

Development, Optimization and Characterization of Amlexanox-Loaded Electrospun Nanofibers for Oral Mucosal Drug Delivery

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Abstract:

Amlexanox-loaded electrospun nanofibers were investigated as a buccal drug-delivery system for local management of aphthous ulceration. Amlexanox was a pale-yellow crystalline, odourless, metallic-tasting powder and showed a practical melting point of 333.0 °C. UV spectrophotometric analysis in phosphate buffer pH 6.8 showed λ_{max} at 244 nm. PVA, PVP K30 and PVP K90 were screened by preliminary electrospinning trials. Drug-loaded PVP K30 did not sustain Taylor-cone formation, whereas PVP K90 supported drug-loaded electrospinning; A29 and A30 showed prolonged Taylor-cone formation and drug entrapment of 73.57% and 76.84%, respectively. A two-factor, three-level optimization study evaluated PVP K90 concentration and tip-to-collector distance. For AF1–AF10, entrapment efficiency ranged from 70.4±2.3% to 81.6±2.0%, 12-h cumulative diffusion from 47.32% to 83.73%, and 24-h cumulative diffusion from 82.16% to 97.82%. The quadratic model for entrapment efficiency was significant ($F=9.15$, $p=0.0260$; $R^2=0.9196$). AF11 showed 59.70% diffusion at 12 h and 91.58% at 24 h. The experimental dataset demonstrates the feasibility of PVP K90-based amlexanox nanofibers, while the coded-versus-actual optimization matrix and prospective stability study require reconciliation/completion before definitive optimized-product claims.

Keywords: Amlexanox; buccal drug delivery; electrospinning; nanofiber; PVP K90; aphthous ulcer; factorial optimization.

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1. Introduction and Rationale

Electrospun nanofibers provide a high specific surface area, high surface-area-to-volume ratio, and porous interconnected networks^{1-5, 15-18}. These characteristics have attracted considerable interest in pharmaceutical drug-delivery applications because electrospinning can produce fibrous matrices capable of incorporating therapeutic agents and modifying their release characteristics^{1, 2, 15-18}.

The development of electrospun drug-delivery systems is influenced by polymer characteristics, solution properties, and processing variables, including polymer concentration, applied voltage, flow rate, needle characteristics, and tip-to-collector distance^{1, 15-18}. Appropriate optimization of these parameters is therefore important for achieving reproducible fiber formation and suitable drug-delivery performance.

Electrospun polymeric systems have been investigated for the delivery of poorly soluble drugs and for mucosal, sublingual, and controlled-release applications⁶⁻⁹. The use of polymeric nanofibers for poorly soluble drugs may provide advantages associated with increased surface area and improved dispersion of the incorporated drug^{6, 18}.

For the amlexanox phase of the present research, the rationale was to develop a local oral/buccal drug-delivery system. Buccal administration provides direct access to the oral mucosa and has been widely investigated using mucoadhesive patches and related polymeric systems^{11, 13, 14}. Electrospun and mucoadhesive fibrous systems may additionally provide prolonged contact of the formulation with the mucosal surface^{7-9, 14}.

Amlexanox is an anti-inflammatory/anti-allergic drug that has been investigated for the treatment of recurrent aphthous ulceration^{10, 12, 19, 20}. Clinical studies of amlexanox oral adhesive formulations have reported its application in recurrent aphthous stomatitis and minor aphthous ulcers^{12, 19, 20}.

The present research therefore investigated amlexanox-loaded electrospun nanofibers as a potential oral mucosal drug-delivery system. PVA, PVP K30, and PVP K90 were initially screened, followed by drug-loading studies and optimization of the selected polymer system.

2. Materials and Methods

Electrospinning and Preliminary Screening Procedure

The in-house electrospinning assembly comprising a high-voltage arc generator, syringe/infusion pump, and spinal needles. Polymer solutions were prepared in the solvent systems specified for each experimental trial.

Polymer concentration, needle gauge, and tip-to-collector distance were varied during preliminary screening. These variables are among the important formulation and processing parameters affecting electrospinning behavior and nanofiber formation^{1, 15-18}.

Taylor-cone formation was used as the primary screening response. Stable Taylor-cone formation is an important practical indicator during the electrospinning process because it reflects the interaction between the applied electrical field and the polymer solution^{1, 15, 16}.

In the amlexanox phase, PVA was screened at 8, 10, and 12% w/v in water; PVP K30 at 30, 35, and 40% w/v in 10 mL ethanol; and PVP K90 at 15, 20, and 25% w/v in 10 mL ethanol. Drug-loading trials were subsequently performed using the selected polymer systems.

Rationale for PVP-Based Nanofibers

Polyvinyl pyrrolidone has been employed as a hydrophilic polymer in electrospun pharmaceutical systems, including formulations intended for rapid or mucosal drug delivery⁷. The suitability of an electrospinning polymer depends on factors such as molecular weight, polymer concentration, chain entanglement, viscosity, solvent compatibility, and processing conditions^{1, 15-18}.

Accordingly, PVP K30 and PVP K90 were experimentally screened rather than assuming equivalent electrospinning performance. The experimental findings demonstrated that drug-loaded PVP K30 did not sustain the required Taylor-cone formation under the tested conditions, whereas PVP K90 showed more favorable drug-loaded electrospinning behavior.

Drug profile and marketed preparations

Table 1. Drug profile of amlexanox.

Parameter	Reported information
Brand name	Aphthera, Aphthasol
Chemical name	2-Amino-7-isopropyl 5-oxo-5H-chromeno[2,3-b]pyridine-3-carboxylic acid
Chemical formula	C ₁₆ H ₁₄ N ₂ O ₄
Molecular weight	298.29 g/mol
Description	Pale yellow crystalline powder
Solubility	Soluble in 95% ethanol, DMF, DMSO and phosphate buffer pH 6.8; sparingly soluble in aqueous buffer
Melting point (profile value)	320.24 °C
Half-life	3.5±1.1 h
Dose	4 mg four times a day
Dose strength	5 mg paste; 2 mg oral tablet
Log P	3.65
Log S	-3.3
Category	Anti-inflammatory; anti-allergic (topical)/anti-allergic
BCS class	Class II
Use	Aphtha's ulcer

Table 2. Market preparations.

Company	Brand	Route	Dosage form	Strength
Uluru Inc., Texas, USA	Aphthasol®	Oral	Paste	5% w/w
Uluru Inc., Texas, USA	OraDisc®	Oral	Erodible mucoadhesive disc	5% w/w
Cayman Chemicals, USA	Solfa®	Oral	Paste	5% w/w
Cayman Chemicals, USA	ELICS®	Teeth paste	Teeth paste	5% w/w

Materials**Table 3. Probable polymers/materials screened or proposed.**

Material/polymer	Supplier
Polyethylene oxide N80	Emcure Pharmaceuticals Ltd., Gandhinagar, Gujarat
Polyethylene oxide N303	Emcure Pharmaceuticals Ltd., Gandhinagar, Gujarat
Polyvinyl alcohol (PVA)	Chemlink Corporation
Polyvinylpyrrolidone K30, K60, K90	Chemlink Corporation
Chitosan	Emcure Pharmaceuticals Ltd., Gandhinagar, Gujarat
Eudragit RS	Chemlink Corporation
Polylactic acid (PLA)	Chemlink Corporation

Table 4. Solvents/Material.

Solvent/material	Supplier
Purified water	In House facility
Chloroform	Chemlink Corporation, Ahmedabad, Gujarat
Acetone	Chemlink Corporation, Ahmedabad, Gujarat
Isopropyl alcohol	Chemlink Corporation, Ahmedabad, Gujarat
Methanol	Chemlink Corporation, Ahmedabad, Gujarat
Chloroform (duplicate entry in source)	Chemlink Corporation, Ahmedabad, Gujarat
Ethanol	Astron Chemicals, Ahmedabad, Gujarat

Equipment**Table 5. Equipment used.**

Equipment	Reported make/supplier
Digital weighing balance	Virgo, Ahmedabad, Gujarat
High-voltage arc generator	Robokits, India
Syringe/infusion pump	Fresenius Kabi, Agilia
Spinal needles, 18/22/25 G	Becton Dickinson
Magnetic stirrer	Emtek, Mumbai
UV–Visible spectrophotometer	Shimadzu UV-1800, Japan
Dissolution apparatus	Veego Instrument Corporation, Mumbai
Franz diffusion cell	Polylab, India

Preformulation studies

Preformulation comprised morphological/organoleptic evaluation, melting-point determination, UV–Visible identification and calibration, and FTIR-based drug–excipient compatibility assessment. For UV identification, a 3.0 µg/mL amlexanox solution in phosphate buffer pH 6.8 was scanned from 200 to 400 nm using the Shimadzu UV-1800; λ_{max} was observed at 244 nm. Calibration standards from 3–8 µg/mL were measured in triplicate at 244 nm.

Electrospinning and preliminary screening procedure

The in-house electrospinning assembly comprising a high-voltage arc generator, syringe/infusion pump and spinal needles. Polymer solutions were prepared in the solvent systems specified for each trial and evaluated by varying polymer concentration, needle gauge and tip-to-collector distance. Taylor-cone formation was the screening response. In the amlexanox phase, PVA was screened at 8, 10 and 12% w/v in water; PVP K30 at 30, 35 and 40% w/v in 10 mL ethanol; and PVP K90 at 15, 20 and 25% w/v in 10 mL ethanol. Drug-loading trials were then performed in the selected polymer systems.

Table 6. Preliminary PVA electrospinning trials (A1–A9).

Batch	PVA (% w/v, water)	Needle (G)	Distance (cm)	Taylor cone
A1	8	18	5	+
A1	8	18	10	+
A1	8	18	15	-
A2	8	20	5	+
A2	8	20	10	+
A2	8	20	15	-
A3	8	22	5	+
A3	8	22	10	+
A3	8	22	15	-

A4	10	18	5	+
A4	10	18	10	+
A4	10	18	15	-
A5	10	20	5	+
A5	10	20	10	+
A5	10	20	15	-
A6	10	22	5	+
A6	10	22	10	-
A6	10	22	15	-
A7	12	18	5	+
A7	12	18	10	+
A7	12	18	15	-
A8	12	20	5	+
A8	12	20	10	+
A8	12	20	15	-
A9	12	22	5	+
A9	12	22	10	+
A9	12	22	15	-

Table 7. Preliminary PVP K30 electrospinning trials (A10–A18).

Batch	PVP K30 (% w/v, ethanol)	Needle (G)	Distance (cm)	Taylor cone
A10	30	18	5	-
A10	30	18	10	-
A10	30	18	15	+
A11	30	20	5	-
A11	30	20	10	+
A11	30	20	15	+
A12	30	22	5	+
A12	30	22	10	+
A12	30	22	15	+
A13	35	18	5	-
A13	35	18	10	+
A13	35	18	15	+
A14	35	20	5	-
A14	35	20	10	+

A14	35	20	15	+
A15	35	22	5	+
A15	35	22	10	++
A15	35	22	15	++
A16	40	18	5	-
A16	40	18	10	-
A16	40	18	15	+
A17	40	20	5	-
A17	40	20	10	+
A17	40	20	15	+
A18	40	22	5	+
A18	40	22	10	++
A18	40	22	15	++

Table 8. Drug-loading trials with PVP K30.

Batch	Drug (mg)	PVP K30 (% w/v)	Needle (G)	Taylor cone
A15	10	35	22	-
A15	5	35	22	-
A15	2	35	22	-
A18	10	40	22	-
A18	5	40	22	-
A18	2	40	22	-

Table 9. Preliminary PVP K90 electrospinning trials (A19–A27).

Batch	PVP K90 (% w/v, ethanol)	Needle (G)	Distance (cm)	Taylor cone
A19	15	18	5	-
A19	15	18	10	+
A19	15	18	15	+
A20	15	20	5	-
A20	15	20	10	++
A20	15	20	15	+
A21	15	22	5	+
A21	15	22	10	++
A21	15	22	15	+

A22	20	18	5	-
A22	20	18	10	+
A22	20	18	15	+
A23	20	20	5	-
A23	20	20	10	++
A23	20	20	15	+
A24	20	22	5	+
A24	20	22	10	++
A24	20	22	15	++
A25	25	18	5	-
A25	25	18	10	-
A25	25	18	15	+
A26	25	20	5	-
A26	25	20	10	+
A26	25	20	15	+
A27	25	22	5	+
A27	25	22	10	++
A27	25	22	15	+

Table 10. Amlexanox-loading trials with PVP K90.

Batch	Drug (mg)	PVP K90 (% w/v)	Ethanol (mL)	Taylor cone	Drug entrapment (%)
A28	10	20	10	-	—
A29	5	20	10	++	73.57
A30	7.5	20	10	++	76.84
A31	8	20	10	+	59.61

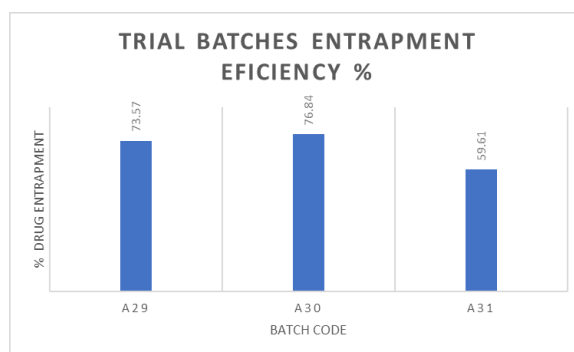


Figure 1. Experimental image embedded in the PVP K90 drug-loading.

Optimization design

A two-factor, three-level design was used with PVP K90 concentration (X1) and tip-to-collector distance (X2) as independent variables. Entrapment efficiency (Y1) and in vitro drug diffusion at 12 h (Y2) were dependent responses. The X1 levels as 15, 20 and 25% w/v and X2 levels as 10, 12.5 and 15 cm for coded levels -1, 0 and +1, respectively.

Table 11. Factor levels reported for optimization.

Coded level	X1: PVP K90 (% w/v)	X2: distance (cm)
-1	15	10
0	20	12.5
+1	25	15

Entrapment efficiency and in vitro diffusion methodology

Entrapment efficiency was used as Y1 and drug diffusion as Y2. The Franz diffusion cell and a dissolution apparatus among the available equipment are used

Organoleptic and melting-point evaluation*Table 12. Morphological/organoleptic evaluation of amlexanox.*

Property	Observation
State	Fine powder
Colour	Pale yellow crystalline powder
Odour	Odourless
Taste	Metallic

Table 13. Melting-point evaluation.

Theoretical range	Practically obtained
330–334 °C	333.0 °C

UV–Visible spectrophotometric analysis

A 3.0 µg/mL amlexanox solution in phosphate buffer pH 6.8 showed an absorption maximum at 244 nm after scanning from 200–400 nm.

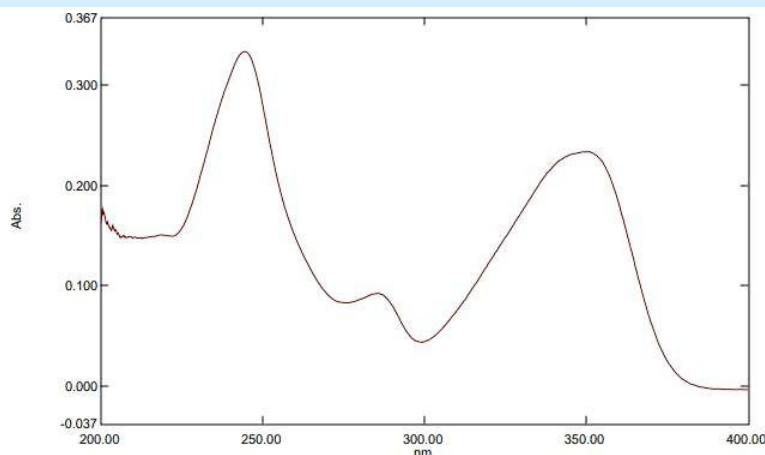


Figure 2. Original UV-range spectrum of amlexanox in phosphate buffer pH 6.8; λ_{max} 244 nm

Table 14. Complete UV calibration at 244 nm.

Conc. ($\mu\text{g/mL}$)	Abs. 1	Abs. 2	Abs. 3	Average absorbance	SD (n=3)
3	0.232	0.225	0.218	0.225	0.0057
4	0.314	0.339	0.331	0.328	0.0104
5	0.421	0.417	0.414	0.417	0.0029
6	0.552	0.549	0.551	0.551	0.0012
7	0.689	0.682	0.679	0.683	0.0042
8	0.787	0.781	0.784	0.784	0.0024

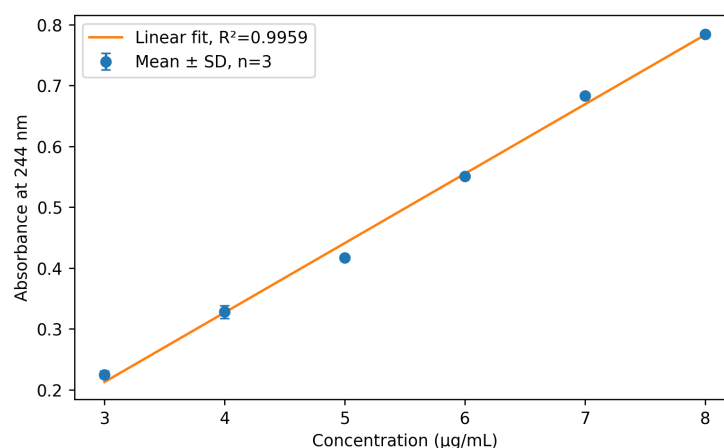


Figure 3. Calibration curve of the triplicate dataset (linear-fit $R^2=0.9959$).

FTIR drug–excipient compatibility assessment

Table 15. FTIR peak interpretation for amlexanox with excipients.

Functional group	Vibration	Standard frequency (cm^{-1})	Observed frequency (cm^{-1})
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Aromatic ring	Stretching	1650–2000	1660
C=O	Stretching	1720–1740	1740
O–H	Stretching	3400–3700	3410
C–H	Stretching	2850–2975	2860
N–H	Stretching	3300–3350	3310
C=C	Stretching	1640–1680	1630

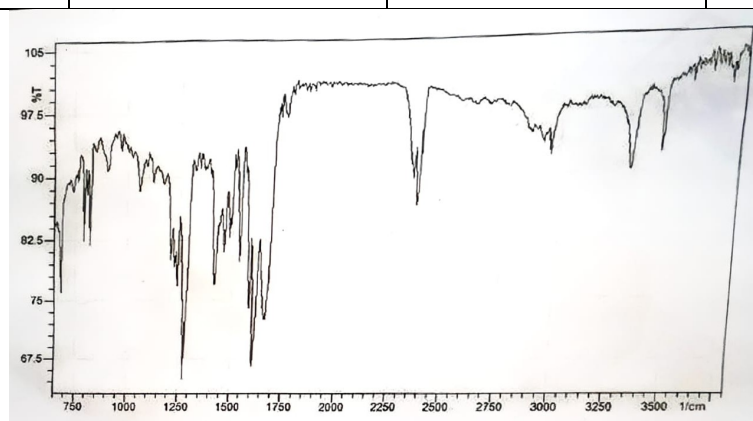


Figure 4. FTIR image for the drug–excipient compatibility.

Optimization results

Table 16. Factorial design of batches.

Batch	Coded X1	Coded X2	Actual X1 (%)	Actual X2 (cm)	Y1 EE (%)	Y2 diffusion at 12 h (%)
AF1	-1	1	15	15	70.4 ± 2.3	56.21
AF2	1	-1	20	10	75.2 ± 2.1	83.73
AF3	1	1	20	15	78.4 ± 1.6	48.95
AF4	0	0	25	12.5	79.8 ± 1.7	80.41
AF5	-1	-1	15	10	71.6 ± 1.8	80.52
AF6	0	-1	25	10	73.4 ± 1.5	76.10
AF7	1	0	20	12.5	81.6 ± 2.0	47.32
AF8	0	-1	25	10	73.9 ± 2.0	77.27
AF9	0	1	25	15	76.2 ± 1.9	61.60
AF10	-1	0	15	12.5	71.4 ± 1.3	68.05

AF7 showed the highest reported entrapment efficiency ($81.6 \pm 2.0\%$), while AF2 showed the highest 12-h diffusion (83.73%). These endpoints therefore identify different high-performing batches and support multi-response optimization rather than selection from a single response.



Figure 5. Entrapment-efficiency graph for AF1–AF10.

Table 17. ANOVA for quadratic model of Y1 entrapment efficiency.

Source	Sum of squares	df	Mean square	F-value	p-value	Interpretation
Model	118.38	5	23.68	9.15	0.0260	Significant
A—Polymer concentration	79.21	1	79.21	30.61	0.0052	
B—Distance	5.06	1	5.06	1.96	0.2345	
AB	4.84	1	4.84	1.87	0.2432	
A ²	5.71	1	5.71	2.21	0.2116	
B ²	24.94	1	24.94	9.64	0.0361	
Residual	10.35	4	2.59	--	--	
Lack of fit	10.23	3	3.41	27.27	0.1396	Not significant
Pure error	0.1250	1	0.1250	--	--	
Cor total	128.73	9	--	--	--	

Table 18. Model-summary statistics for Y1.

Statistic	Value	Statistic	Value
Std. Dev.	1.61	R ²	0.9196
Mean	75.19	Adjusted R ²	0.8191
C.V. %	2.14	Predicted R ²	0.2452
		Adeq Precision	8.8010

Reported coded-factor equation: Entrapment efficiency = 78.64 + 3.63A + 0.867B + 1.10AB – 1.56A² – 3.46B².

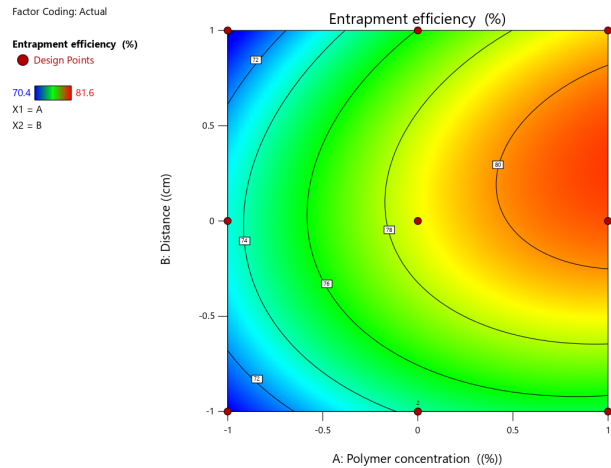


Figure 6. Original 2D contour plot for entrapment efficiency.

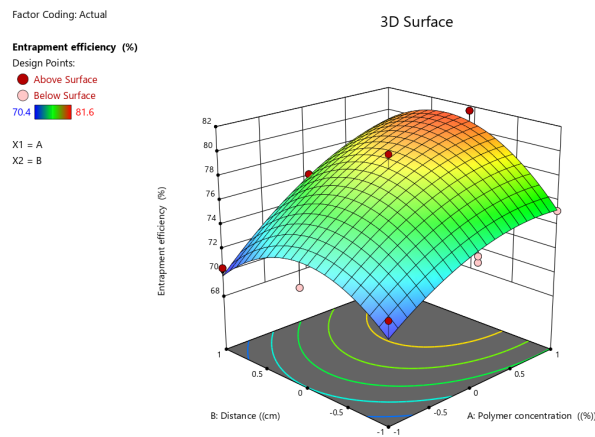


Figure 7. Original 3D response-surface plot for entrapment efficiency.

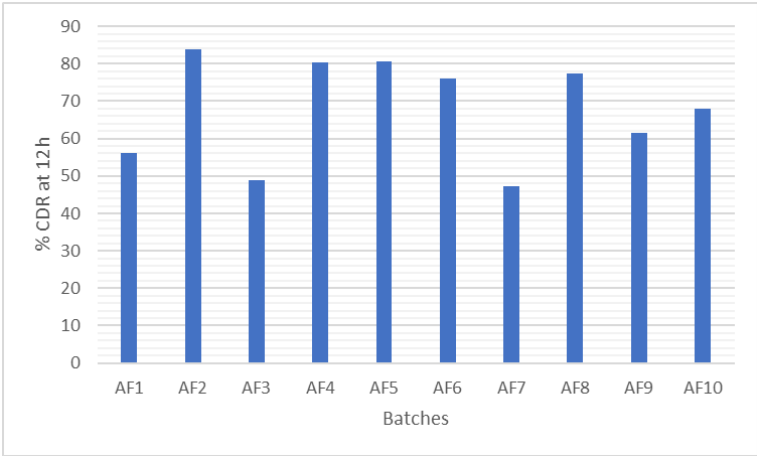


Figure 8. Y2, 12-h cumulative drug diffusion,,
In vitro drug-diffusion data

Table 19. cumulative drug-diffusion data for AF1–AF10.

Time	AF1	AF2	AF3	AF4	AF5	AF6	AF7	AF8	AF9	AF10
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(h)										
0	0	0	0	0	0	0	0	0	0	0
0.5	2.11	4.05	2	4.01	4.1	3.51	1.81	3.91	2.51	2.82
1	4.34	9	3.91	8.22	8.4	7.21	3.42	7.82	5.03	6.15
2	8.51	18.08	7.42	17.11	17.32	15.9	6.91	16.23	9.51	12.86
4	13.7	30.51	11.61	27.52	27.6	23.53	10.81	25	15.22	20.41
6	34.51	63.57	29.62	60.02	59.52	52.61	28.26	55.01	37.91	45.08
8	41.62	75.21	32.91	66.48	73.61	69.82	33.51	63.14	45.97	51.5
10	52.62	81.7	45.81	78.42	78.01	72.42	44.21	70.55	50.81	64.93
12	56.21	83.73	48.95	80.41	80.52	76.1	47.32	77.27	61.6	68.05
24	88.63	97.82	85.84	96.41	96.81	94.61	82.16	95.12	90.41	91.81

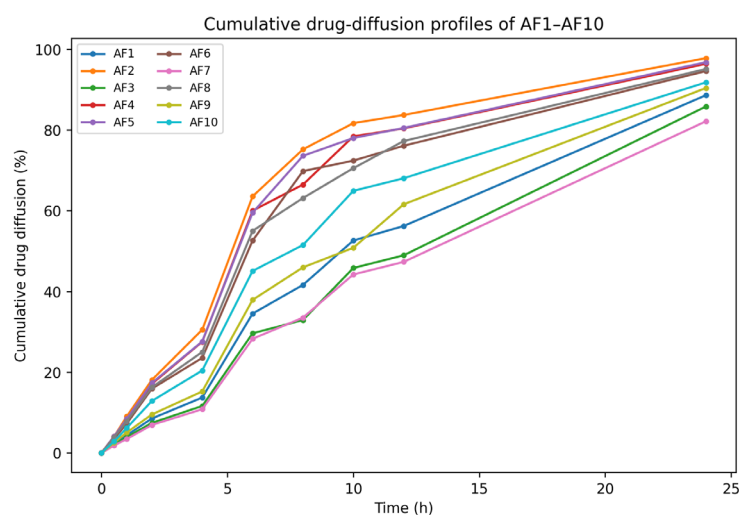


Figure 9. Cumulative drug-diffusion profiles of AF1–AF10.

At 24 h, diffusion ranged from 82.16% (AF7) to 97.82% (AF2). AF5 and AF4 reached 96.81% and 96.41%, respectively. The full profiles show formulation-dependent separation particularly between 6 and 12 h.

Table 20. AF11 drug-diffusion.

Time (h)	AF11 cumulative diffusion (%)
0	0
0.5	2.01
1	4.23
2	8.51
4	16.23
6	30.04
8	39.64

10	49.81
12	59.7
24	91.58

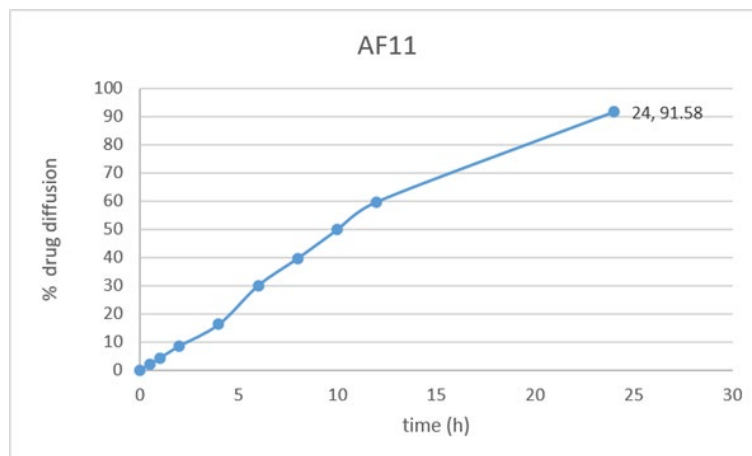


Figure 10. AF11 drug-diffusion graph.

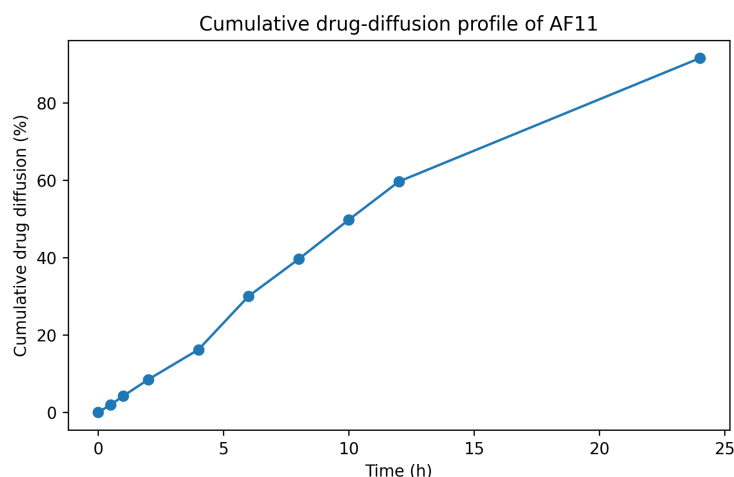


Figure 11. AF11 cumulative drug-diffusion profile.

Stability study status

The stability data describes an accelerated stability study of the amlexanox sublingual/buccal nanofiber strip at 40 ± 2 °C/ $75 \pm 5\%$ RH for six months in aluminium foil, with intended sampling at 3 and 6 months for entrapment efficiency, drug diffusion and SEM analysis.

3. Discussion

The preliminary screening clearly differentiated the three polymer systems. PVA generated Taylor cones over several conditions but generally lost cone formation at 15 cm. PVP K30 showed stronger cone persistence at higher concentration and smaller needle diameter, yet the selected drug-loaded A15/A18 trials failed to maintain a Taylor cone at 2–10 mg drug loading. In contrast, PVP K90 retained drug-loaded electrospinning behavior: A29 (5 mg) and A30 (7.5

mg) both showed prolonged cone formation (++) and entrapment of 73.57% and 76.84%, respectively.

In the factorial study, the Y1 quadratic model was statistically significant. Polymer concentration was significant ($p=0.0052$), while the linear distance term and AB interaction were not significant; the B^2 term was significant ($p=0.0361$), indicating a nonlinear distance effect. However, the predicted R^2 (0.2452) was substantially lower than the adjusted R^2 (0.8191), and the run table contains coded/actual-level inconsistencies.

Drug diffusion varied more widely than entrapment efficiency. AF2 produced the highest 12-h and 24-h values (83.73% and 97.82%), whereas AF7 showed the highest entrapment efficiency but comparatively low diffusion (47.32% at 12 h and 82.16% at 24 h). This demonstrates that loading and release behavior should be optimized simultaneously. AF11 reached 59.70% at 12 h and 91.58% at 24 h, its exact status as an optimized or confirmatory formulation has been supported by the optimization data.

4. Conclusion

The complete dataset supports PVP K90 as the most suitable of the reported screened polymers for drug-loaded amlexanox electrospinning. The study documents preformulation, UV and FTIR assessment, systematic preliminary polymer screening, PVP K90 drug-loading trials, factorial optimization, ANOVA for entrapment efficiency, response-surface/contour outputs and full 24-h diffusion profiles.

5. Acknowledgement

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6. Conflict of Interest

The authors declare no conflict of interest.

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