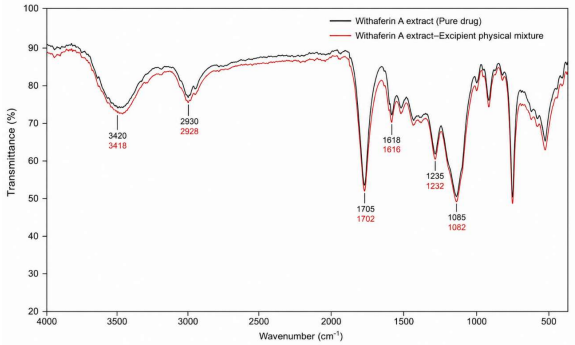


Figure 4: FTIR spectrum of Withaferin A extract–excipient physical mixture

The FTIR spectrum of Withaferin A extract showed characteristic absorption bands corresponding to its major functional groups. The prominent bands were observed around 3420, 2930, 1705, 1618, 1235, and 1085 cm^{-1}. These bands were associated with O–H, C–H, carbonyl, C=C, and C–O/C–O–C functional groups, respectively.

The FTIR spectrum of the physical mixture showed the characteristic absorption bands of Withaferin A extract with only minor changes in their positions and intensities. No significant disappearance of the characteristic peaks or appearance of a prominent new peak was observed.

The FTIR findings indicated no major chemical interaction between Withaferin A extract and the selected excipients, supporting their compatibility for formulation of the transethosomal buccal film.

Differential Scanning Calorimetry (DSC) Study

Differential Scanning Calorimetry (DSC) was performed to investigate the thermal behaviour of Withaferin A extract and to further assess its compatibility with the selected excipients. The DSC thermograms of the pure Withaferin A extract and Withaferin A extract–excipient physical mixture were recorded under controlled conditions.

The thermograms were examined with respect to the onset temperature, peak temperature, endset temperature, and enthalpy of the major thermal transition.

Table 7: DSC Thermal Parameters of Withaferin A Extract

Sr. No.	Parameter	Result
1	Onset temperature	235.2°C
2	Peak temperature	238.6°C
3	Endset temperature	241.8°C
4	Enthalpy	82.4 J/g

Table 8: DSC Thermal Parameters of Withaferin A Extract–Excipient Physical Mixture

Sr. No.	Parameter	Result
1	Onset temperature	233.8°C
2	Peak temperature	237.4°C
3	Endset temperature	242.1°C
4	Enthalpy	78.9 J/g

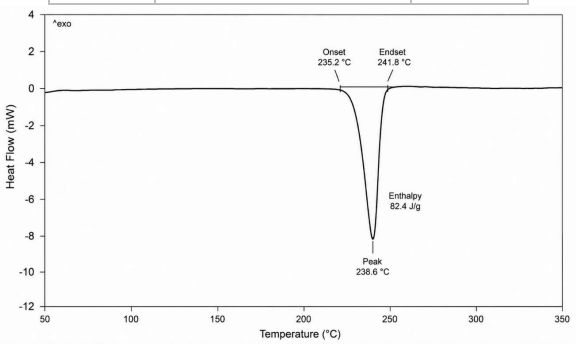


Figure 5: DSC thermogram of pure Withaferin A extract

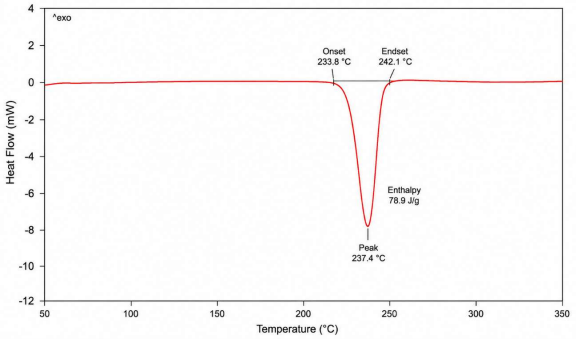


Figure 6: DSC thermogram of Withaferin A extract–excipient physical mixture

The DSC thermogram of Withaferin A extract exhibited a characteristic endothermic thermal transition with an onset temperature of approximately 235.2°C, a peak temperature of 238.6°C, and an endset temperature of approximately 241.8°C. The corresponding enthalpy was found to be 82.4 J/g.

The physical mixture exhibited a thermal transition in a comparable temperature region, with an onset

temperature of 233.8°C, peak temperature of 237.4°C, and endset temperature of 242.1°C. A slight change in the thermal parameters was observed after mixing with the excipients; however, the major thermal event was retained without the appearance of a significant new thermal transition.

Formulation and Characterization of Withaferin A Extract-Loaded Transethosomes

Formulation Composition

Table 9: Formulation composition of trial batch

Formulation	Withaferin A Extract (mg)	Soya Lecithin (mg)	Sodium Deoxycholate (mg)	Ethanol (% v/v)	Final Volume
T1	10	100	10	20	10 mL
T2	10	100	20	20	10 mL
T3	10	100	30	20	10 mL
T4	10	150	10	20	10 mL
T5	10	150	20	20	10 mL
T6	10	150	30	20	10 mL
T7	10	200	10	20	10 mL
T8	10	200	20	20	10 mL
T9	10	200	30	20	10 mL

Physical Appearance of Transethosomal Formulations

All prepared transethosomal formulations were observed for colour, appearance, homogeneity and presence of visible aggregates. The formulations produced a uniform, translucent to slightly opalescent vesicular dispersion without visible phase separation or large aggregates.

Table 10: Formulation parameter evolution

Formulation	Colour/Appearance	Homogeneity	Phase Separation
T1	Slightly opalescent	Good	Absent
T2	Slightly opalescent	Good	Absent
T3	Opalescent	Good	Absent
T4	Slightly opalescent	Very good	Absent
T5	Opalescent	Very good	Absent
T6	Opalescent	Very good	Absent
T7	Slightly opalescent	Good	Absent
T8	Opalescent	Very good	Absent
T9	Opalescent	Good	Absent

Optimization of Transethosomal Formulation

The prepared formulations were evaluated for vesicle size, polydispersity index, zeta potential, entrapment efficiency, deformability index and drug content. Sample/illustrative results are shown below.

Table 11: Optimization of Transethosomal Formulation

Formulations	Vesicle Size (nm)	PDI	Zeta Potential (mV)	Entrapment Efficiency (%)	Deformability Index	Drug Content (%)
T1	184.6	0.312	−21.4	76.8	18.6	94.2
T2	172.3	0.286	−24.8	80.5	22.4	95.1
T3	161.8	0.264	−27.6	83.7	26.8	96.0
T4	168.5	0.251	−23.7	82.4	24.7	96.3
T5	142.8	0.198	−31.6	91.6	35.8	98.2
T6	151.4	0.217	−29.8	89.3	32.6	97.5
T7	176.2	0.278	−22.9	84.1	25.3	96.7
T8	158.7	0.229	−28.4	88.2	31.4	97.1
T9	165.9	0.243	−30.2	90.1	33.2	97.8

The formulation variables showed a noticeable effect on the characteristics of the transethosomal vesicles. Increasing the concentration of sodium deoxycholate generally improved vesicle deformability and increased drug entrapment up to an optimum concentration. Excessive edge activator concentration may, however, disturb the vesicular membrane and affect vesicle stability.

Among the preliminary formulations, T5 exhibited the most desirable overall characteristics, with a

vesicle size of approximately 142.8 nm, PDI of 0.198, zeta potential of −31.6 mV, entrapment efficiency of 91.6%, deformability index of 35.8 and drug content of 98.2%.

Evaluation of Transethosomes

The prepared Withaferin A extract-loaded transethosomal formulations were evaluated for particle size, polydispersity index, zeta potential, entrapment efficiency, drug loading, and vesicle morphology. The results obtained for the different formulations are presented in Table 7.12.

Table 12: Evaluation Parameters of Withaferin A Extract-Loaded Transethosomes

Formulation	Particle Size (nm)	PDI	Zeta Potential (mV)	Entrapment Efficiency (%)	Drug Loading (%)
T1	184.6 ± 2.31	0.312 ± 0.012	−21.4 ± 1.2	76.8 ± 1.54	5.82 ± 0.21
T2	172.3 ± 2.18	0.286 ± 0.010	−24.8 ± 1.1	80.5 ± 1.32	6.14 ± 0.18
T3	161.8 ± 1.96	0.264 ± 0.009	−27.6 ± 1.3	83.7 ± 1.27	6.48 ± 0.23
T4	168.5 ± 2.42	0.251 ± 0.011	−23.7 ± 1.4	82.4 ± 1.46	6.31 ± 0.20
T5	142.8 ± 1.74	0.198 ± 0.008	−31.6 ± 1.0	91.6 ± 1.18	7.24 ± 0.16
T6	151.4 ± 2.06	0.217 ± 0.009	−29.8 ± 1.2	89.3 ± 1.25	7.02 ± 0.19
T7	176.2 ± 2.27	0.278 ± 0.013	−22.9 ± 1.3	84.1 ± 1.38	6.56 ± 0.22
T8	158.7 ± 1.83	0.229 ± 0.010	−28.4 ± 1.1	88.2 ± 1.21	6.91 ± 0.17
T9	165.9 ± 2.14	0.243 ± 0.011	−30.2 ± 1.2	90.1 ± 1.16	7.13 ± 0.20

Particle Size

The particle size of the prepared transethosomal formulations was found to be in the range of 142.8 ± 1.74 to 184.6 ± 2.31 nm. Formulation T5 showed the lowest particle size (142.8 nm), indicating the formation of relatively small vesicles.

The reduction in vesicle size may be attributed to the appropriate balance between phospholipid and edge activator concentration. Smaller vesicles are generally desirable for efficient interaction with the buccal mucosal surface and may facilitate improved drug permeation.

Polydispersity Index

The PDI values ranged from 0.198 ± 0.008 to 0.312 ± 0.012. T5 exhibited the lowest PDI of 0.198, indicating a relatively narrow vesicle-size distribution and good uniformity.

The comparatively higher PDI observed in some formulations may be associated with variations in phospholipid and edge activator concentrations affecting vesicle formation.

Zeta Potential

The zeta potential values ranged from −21.4 ± 1.2 to −31.6 ± 1.0 mV. Formulation T5 showed a zeta potential of −31.6 ± 1.0 mV, suggesting good surface charge and favourable physical stability of the vesicular dispersion.

The negative surface charge may also contribute to reducing vesicle aggregation through electrostatic repulsion.

Entrapment Efficiency

The entrapment efficiency of the formulations ranged from 76.8 ± 1.54% to 91.6 ± 1.18%. T5 demonstrated the highest entrapment efficiency (91.6%).

The higher entrapment observed with T5 may be attributed to the optimum concentration of phospholipid and sodium deoxycholate, which provided a suitable vesicular environment for incorporation of Withaferin A extract.

Drug Loading

The drug-loading capacity ranged from 5.82 ± 0.21% to 7.24 ± 0.16%. Formulation T5 showed the highest drug loading of 7.24 ± 0.16%, which was consistent with its higher entrapment efficiency.

Vesicle Morphology

The morphology of the optimized transethosomal formulation T5 was examined using SEM. The vesicles appeared predominantly spherical to nearly

spherical, with a relatively smooth surface and no significant aggregation.

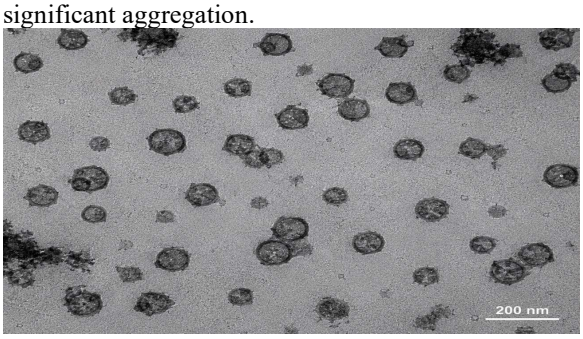


Figure 7: SEM image of optimized Withaferin A extract-loaded transethosomes (T5)

Formulation and Evaluation of Transethosomal Buccal Films

Physical Appearance

The prepared Withaferin A extract-loaded transethosomal buccal films were evaluated visually for colour, surface appearance, flexibility, uniformity, air bubbles, cracks and ease of removal from the casting surface. All formulations produced smooth and uniform films without significant visible defects.

Formulation	Appearance	Surface	Flexibility	Air Bubbles/Cracks
F1	Slightly opaque	Smooth	Moderate	Absent
F2	Slightly opaque	Smooth	Good	Absent
F3	Uniform translucent	Very smooth	Very good	Absent
F4	Slightly opaque	Smooth	Moderate	Absent
F5	Uniform translucent	Smooth	Very good	Absent
F6	Opaque	Smooth	Good	Slight
F7	Uniform translucent	Very smooth	Good	Absent
F8	Slightly opaque	Smooth	Good	Absent
F9	Opaque	Slightly rough	Moderate	Slight

Formulation Composition

Table 13: Composition of Withaferin A Extract-Loaded Transethosomal Buccal Films

Formulation	HPMC K15	Carbopol 934	PEG 40	Transethosomal Dispersio	Water
	M (mg)	**(mg)**	**0 (mg)**	**n**	
F1	100	10	20	Equivalent to 10 mg extract	q.s. to 10 mL
F2	100	20	20	Equivalent to 10 mg extract	q.s. to 10 mL
F3	100	30	20	Equivalent to 10 mg extract	q.s. to 10 mL
F4	150	10	20	Equivalent to 10 mg extract	q.s. to 10 mL
F5	150	20	20	Equivalent to 10 mg extract	q.s. to 10 mL
F6	150	30	20	Equivalent to 10 mg extract	q.s. to 10 mL
F7	200	10	20	Equivalent to 10 mg extract	q.s. to 10 mL
F8	200	20	20	Equivalent to 10 mg extract	q.s. to 10 mL
F9	200	30	20	Equivalent to 10 mg extract	q.s. to 10 mL

Optimization of Buccal Film Formulation

The prepared formulations F1–F9 were evaluated for thickness, folding endurance, swelling index, drug content, mucoadhesive strength and in-vitro drug release. The results are presented in Table 14.

Table 14: Evaluation and Optimization Data of Transethosomal Buccal Films

Formulation	Thickness (mm)	Folding Endurance	Swelling Index (%)	Drug Content (%)	Mucoadhesive Strength (g)	Drug Release at 8 h (%)
F1	0.11	112	168	93.	18.4 ±	92.

	8 ± 0.004	± 3	.4 ± 4.2	6 ± 1.2	0.8	6 ± 1.4
F2	0.126 ± 0.003	126 ± 4	186 .7 ± 3.8	95. 1 ± 1.1	22.7 ± 0.9	89. 8 ± 1.3
F3	0.134 ± 0.004	148 ± 3	214 .6 ± 4.1	98. 2 ± 0.8	29.6 ± 0.7	84. 7 ± 1.1
F4	0.142 ± 0.005	118 ± 4	176 .8 ± 3.6	94. 2 ± 1.3	20.1 ± 0.8	91. 4 ± 1.2
F5	0.151 ± 0.004	156 ± 3	226 .8 ± 4.5	98. 7 ± 0.7	31.8 ± 0.8	82. 9 ± 1.0
F6	0.164 ± 0.005	139 ± 4	241 .5 ± 4.8	97. 1 ± 0.9	34.2 ± 0.9	78. 6 ± 1.2
F7	0.176 ± 0.004	143 ± 3	192 .6 ± 3.9	97. 8 ± 0.9	25.6 ± 0.8	86. 8 ± 1.3
F8	0.184 ± 0.005	151 ± 4	218 .3 ± 4.2	96. 9 ± 1.0	32.4 ± 0.9	81. 7 ± 1.1
F9	0.196 ± 0.006	137 ± 4	253 .7 ± 5.1	95. 8 ± 1.2	38.6 ± 1.0	74. 9 ± 1.4

The results demonstrated that increasing the concentration of HPMC K15M increased film thickness and generally enhanced the matrix strength. However, excessive polymer concentration resulted in thicker films and slower drug release.

An increase in Carbopol 934 concentration improved swelling and mucoadhesive strength because of its high water-uptake and mucoadhesive properties. However, excessive Carbopol concentration resulted in excessive swelling and a slower drug-release rate.

Among the prepared formulations, F5, F7 and F3 demonstrated comparatively favourable overall characteristics and were considered promising formulations during optimization.

F5 showed excellent folding endurance, high drug content, good swelling behaviour and strong mucoadhesion. However, its relatively higher HPMC concentration produced a somewhat slower drug-release profile. F7 showed good mechanical properties and drug content, but its lower Carbopol concentration resulted in comparatively lower mucoadhesive strength than F3 and F5.

Although F5 and F7 demonstrated favourable individual characteristics, F3 provided the best overall balance between mechanical properties, swelling, mucoadhesion, drug content and controlled drug release.

Evaluation of Transethosomal Buccal Films

The optimized and developed Withaferin A extract-loaded transethosomal buccal films were evaluated for their physical, mechanical, physicochemical and mucoadhesive characteristics. The results obtained for formulations F1–F9 are presented in Table 7.15.

Table 15: Evaluation of Withaferin A Extract-Loaded Transethosomal Buccal Films

Formulation	**Thickness (mm)**	**Weight (mg)**	**Folding Endurance**	**Surface pH**	**Moisture Content (%)**	**Drug Content (%)**	**Tensile Strength (N/mm²)**	**Elongation (%)**	**Mucoadhesive Strength (g)**
F1	0.118 ± 0.004	42.6 ± 1.2	112 ± 3	6.42 ± 0.08	5.82 ± 0.24	93.6 ± 1.2	1.82 ± 0.09	48.6 ± 2.1	18.4 ± 0.8
F2	0.126 ± 0.003	44.1 ± 1.1	126 ± 4	6.48 ± 0.07	5.64 ± 0.21	95.1 ± 1.1	1.94 ± 0.08	46.2 ± 1.8	22.7 ± 0.9
F3	0.134 ± 0.004	46.8 ± 1.0	148 ± 3	6.51 ± 0.06	5.46 ± 0.19	98.2 ± 0.8	2.08 ± 0.07	52.4 ± 2.0	29.6 ± 0.7
F4	0.142 ± 0.005	48.2 ± 1.3	118 ± 4	6.45 ± 0.07	5.91 ± 0.23	94.2 ± 1.3	2.16 ± 0.10	44.8 ± 1.9	20.1 ± 0.8

F5	0.151 ± 0.004	51.4 ± 1.2	156 ± 3	6.49 ± 0.06	5.38 ± 0.18	98.7 ± 0.7	2.34 ± 0.08	50.6 ± 1.7	31.8 ± 0.8
F6	0.164 ± 0.005	54.8 ± 1.4	139 ± 4	6.56 ± 0.08	6.12 ± 0.25	97.1 ± 0.9	2.52 ± 0.11	42.7 ± 1.8	34.2 ± 0.9
F7	0.176 ± 0.004	57.3 ± 1.3	143 ± 3	6.43 ± 0.07	5.72 ± 0.22	97.8 ± 0.9	2.68 ± 0.09	45.8 ± 1.9	25.6 ± 0.8
F8	0.184 ± 0.005	59.1 ± 1.4	151 ± 4	6.52 ± 0.07	5.96 ± 0.23	96.9 ± 1.0	2.81 ± 0.10	43.2 ± 1.7	32.4 ± 0.9
F9	0.196 ± 0.006	62.4 ± 1.5	137 ± 4	6.58 ± 0.08	6.28 ± 0.26	95.8 ± 1.2	2.96 ± 0.12	39.6 ± 1.6	38.6 ± 1.0

Appearance and Surface Characteristics

All prepared buccal films were visually examined for colour, surface smoothness, flexibility, homogeneity, transparency and visible defects. The films were found to be smooth, uniform and flexible, with no significant cracks, wrinkles, air bubbles, pinholes or visible aggregation.

F3, F5 and F7 exhibited particularly satisfactory surface characteristics. The optimized F3 film showed a smooth, uniform and translucent appearance, indicating good distribution of the transethosomal dispersion throughout the polymeric matrix.

Thickness

The thickness of the prepared films ranged from 0.118 ± 0.004 to 0.196 ± 0.006 mm. F3 showed a thickness of 0.134 ± 0.004 mm.

The gradual increase in thickness with increasing HPMC K15M concentration was attributed to the greater polymer content in the casting solution. The relatively uniform thickness of F3 indicated satisfactory casting and distribution of formulation components.

Weight Variation

The average weight of the film patches ranged from 42.6 ± 1.2 to 62.4 ± 1.5 mg. F3 exhibited a mean weight of 46.8 ± 1.0 mg.

The relatively low variation in individual film weights indicated uniformity of the casting process and satisfactory distribution of the formulation components.

Folding Endurance

Folding endurance ranged from 112 ± 3 to 156 ± 3 folds. F5 showed the highest folding endurance, whereas F3 exhibited 148 ± 3 folds.

The high folding endurance indicated that the films possessed adequate flexibility and mechanical resistance for handling and buccal administration.

Surface pH

The surface pH values ranged from 6.42 ± 0.08 to 6.58 ± 0.08. F3 showed a surface pH of 6.51 ± 0.06.

The surface pH values were close to the physiological pH range of the buccal mucosa, indicating that the prepared films were unlikely to cause significant irritation due to pH.

Moisture Content

The moisture content of the films ranged from 5.38 ± 0.18 to 6.28 ± 0.26%. F3 showed a moisture content of 5.46 ± 0.19%.

The moderate moisture content suggested adequate drying of the films while maintaining sufficient flexibility. Excessively low moisture content may produce brittle films, whereas excessive moisture can adversely affect film stability.

Drug Content

The drug content ranged from 93.6 ± 1.2 to 98.7 ± 0.7%. F3 showed a drug content of 98.2 ± 0.8%, indicating good uniformity of Withaferin A extract throughout the film matrix.

The relatively high drug content of F3 confirmed that incorporation of the optimized transethosomal dispersion into the polymeric film did not result in significant drug loss during film preparation.

Tensile Strength

The tensile strength of the films ranged from 1.82 ± 0.09 to 2.96 ± 0.12 N/mm². F3 exhibited a tensile strength of 2.08 ± 0.07 N/mm².

The results indicated that the films possessed adequate mechanical strength to withstand handling, cutting and application to the buccal mucosa without premature rupture.

Percentage Elongation

Percentage elongation ranged from 39.6 ± 1.6 to 52.4 ± 2.0%. F3 exhibited 52.4 ± 2.0% elongation, indicating good flexibility.

The balance between tensile strength and elongation is important because an excessively rigid film may be

uncomfortable during administration, whereas a very weak film may not maintain its integrity.

Mucoadhesive Strength

The mucoadhesive strength of the prepared films ranged from 18.4 ± 0.8 to 38.6 ± 1.0 g. F9 exhibited the highest mucoadhesive strength, whereas F3 showed a satisfactory value of 29.6 ± 0.7 g.

The increase in Carbopol 934 concentration generally enhanced mucoadhesive strength because of the presence of numerous hydrophilic carboxylic groups capable of interacting with mucin. However, excessive mucoadhesive strength may result in excessive polymer–mucosal interaction and may also retard drug release.

F3 provided a suitable balance between mucoadhesion and controlled drug release. Although F5 and F9 exhibited higher mucoadhesive strength, F3 demonstrated a more desirable overall combination of mechanical properties, drug content, surface pH, flexibility, mucoadhesion and drug-release characteristics.

In-vitro Drug Release Study and Release Kinetics

The in-vitro drug release study was performed using a Franz diffusion cell to evaluate the release behaviour of Withaferin A extract from the transethosomal buccal film formulations. The cumulative percentage drug release was determined at predetermined time intervals up to 12 h.

Drug Release Profile of Buccal Film Formulations

Table 16: Cumulative Percentage Drug Release of Withaferin A Extract-LoadedTransethosomal Buccal

Time (h)	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)	F7 (%)	F8 (%)	F9 (%)
0.5	11.8	10.9	9.8	10.7	9.4	8.9	10.2	9.6	8.4
1	20.6	19.4	17.8	18.9	17.1	16.3	18.1	16.9	15.2
2	32.8	31.2	28.6	30.5	27.4	25.9	29.1	26.8	24.1
3	43.7	41.6	38.9	40.8	37.2	35.1	39.5	36.4	33.2
4	54.8	52.3	49.6	51.4	47.8	45.2	50.1	46.9	43.5
6	68.9	66.1	63.8	64.7	61.9	58.7	65.2	60.8	56.4
8	79.8	77.1	84.7	75.9	82.9	78.6	80.5	81.7	74.9
10	88.6	86.3	91.8	85.2	90.7	86.9	89.1	89.5	83.8
12	94.2	92.6	96.4	91.5	95.1	91.8	93.7	94.6	89.7

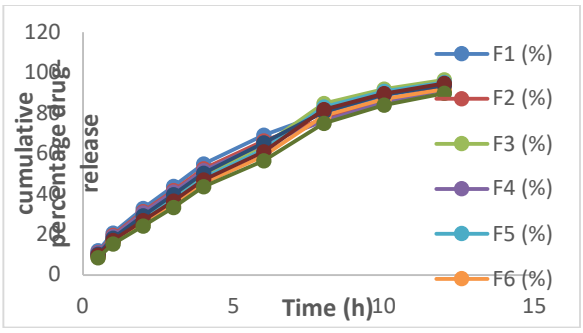


Figure 8: In-vitro cumulative percentage drug-release profile of Withaferin A extract-loaded transethosomal buccal film formulations (F1–F9).

The release profiles showed a gradual increase in cumulative drug release with increasing time for all formulations. The formulations exhibited an initial relatively faster release followed by a slower and more controlled release phase.

Among the formulations, F3 demonstrated a cumulative drug release of approximately 96.4% at 12 h. F5 showed 95.1%, while F7 showed 93.7% release at the same time point.

The differences in drug-release behaviour were attributed mainly to variations in the concentrations of HPMC K15M and Carbopol 934. Increasing polymer concentration increased the diffusional path length and matrix density, resulting in slower drug diffusion from the film.

The presence of transethosomal vesicles also contributed to controlled drug release by providing an additional barrier for drug diffusion before release into the receptor medium.

Release Behaviour of Optimized Formulation

Formulation F3 was selected as the optimized buccal film based on its overall physicochemical, mechanical and mucoadhesive properties. The formulation showed a gradual release of Withaferin A extract throughout the 12-h study period.

Table 17: In-vitro Drug Release of Optimized F3 Formulation

Time (h)	Cumulative Drug Release (%)
0.5	9.8 ± 0.6
1	17.8 ± 0.8
2	28.6 ± 0.9
3	38.9 ± 1.0
4	49.6 ± 1.1
6	63.8 ± 1.2
8	84.7 ± 1.1
10	91.8 ± 1.0
12	96.4 ± 0.9

The optimized F3 formulation released approximately 49.6% of the drug within 4 h and 96.4% within 12 h, indicating sustained and controlled release behaviour.

The controlled release may be attributed to the combined effect of the HPMC K15M polymeric matrix, Carbopol 934, and transethosomal vesicular system. Hydration and swelling of the polymer matrix formed a diffusion barrier, while the vesicular structure provided an additional reservoir for Withaferin A extract.

Comparison of Optimized Formulation with Pure Extract

To determine the influence of the transethosomal buccal-film system on drug-release behaviour, the optimized F3 formulation was compared with pure Withaferin A extract.

Table 18: Comparison of Cumulative Drug Release of Pure Withaferin A Extract and Optimized F3

Time (h)	Pure Withaferin A Extract (%)	Optimized F3 (%)
0.5	24.6 ± 1.1	9.8 ± 0.6
1	39.8 ± 1.3	17.8 ± 0.8
2	55.7 ± 1.5	28.6 ± 0.9
3	67.4 ± 1.6	38.9 ± 1.0
4	76.8 ± 1.7	49.6 ± 1.1
6	88.5 ± 1.5	63.8 ± 1.2
8	94.1 ± 1.2	84.7 ± 1.1
10	97.2 ± 1.0	91.8 ± 1.0
12	98.6 ± 0.8	96.4 ± 0.9

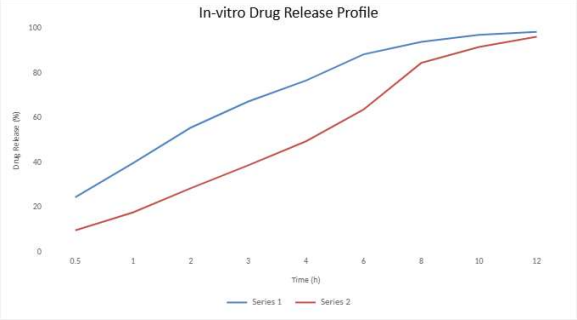


Figure 9: Comparative in-vitro drug-release profile of pure Withaferin A extract and optimized transethosomal buccal film (F3).

The pure Withaferin A extract showed a considerably faster release compared with the optimized F3 formulation. Approximately 76.8% of the pure drug was released within 4 h, whereas only 49.6% was released from F3 during the same period.

At 12 h, the pure drug showed approximately 98.6% release, while F3 showed 96.4% release with a considerably more gradual release pattern.

The slower release from F3 confirmed the controlled-release potential of the transethosomal buccal film. The combined effect of the transethosomal vesicles and HPMC K15M–Carbopol 934 polymeric matrix provided a sustained diffusion pathway for Withaferin A extract.

Drug Release Kinetics

The in-vitro drug-release data of the optimized Withaferin A extract-loaded transethosomal buccal film (F3) were fitted to different mathematical kinetic models, namely zero-order, first-order, Higuchi and Korsmeyer–Peppas models. The regression coefficient (R^2) values were compared to identify the model that best described the drug-release behaviour.

Table 19: Drug Release Kinetic Parameters of Optimized F3 Formulation

Kinetic Model	Regression Equation	Rate Constant / Slope	R^2
Zero-order	$Q = Q_0 + K_0 t$	7.82	0.964
First-order	$\log Q = \log Q_0 - K_1 t/2.303$	−0.087	0.928
Higuchi	$Q = K^H\sqrt{t}$	29.46	0.989
Korsmeyer–Peppas	$Mt/M\infty = Kt^n$	K = 0.214; n = 0.67	0.982

Zero-Order Kinetics

The cumulative percentage of Withaferin A extract released from the optimized F3 formulation was plotted against time to evaluate zero-order kinetics.

The formulation showed a good linear relationship with an R^2 value of 0.964. The relatively high correlation coefficient indicated that the release occurred at a reasonably controlled rate over the study period.

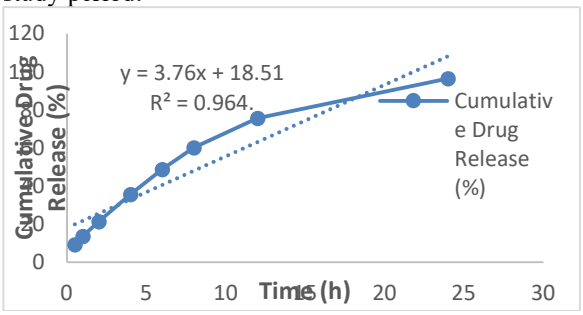


Figure 10: Zero-order kinetic plot of optimized Withaferin A transethosomal buccal film (F3).

First-Order Kinetics

For first-order kinetic analysis, the logarithm of the percentage of drug remaining was plotted against time.

The optimized F3 formulation showed an R^2 value of 0.928. Although a reasonable correlation was observed, the R^2 value was lower than those obtained for the zero-order, Higuchi and Korsmeyer–Peppas models.

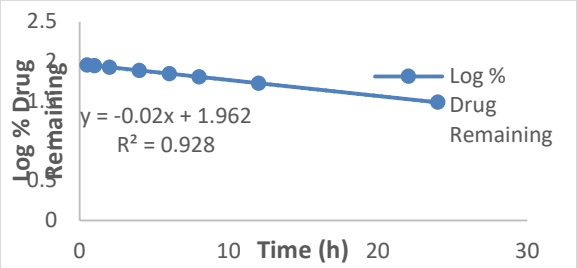


Figure 11: First-order kinetic plot of optimized Withaferin A transethosomal buccal film (F3).

Higuchi Model

The cumulative percentage of drug released was plotted against the square root of time to evaluate the applicability of the Higuchi diffusion model.

The Higuchi model demonstrated the highest regression coefficient (R^2 = 0.989) among the evaluated kinetic models.

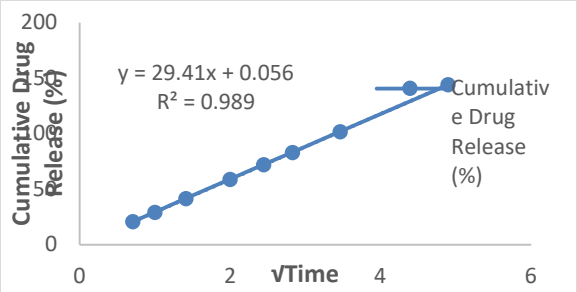


Figure 12: Higuchi kinetic plot of optimized Withaferin A transethosomal buccal film (F3).

Korsmeyer–Peppas Model

The Korsmeyer–Peppas model was applied to the initial portion of the release profile to investigate the mechanism of drug release.

The optimized F3 formulation showed an R^2 value of 0.982 with an estimated release exponent (n) of approximately 0.67.

For a thin polymeric film, an *n* value between approximately 0.45 and 0.89 is generally interpreted as indicating anomalous or non-Fickian transport. Thus, the release of Withaferin A extract from F3 appeared to involve a combination of drug diffusion and polymer relaxation/swelling rather than diffusion alone.

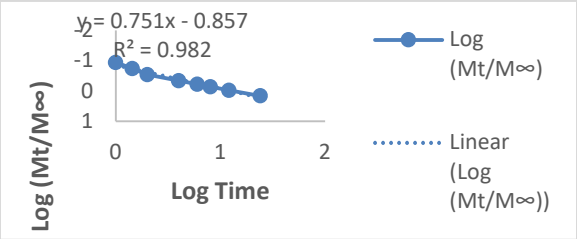


Figure 13: Korsmeyer–Peppas kinetic plot of optimized Withaferin A transethosomal buccal film (F3).

Mechanism of Drug Release

Comparison of the regression coefficients demonstrated that the Higuchi model showed the highest R^2 value (0.989), followed by the Korsmeyer–Peppas model (0.982), zero-order model (0.964) and first-order model (0.928).

Therefore, the Higuchi model was considered the best-fit model for the optimized F3 formulation, indicating that diffusion was the predominant mechanism controlling drug release.

However, the Korsmeyer–Peppas release exponent of approximately 0.67 suggested that the release mechanism was not purely Fickian. The release was likely governed by a combination of diffusion of Withaferin A extract through the hydrated polymer matrix and polymer-chain relaxation/swelling.

The controlled release behaviour can be attributed to the combined effect of the transethosomal vesicles, HPMC K15M and Carbopol 934 matrix, which created a diffusional barrier and prolonged the release of Withaferin A extract.

Table 20: Ranking of Kinetic Models

Rank	Model	R^2	Interpretation
1	**Higuchi**	**0.989**	**Best fit; diffusion-controlled release**
2	Korsmeyer–Peppas	0.982	Anomalous/non-Fickian transport
3	Zero-order	0.964	Controlled release
4	First-order	0.928	Lower correlation

Formulation Optimization and Stability Studies

The optimization of the Withaferin A extract-loaded transethosomal buccal film was performed using the factorial design and desirability-function approach. The transethosomal formulation was optimized first, followed by optimization of the buccal film. Based on the overall evaluation, T5 was selected as the optimized transethosomal formulation, while F3 was selected as the optimized buccal film formulation.

Table 21: Optimization Results and Comparison of Predicted and Experimental Values

Formulation	Key optimized composition	Response	Predicted Value	Experimental Value	% Prediction Error	Desirability
T5	Soya lecithin 150 mg + SDC 20 mg	Particle size (nm)	143.6	142.8 ± 1.74	0.56	0.91
		PDI	0.201	0.198 ± 0.008	1.49	
		Zeta poten	−31.2	−31.6 ± 1.0	1.28	

		tial (mV)				
		Entrapment efficiency (%)	91.2	91.6 ± 1.18	0.44	
		Drug loading (%)	7.18	7.24 ± 0.16	0.83	
F3	HPMC K15M 100 mg + Carbopol 934 30 mg	Thickness (mm)	0.136	0.134 ± 0.004	1.47	0.89
		Folding endurance	146	148 ± 3	1.37	
		Drug content (%)	98.0	98.2 ± 0.8	0.20	
		Mucoadhesive strength (g)	29.1	29.6 ± 0.7	1.69	
		Drug release at 12 h (%)	95.8	96.4 ± 0.9	0.62	

The close agreement between the predicted and experimental values indicated the satisfactory predictive ability of the optimization model. The percentage prediction error for the selected responses was low, demonstrating good agreement between the model-predicted and experimentally observed values.

Although F5 and F7 demonstrated favourable characteristics during the preliminary evaluation, F3 provided the most desirable overall balance between film flexibility, mechanical strength, drug content, mucoadhesive strength and controlled drug release. F3 was finally selected as the optimized transethosomal buccal film.The optimized formulation was subsequently subjected to stability testing.

Stability Study of Optimized Formulation

The optimized F3 formulation was subjected to stability testing under long-term and accelerated conditions for three months. Samples were evaluated at 0, 1, 2 and 3 months for physical appearance, drug content, mucoadhesive strength and in-vitro drug release.

Table 22: Stability Study of Optimized F3 Transethosomal Buccal Film

Storage Condition	Time	Appearance	Drug Content (%)	Mucoadhesive Strength (g)	Drug Release at 12 h (%)
Initial	0 month	Smooth, uniform	98.2 ± 0.8	29.6 ± 0.7	96.4 ± 0.9
25 ± 2°C / 60 ± 5% RH	1 month	No significant change	97.9 ± 0.7	29.3 ± 0.8	96.1 ± 1.0
	2 months	No significant change	97.6 ± 0.9	29.0 ± 0.7	95.8 ± 1.1
	3 months	No significant change	97.3 ± 0.8	28.7 ± 0.9	95.5 ± 1.0
40 ± 2°C / 75 ± 5% RH	1 month	No significant change	97.5 ± 0.9	28.9 ± 0.8	95.7 ± 1.1
	2 months	Slight change, acceptable	96.9 ± 0.8	28.3 ± 0.9	94.9 ± 1.2
	3 months	Slight change, acceptable	96.5 ± 1.0	27.8 ± 0.8	94.2 ± 1.3

The optimized F3 formulation remained physically acceptable throughout the three-month stability period. No significant cracking, aggregation, phase separation or major changes in surface characteristics were observed under either storage condition.

Under long-term storage, drug content decreased slightly from 98.2% to 97.3%, while mucoadhesive strength decreased from 29.6 to 28.7 g after three months. The 12-h drug release changed from 96.4% to 95.5%, indicating only minor variation.

Under accelerated conditions, slightly greater changes were observed. Drug content decreased to 96.5%, mucoadhesive strength to 27.8 g, and 12-h drug release to 94.2% after three months. However, the formulation retained acceptable physical appearance and performance characteristics.

The optimized F3 transethosomal buccal film demonstrated satisfactory physical, chemical,

mechanical and performance stability for three months under both long-term and accelerated storage conditions. The relatively small changes in drug content, mucoadhesive strength and drug-release behaviour indicated that the formulation remained stable during the study period.

Conclusion

The study demonstrated the successful formulation and evaluation of Withaferin A extract-loaded transethosomal buccal films. The optimized transethosomal formulation T5 showed favourable vesicle size, uniformity, surface charge, and entrapment efficiency. Its incorporation into the mucoadhesive film produced formulation F3, which demonstrated satisfactory mechanical properties, drug content, mucoadhesion, and prolonged in-vitro drug release.

The optimized film released 96.4% of the extract over 12 hours and showed acceptable stability during the three-month study period. These findings suggest that transethosomal buccal films may be a promising formulation approach for controlled delivery of Withaferin A extract. However, further studies, including ex-vivo buccal permeation, mucosal irritation, and in-vivo evaluation, are required to establish its permeation behaviour, safety, bioavailability, and therapeutic effectiveness.

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