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QbD-steered fabrication of lisinopril ion-pair gel for improved skin permeability and bioavailability in rabbits

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ABSTRACT: Lisinopril belongs to BCS class III having high solubility and low permeability. Reportedly, the oral bioavailability of lisinopril is 25 to 30% and its effectiveness is limited due to poor permeability. Hence, the current investigation is aimed to formulate transdermal ion-pair gel using a permeation enhancer for enhanced delivery of lisinopril across the *stratum corneum* and evaluate pharmacokinetics of lisinopril in rabbits. The formation of ion-pair is corroborated using Fourier Transform Infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC), X-ray Diffraction (XRD), zeta potential, particle size analysis, oil/water partition coefficient study, etc. Optimization of the formulation was done using 3^2 factorial designs. Total nine batches (F1-F9) were prepared and the effect of propylene glycol (mL; X_1) and carbopol 934 (%; X_2) was investigated on gel viscosity (Y_1) and permeability through rabbit's skin at 8h (Y_2). Propylene glycol exhibited a non-significant ($p>0.05$) effect on both gel viscosity and skin permeability whereas carbopol 934 demonstrated significant ($p<0.05$) positive and negative effects on both, respectively. The viscosity of all the lisinopril ion-pair gel (F1-F9) was ranging between 17.24 ± 2.16 Pa.s (Batch F9) to 7.54 ± 1.34 Pa.s (Batch F4). *Ex-vivo* permeability of all the prepared batches (F1-F9) across excised rabbit's skin was ranging between 85.93 ± 1.26% (Batch F4) to 62.17 ± 1.57% (Batch F9). Remarkably, optimized formulation (F4) exhibited 1.7 folds improvement in skin permeability and 2.4 folds improvement in bioavailability than plain lisinopril gel. These findings demonstrate that ion pair formation is a promising strategy for significantly improving the skin permeability of lisinopril.

KEYWORDS: Ion-pair formation; permeation enhancer; topical drug delivery; Carbopol 934; Angiotensin Converting Enzyme.

1. INTRODUCTION

Since the 1970s, the Angiotensin Converting Enzyme (ACE) inhibitor class of drugs is serving mankind to effectively treat all grades of heart failure, hypertension, diabetic nephropathy, and prophylaxis of cardiovascular events [1]. Lisinopril [(*S*)-1-[*N*2-(1-carboxyl-3-phenylpropyl)-*L* -lysyl]-*L*-proline dehydrate] is one of the preferred ACE inhibitors. It is primarily recommended for the management of high blood pressure, congestive heart failure, and the development of survival rate following myocardial infarction [2]. After oral administration, about 30% of the lisinopril is absorbed and peak serum concentration is reached within 6–8 h [1]. For hypertension patients allied with diabetes mellitus and microalbuminuria or overt nephropathy, lisinopril is exceedingly recommended to avert the progression of renal disease [3, 4].

Albeit effectual, lisinopril is a BCS class III drug having high solubility and low permeability. Currently, the oral route is preferred for lisinopril administration. However, limited benefits are achieved on oral delivery due to hepatic first-pass metabolism, unchanged excretion of drug, high and frequent dosing requirement, which leads to both inconvenience and high cost of therapy. Lisinopril is associated with marred oral bioavailability (25-30%), attributed to incomplete and slow absorption through the oral route [5]. These potential drawbacks of lisinopril urge an alternative drug delivery system or route for its systemic delivery and to achieve desired health benefits.

The transdermal drug delivery system has been widely investigated for the administration of a diverse class of therapeutic agents, particularly in pain management, hormonal therapy, and the treatment of cardiovascular and central nervous systems diseases [6]. The potential of ample pharmaceutical dosage forms has been explored to deliver drugs across the skin. The most preferred ones are gels, creams, and ointments, followed by sprays and liquid preparations [7–9]. Gels are capable of increasing the resistance time of the therapeutic agents and also facilitating their delivery across the skin [9, 10].

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Permeability of a wide variety of therapeutic agents across the skin is restrained due to barrier properties of the stratum corneum [11]. This highlights the necessity for the inclusion of penetration enhancers into transdermal dosage forms that could effectively deliver a wide variety of active pharmaceutical agents [12]. Over the years, different types of chemical penetration enhancers have been investigated as a promising approach to diminish barrier properties of intact skin, leading to drug permeation through the skin at an apt rate for a suitable time [13]. Ideal penetration enhancers should be pharmacologically inert, compatible with drugs and formulation additives, nonirritant, and potent [12, 13]. Propylene glycol has been widely investigated as a skin penetration enhancer [14, 15].

In recent times, ion-pairing has emerged as a promising approach to enhance the permeation of topically applied ionized drugs through the stratum corneum. This approach involves the formation of neutral species once the electrostatic energy of attraction between oppositely charged ions exceeds their mean thermal energy [16]. Notably, polar counter ions are replaced stoichiometrically with more hydrophobic ones to achieve hydrophobic ion pairing. This system is been successfully implemented for enhancing the solubility of ionic molecules in nonpolar solvents, augmentation of protein, and addressing the issues of poor bioavailability of hydrophilic drugs [17].

Carbopol is extensively used by researchers as a gelling agent to impart viscosity to formulations. Carbomers, acrylic acid-based polymers are crosslinked with allyl sucrose or allyl pentaerythritol and the degree of cross-linking determines their viscosity [18]. Carbomers offer several advantages namely, high viscosity at low concentrations, heat stability, pleasant texture, non-irritancy, etc [12].

Statistical design of experiments (DOE) is useful for the construction of a predictive model of the critical response variables. It mainly assists in simultaneously identifying the significant factors and interactions amongst several independent variables [19].

In this study, an attempt has been made to investigate the suitability of the ion-pair approach combined with a penetration enhancer as a dual strategy to improve the topical delivery of lisinopril through the stratum corneum. Prepared lisinopril ion-pair system was incorporated into the gel. Optimization of gel formulation was done using the design of experiments approach (DOE) where the effect of propylene glycol (permeation enhancer) and carbopol 934 (gelling agent) was systemically investigated on the viscosity of gel and in-vitro permeation of lisinopril across the rabbit's skin. Further, the in-vivo performance of gel formulation was investigated using rabbits.

2. RESULTS

2.1. Preparation of lisinopril ion-pair

Figure 1 indicates the probable 2D structure (Figure 1A), ball and sticks model representation (Figure 1B), and space field model (Figure 1C) for synthesized lisinopril ion-pair. While preparing the lisinopril ion pair, both positive and negative charged counter ions from lisinopril were used.

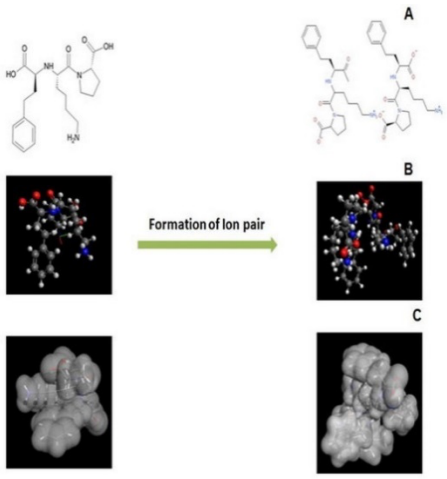

Figure 1. Formation of lisinopril ion-pair, (A) 2D structure, (B) ball and stick model and (C) space field model

2.2. FTIR spectroscopy

FTIR spectrum of lisinopril revealed characteristic peaks representing the presence of functional groups present in its chemical structure as shown in Figure 2. From this, the identity and purity of lisinopril get confirmed. As shown in Figure 2A, lisinopril exhibited characteristics peak of the carbonyl group at 1653 cm^{-1} while two distinct peaks at 1651 and 1729 cm^{-1} indicate a change in IR spectrum of the carbonyl group participating in ion pairing. Both peaks being in the region of the carbonyl group, IR absorption band confirms a change in electronic environment but no chemical reaction leading to a change in the covalent bonding. Peaks of benzene ring at 700 cm^{-1}, 1449 cm^{-1}, and 1137 cm^{-1} are assigned to C-H bending, C-C stretching, and C-N stretching respectively. A stronger broader N-H stretching band in the region between 2865 and 3086 cm^{-1} supports the presence of quaternary nitrogen of the ion pair. Similarly, the sharp IR absorption band of primary amine at 1607 cm^{-1} in lisinopril changes to a relatively weaker peak in the ion pair, indicates the proposed chemical change.

FT-IR Spectra of drug and its ion-pair (Figure 2B) corroborates its identity as all the characteristic peaks corresponding to the functional group present in the molecular structure of lisinopril were found within the reference range. Characteristic differences stated above related to the carbonyl group and the quaternary nitrogen in the spectrum of the ion pair confirm the presence of the proposed ion pair.

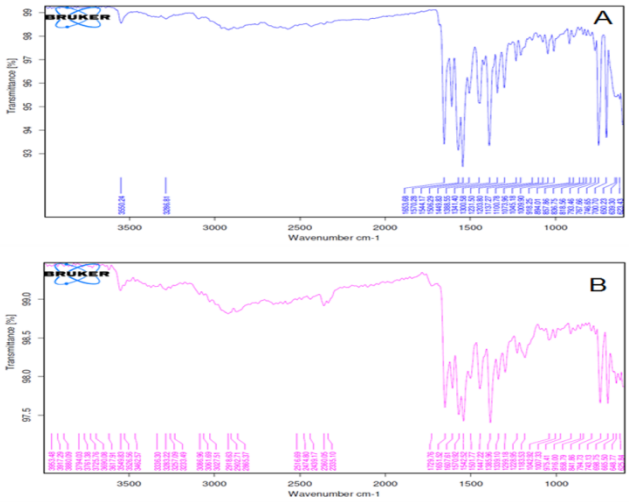

Figure 2. FTIR Spectra of (A) lisinopril (B) lisinopril ion pair

2.3. Differential scanning calorimetry (DSC) analysis

DSC thermogram of lisinopril (Figure 3A) showed a sharp endothermic peak at 180 ^oC, corresponding to its melting point while lisinopril ion-pair exhibited the endothermic peak at 172.7^oC (Figure 3B). Thus shifting in melting peak corroborates the formation of ion-pair in between lisinopril molecules.

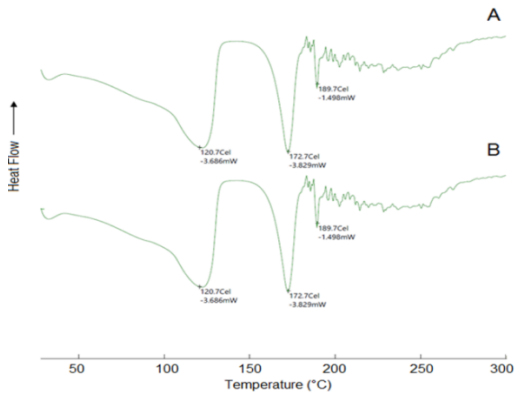

Figure 3. DSC thermogram of (A) lisinopril (B) lisinopril ion pair

2.4. X-ray diffraction analysis (XRD)

The X-ray diffraction pattern of pure lisinopril and lisinopril ion-pair is depicted in Fig. 4. XRD spectrum of lisinopril (Figure 4A) exhibited strong diffraction peaks at 10.90°, 11.45°, 17.71°, indicating its crystalline nature. XRD spectrum of lisinopril ion pair (Figure 4B) exhibited diffraction peaks with somewhat reduced intensities indicating its partial crystalline nature.

2.5. Zeta potential and particle size analysis

The particle size of lisinopril and its ion-pair was observed to be 159.1 nm and 242.5 nm, respectively (Figure 5A; Figure 5B). The difference in particle size of lisinopril and its ion-pair confirms the formation of ion-pair. The Zeta potential of lisinopril and lisinopril ion-pair was found to be -31 mV and -27.5 mV, respectively (Figure 5C; Figure 5D). A shift in zeta potential values indicates the formation of ion-pair.

2.6. Oil-water partition coefficient study

Log P value for lisinopril determined using N-octanol-water system was found to be -3.1. This indicates the hydrophilic nature of lisinopril, attributed to its amino acid moiety and sulfonamide group. Notably, the log p-value for the lisinopril ion-pair complex was determined to be -0.583. Thus, it was seen that the hydrophobicity of lisinopril was increased due to ion-pair. This enhancement might be responsible for the permeation of the drug through the *stratum corneum*.

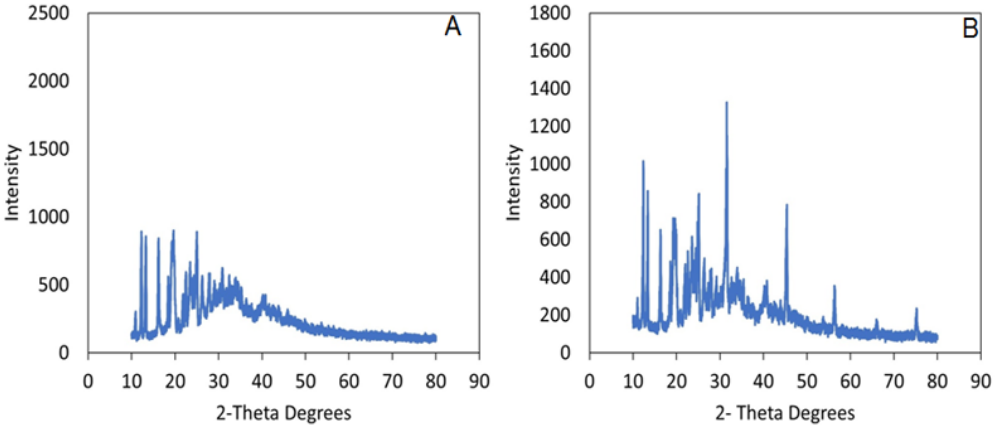

Figure 4. XRD Spectra of (A) lisinopril (B) lisinopril ion pair

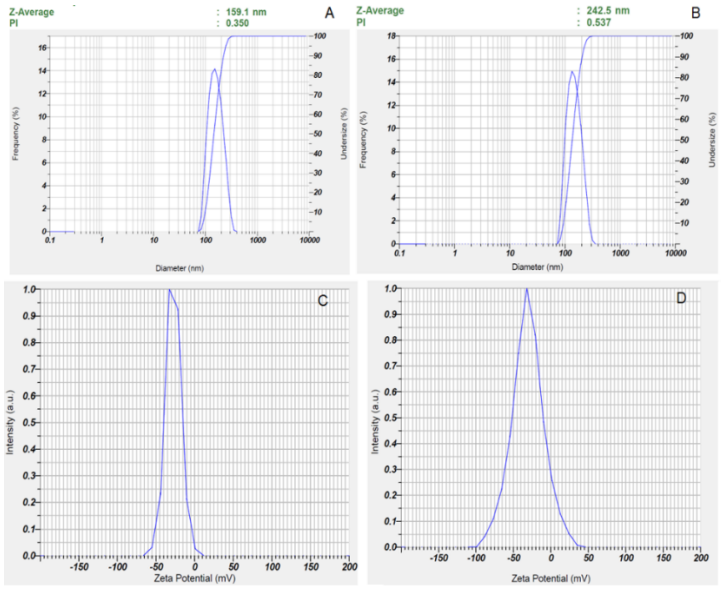

Figure 5. Particle size analysis of (A) lisinopril (B) lisinopril ion pair. Zeta potential of (C) lisinopril (D) lisinopril ion pair

2.7. Formulation of lisinopril gel formulation

The detailed composition of different batches of gel is shown in Table 1.

Table 1. Composition of different gel formulations

Formulation	Drug	Drug ion-pair	Carbopol 934 (%)	Propylene Glycol (mL)	TEA	Glycerine
Lisinopril Gel	10 mg	-	0.5	400	q. s.	up to 1000 mg
Lisinopril Ion-pair Gel	-	10 mg	0.5	400	q. s.	up to 1000 mg

2.7.1. Physical examination

The prepared lisinopril and lisinopril ion-paired gel were inspected visually for their color, homogeneity, and consistency. Prepared gel formulations were colorless, transparent, homogeneous, and consistent.

2.7.2. Determination of pH

The pH of lisinopril and lisinopril ion-paired gel was found to be 6.45 ± 0.15 and 6.56 ± 0.21 respectively.

2.7.3. Determination of drug content

The drug content of lisinopril gel formulation and lisinopril ion pair gel formulation was found to be 99.11 ± 0.11 and 100.67 ± 0.56, respectively.

2.7.4. Viscosity measurement

The viscosity of lisinopril gel and lisinopril ion pair gel was found to be 7.7892 Pa.s and 7.1478 Pa.s, respectively.

2.7.5. Spreadability

Lisinopril ion-pair gel exhibited higher spreadability (30.08 ± 1.16 g.cm/sec) than plain lisinopril based gel (28.47 ± 1.19 g.cm/sec). This could be attributed to ion-pair formation.

2.7.6. Ex-vivo permeation study

Lisinopril ion-pair gel significantly ($p < 0.05$) improved the permeation of lisinopril (80.22 ± 3.19 %) across the excised rabbit's skin compared to plain lisinopril-based gel (51.18 ± 4.87 %). Results of the comparative permeation study are shown in Fig. 6. Approximately 1.5 fold improvement in permeation was observed. This reveals prepared ion-pair topical gel formulation would be a novel formulation to enhance the permeability of lisinopril.

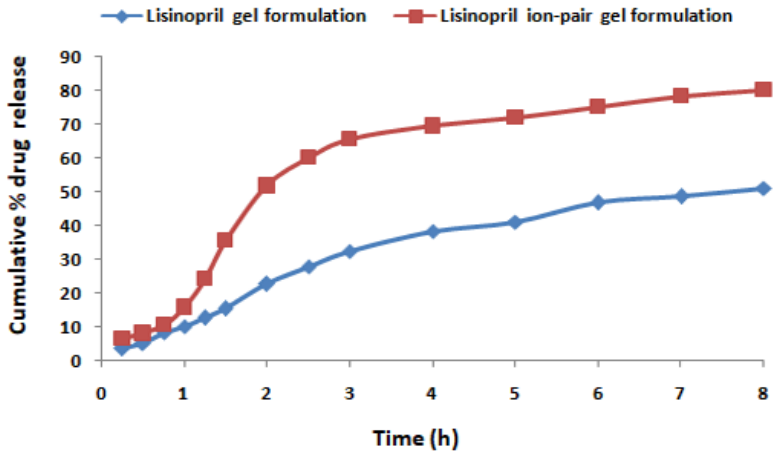

Figure 6. Comparative *Ex-vivo* permeation of lisinopril gel and lisinopril ion pair gel

2.8. Optimization of lisinopril ion-pair topical gel

Total nine formulations (F1-F9) of lisinopril ion pair topical gel were successfully formulated using 3^2 factorial design and the effect of propylene glycol and carbopol 934 was investigated on viscosity and *ex-vivo* permeability. Detailed results are summarized in Table 2.

Table 2. Composition of lisinopril ion pair gel and its evaluation parameters*

Batch	Lisinopril ion pair (mg)	Propylene glycol (mL)	Carbopol 934 (%)	Drug Content (%)	pH	Viscosity (Pa.s)	Permeability after 8 h (%)	Spreadability (g.cm/sec)
F1	10	472.5	0.75	98.90±0.56	6.20±0.18	11.3589±1.89	71.74±2.16	27.73±1.11
F2	10	480	0.75	98.43±0.47	6.43±0.21	12.1453±2.14	68.26±2.68	25.66±1.05
F3	10	472.5	1	98.85±0.36	6.19±0.32	16.8456±2.63	63.85±1.98	22.38±1.02
F4	10	465	0.5	99.98±0.23	6.52±0.09	7.5414±1.34	85.93±1.26	32.56±0.98
F5	10	472.5	0.5	98.72±0.58	6.76±0.11	7.8952±1.25	82.88±1.39	31.29±1.15
F6	10	480	1	98.24±0.45	6.49±0.31	15.8963±2.66	65.67±1.47	24.76±1.32
F7	10	465	0.75	99.15±0.91	6.38±0.14	10.8549±1.96	75.46±2.16	28.69±1.09
F8	10	480	0.5	98.31±0.72	6.44±0.32	8.1278±1.58	79.69±1.89	30.48±1.41
F9	10	465	1	99.27±0.63	6.59±0.15	17.2368±2.16	62.17±1.57	21.46±1.08

* All values represent mean ± standard deviation (n=3). Volume of each gel formulation was adjusted with glycerine q.s. 1000 mg.

2.9. Characterization of optimized lisinopril ion-pair topical gel

2.9.1. Lisinopril ion-pair and excipient compatibility

FTIR spectrum of lisinopril is shown in Figure 7, revealing characteristic peaks representing the presence of functional groups claimed by its chemical structure as shown in Figure 1. From this, we can consider that the lisinopril was of pure quality.

As shown in Fig. 7. lisinopril exhibited characteristics peaks of a carbonyl group at 1341 cm^{-1} and 1300 cm^{-1} (C=O stretching), the peak of benzene ring at 700 cm^{-1}(C-H bending), 1449 cm^{-1} (C-C stretching), and 1137 cm^{-1} (C-N stretching). After interpretation of FT-IR spectrum of a drug, it was concluded that all the characteristic peaks corresponding to the functional group present in the molecular structure of lisinopril were found within the reference range and confirmed its identity.

FTIR spectrum of carbopol showed characteristics peaks of the carbonyl group at 1700 cm^{-1} (C=O stretching) and 2927 cm^{-1} (C-H stretching). FTIR spectra of a physical mixture (lisinopril ion-pair and carbopol) and optimized formulation (F4) retained the characteristic peaks of lisinopril, indicating no chemical interaction occurred during formulation.

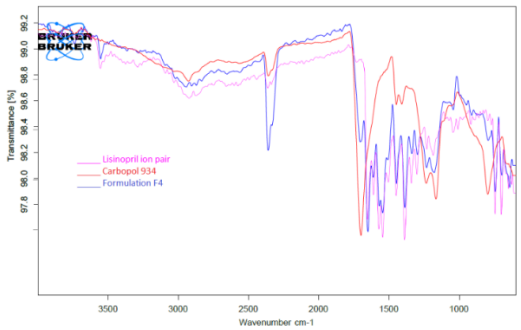

Figure 7. FTIR spectra of lisinopril ion pair, carbopol 934 and formulation F4

2.9.2. *Physical evaluation*

All formulations of lisinopril ion-paired gel (F1-F9) were found to be colorless, transparent, homogeneous, and consistent.

2.9.3. *Drug content, pH, and spreadability*

The drug content of all lisinopril ion-pair gel (F1-F9) was ranging from 98.24 ± 0.45 to 99.98 ± 0.23. the pH of all formulations was found to be between 6.19 ± 0.32 to 6.76 ± 0.11 whereas spreadability was ranging between 21.46 ± 1.08 to 32.56 ± 0.98. The concentration of Carbopol 934 exhibited a synergistic effect on viscosity while an antagonistic effect on spreadability. Detailed results are summarized in Table 2.

2.9.4. *Viscosity*

Viscosity of all lisinopril ion-pair gel (F1-F9) was ranging between 17.2368 ± 2.16 Pa.s (Batch F9) to 7.5414 ± 1.34 Pa.s (Batch F4). Results of the viscosity diagram of optimized batch and plain lisinopril gel are shown in Figure 8. Viscosity of optimized formulation (Figure 8A) and plain lisinopril gel (Figure 8B) was found to be diminished with sheare rate increment. F4 gel formulation exhibited combination of hysteresis loops, i.e., small anti-clockwise loop at high shear rates (8–100 s^{-1}) and a moderately large clockwise loop at low shear rates (0–8 s^{-1}). Plain lisinopril gel showed anti-clockwise hysteresis loop. For both gels, low area of hysteresis loops corroborates less damage of gel structure at high shear rates. Results of viscosity are significantly ($p<0.001$) higher than previously prepared lisinopril dihydrate transdermal proniosomal gels prepared by Shamsheer et al., (2011) [20]. According to their reports, the viscosity of gel was within the range of 1375 cps to 1625 cps.

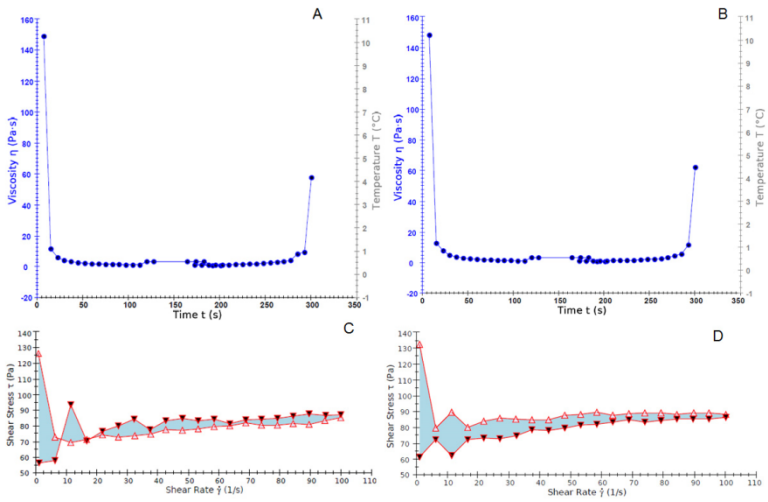

Figure 8. Viscosity diagram of (A) F4 formulation and (B) plain lisinopril gel. Thixotropy analysis of (C) F4 formulation and (D) plain lisinopril gel

2.9.5. *Effect of formulation variables on viscosity (Y_1)*

A linear model was found appropriate when viscosity was considered as a response. Both independent variables (X_1 and X_2) exhibited a linear effect on Y_1. ANOVA results for viscosity as a response based on 9 experimental runs are summarized in Table 3.

Table 3 ANOVA results showing effect of variables on responses

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Response: Viscosity (Y_1)						
Model	116.33	2	58.17	108.52	< 0.0001	significant
X_1-Propylene gylcol (mL)	0.0479	1	0.0479	0.0894	0.7750	
X_2-Carbopol 934 (%)	116.29	1	116.29	216.95	< 0.0001	
Residual	3.22	6	0.5360			
Cor Total	119.55	8				
Response: Permeability at 8h (Y_2)						
Model	578.08	3	192.69	59.77	0.0002	significant
X_1-Propylene gylcol (mL)	16.47	1	16.47	5.11	0.0733	
X_2-Carbopol 934 (%)	537.90	1	537.90	166.84	< 0.0001	
X_1X_2	23.72	1	23.72	7.36	0.0422	
Residual	16.12	5	3.22			
Cor Total	594.20	8				

The Model F-value of 108.52 confirms the significance of the model. There is only a 0.01% chance that an F-value this large could occur due to noise. *P<0.05* indicates model terms are significant. In this case, X_2 is a significant model term.

The full mathematical model for viscosity is given by the following equation,

$$Y_1 = -3.91424 + 0.011918\ X_1 + 0.586984\ X_2 \qquad \text{Eq. 1}$$

where Y_1 is the viscosity, X_1, is the concentration of propylene glycol (mL) and X_2 is the concentration of carbopol 934 (%). Based on the polynomial equation, it can be confirmed that X_1 exhibited a non-significant (*P>0.05*) positive effect while X_2 showed a significant (*P<0.05*) positive effect on viscosity. The positive coefficient of the concentration of propylene glycol and concentration of carbopol 934 indicates that the viscosity of lisinopril ion-pair gel increases with an increase in their concentration. Despite showing a similar effect, carbopol 934 showed a significantly prominent main effect on Y_1 than the concentration of propylene glycol, indicated by its relatively larger F value (216.95). The probable interactions between propylene glycol carbopol 934 were also investigated systematically. As per the interaction plot (Figure 9A), parallelism was observed in the lines. This indicated the no interactions amongst variables and signifies individual effect.

For the model, the value of the determination coefficient (R^2 = 0.9731) implies that 97.31% of the variation in response Y_1 was precisely explained by independent variables. The Predicted R^2 (0.9349) was in reasonable agreement with the Adjusted R^2 (0.9641). The difference between the two values was < 0.2, which confirms a good correlation between the obtained and predicted value. Adeq Precision of 21.253 indicated an adequate signal and confirms the suitability of this model to navigate the design space. The significance of the suggested model can further be justified using the plot of predicted versus actual values of viscosities (Figure 9B).

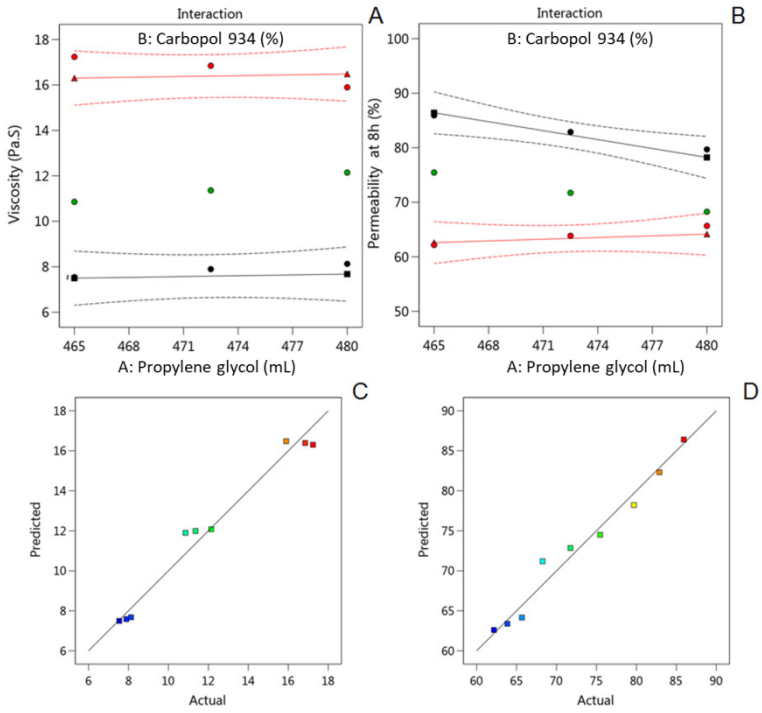

Figure 9. Interaction plot showing effect of propylene glycol and carbopol 934 on (A) viscosity and (B) permeability at 8h. Predicted Vs actual values plot for (C) viscosity and (D) permeability at 8h

Three-dimensional response surface plots and two-dimensional counter plots were generated and used to study the simultaneous effect of two variables on response parameters. These plots assist in visualizing the qualitative effect of each variable on each response parameter.

Figure 10(A) & 10(B) depicts the interaction between concentrations of propylene glycol (X_1) and carbopol 934 (X_2) when viscosity was considered as a response. It can be seen that increasing the concentration of propylene glycol and carbopol 934 increased the viscosity of lisinopril ion-pair gel. This could be attributed to the cross-linked structure of carbopol that absorbs water readily once added to water, becomes hydrated, and is followed by swelling that eventually impart viscosity to the dispersion.

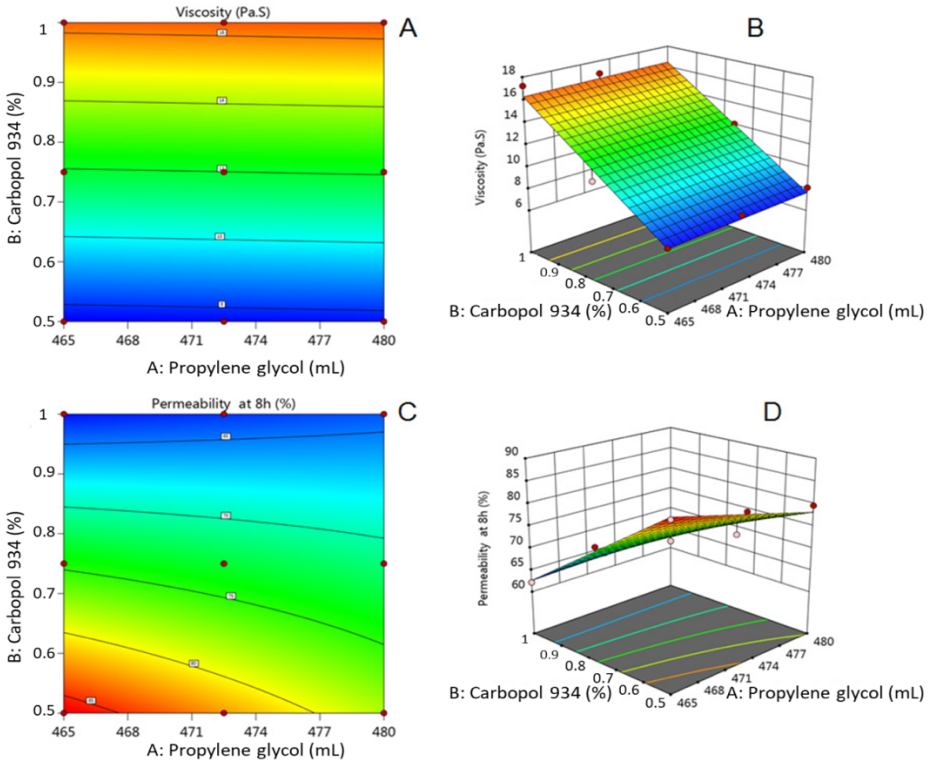

Figure 10. Counter plots (A&C) and 3D response surface plots (B&D) showing effect of effect of propylene glycol and carbopol 934 on viscosity and permeability at 8h

2.9.6. Thixotropy analysis

Comparative rheological study of lisinopril ion-Pair gel (F4 formulation) and plain lisinopril gel (0.5g each) were carried out. Thixotropy analysis of both formulations is shown in Figure 8(C) and Figure 8(D) respectively. The system was observed to be shear thinning.

2.9.7. Ex-vivo skin permeability at 8h

Ex-vivo permeability of all the prepared batches (F1-F9) across excised rabbit's skin was ranging between 62.17 ± 1.57% (Batch F9) to 85.93 ± 1.26% (Batch F4). Optimized formulation (F4) exhibited 1.7 folds improvement in skin permeability than plain lisinopril gel. Results of e*x-vivo* skin permeability are shown in Figure 11.

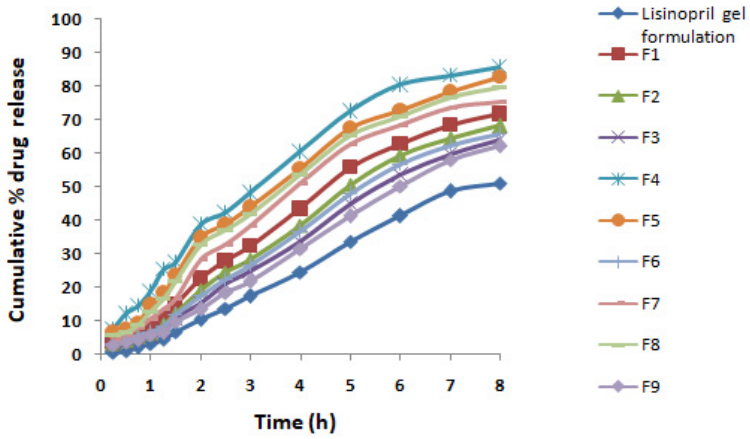

Figure 11. Comparative *Ex-vivo* permeation of different batches of lisinopril ion pair gels (F1-F9) produced using factorial design and lisinopril gel

2.9.8. Effect of formulation variables on Ex-vivo skin permeability at 8h (Y_2)

Using % permeability of gel after 8h as a response and based upon ANOVA F value, two-factor interaction was suggested as the best model to fit the data. ANOVA results for permeability as a response based on 9 experimental runs are summarized in Table 3.
The full mathematical model for permeability at 8h is given by Eq. 2,

$$Y_2 = +72.85 - 1.66\ X_1 - 9.47 + 2.43\ X_1X_2 \qquad \text{Eq. 2}$$

where Y_2 is the permeability (%) of gel determined after 8h of topical application, X_1 is a concentration of propylene glycol and X_2 is a concentration of carbopol 934.

The Model F-value of 59.77 confirms the significance of the model. There is only a 0.01% chance that an F-value this large could occur due to noise. *P<0.05* indicates model terms are significant. In this case, X_2 and X_1X_2 are significant model terms. Based on the polynomial equation, it can be confirmed that X_1 exhibited a non-significant (*P>0.05*) negative effect while X_2 showed a significant (*P<0.05*) negative effect on the transdermal permeability of a gel. Notably, both propylene glycol and carbopol 934 togetherly exhibited a significant (*P<0.05*) synergistic effect on the transdermal permeability of gel.

A negative coefficient of the concentration of propylene glycol and concentration of carbopol 934 indicates their antagonistic effect on the permeability of lisinopril ion-pair gel across the *stratum corneum*. Despite showing a similar antagonistic effect on permeability, carbopol 934 showed a significantly prominent effect than the concentration of propylene glycol, indicated by its relatively larger F value (166.84). The concentration of propylene glycol and carbopol 934 (X_1X_2) had a significant positive effect (F = 7.36), confirmed by the absence of parallel lines in the interaction plot (Fig. 9B).

For the model, the value of the determination coefficient (R^2 = 0.9729) implies that 97.29% of the variation in response Y_2 was precisely explained by independent variables. The Predicted R^2 (0.8777) was in reasonable agreement with the Adjusted R^2 (0.9566). The difference between the two values was < 0.2, which confirms a good correlation between the obtained and predicted value. Adeq precision of 19.888 indicated an adequate signal and confirms the suitability of this model to navigate the design space. The significance of the suggested model can further be justified using the plot of predicted versus actual values of permeability at 8h (Fig 9D).

Three-dimensional response surface plots and two-dimensional counter plots were generated and used to study the simultaneous effect of the concentration of propylene glycol and carbopol 934 on the permeability of developed gel across the rabbit skin.

Fig. 10(C) & Fig. 10(D) depicts the interaction between concentrations of propylene glycol (X_1) and carbopol 934 (X_2) when permeability at 8h was considered as a response. It can be seen that increasing the concentration of propylene glycol and carbopol 934 diminished the permeability of lisinopril ion-pair gel through the rabbit's skin. This could be attributed to swollen network-like structuring of a system that might have held the drug and retarded its release.

3.9.9. Stability study of optimized lisinopril ıon-pair gel

The selected lisinopril ion-Pair gel of F4 formulation was placed in a humidity chamber at 40°C ± 2°C/75% RH ± 5% RH. Samples were evaluated for change in drug content, spreadability, pH, and viscosity before and after stability. Results are shown in Table 4. There were no major changes in drug content, spreadability, pH, and viscosity during the stability study.

Table 4. Stability study of lisinopril ion-pair gel of F4 formulation

Evaluation parameter	Before stability	After stability
Drug content (%)	99.98±0.23	98.96±0.23
Spreadability (g.cm/sec)	32.56±0.98	33.42±0.29
pH	6.52±0.09	6.50±0.07
Viscosity (Pa.S)	7.5414±1.34	7.4652±1.12

3.9.10. Skin ırritation test

Skin irritation results are reported in Table 5. No evidence of any visible skin irritation (edema and erythema) and inflammation was observed during the experimentation period (72 h) in comparison with

control. Throughout the studies, in all rabbits, edema and erythema score was found to be "0". This reflects the suitability of prepared gel formulation by topical route.

Table 5. Primary irritation index values of lisinopril ion pair gel (F4) on the skin at the end of 24, 48 and 72 h

Formulation	Time of Application		
	24h	48h	72h
	Irritation Score		
F4	0	0	0
Plain lisinopril gel	0	0	0

3.9.11. In-vivo bioavailability study

The study found that there was an increase in Cmax, AUC, $t_{1/2}$, and MRT of Lisinopril ion pair gel (F4) as compared to plain lisinopril gel. Topically applied lisinopril ion-pair gel showed 2.13 and 2.42 folds improvement in C_{max} and AUC_{0-24} than plain lisinopril gel. The relative bioavailability of lisinopril ion pair gel was found to be 242.93% as compared to plain lisinopril gel. Results are mentioned in Table 6. Plasma drug concentrations vs. time profile for lisinopril ion-pair gel and plain lisinopril gel is shown in Figure 12.

Table 6. Comparative pharmacokinetic parameters of lisinopril ion pair gel (F4) and lisinopril gel

Parameters	Formulations	
	Lisinopril ion pair gel (F4)	Lisinopril gel
Cmax (ng/L)	45.89±3.62	21.5±2.89
Tmax (h)	6	6
AUC_{0-24} (ng h/L)	1170.2	481.7
K_{el} (/h)	0.0541	0.0916
t ½ (h)	12.80	7.56
MRT (h)	18.48	10.91
Relative bioavailability (F_{rel} %)	242.93	-

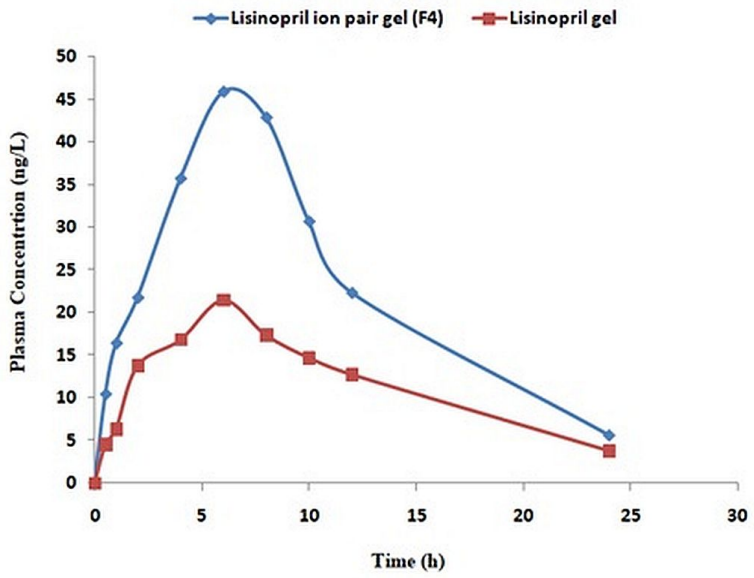

Figure 12. Plasma concentration-time profiles of lisinopril ion pair gel (F4) and lisinopril gel

3. DISCUSSION

Lisinopril has been prescribed for many years by physicians and is one of the preferred ACE inhibitors to combat high blood pressure, congestive heart failure, etc. As it belongs to BCS class III, its effectiveness is

limited due to poor permeability [1, 2]. This study was aimed to improve the transdermal delivery of lisinopril by using the ion-pair approach. Our data demonstrate that the ion-pair approach improved the permeability of lisinopril ex-vivo. There are many studies claiming the improvement of permeability of active pharmaceutical ingredients through the ion-pair approach but most of them fail to achieve *in-vivo* efficacy [21]. For this reason, we evaluated the effectiveness of optimized gel formulation by *in-vivo* studies. With the *in-vivo* study, significant improvement in bioavailability in rabbits was observed compared to plain drug-based gel. This was corroborated by the estimated pharmacokinetic parameters such as Cmax, AUC, $t_{1/2}$, and MRT after the application of ion-pair-based gel to rabbits. An increase in permeability and bioavailability of lisinopril might emerge as an innovative alternative to other permeability improvement technologies.

4. CONCLUSION

In summary, this study corroborates the prospect to increase transdermal permeability of lisinopril with ion-pair approach. Herein, lisinopril ion pair-based gel is suggested as an alternative approach to surpass the permeability issue associated with lisinopril. 3^2 factorial design was successfully implemented to identify the effect of formulation variables on the viscosity and permeation of gel across the skin. The use of the design of experiment allowed us to facilitate the fabrication and optimization of lisinopril ion-pair gel. The concentration of carbopol 934 significantly affected the viscosity of gel and permeation of the drug across the rabbit's skin. After optimization of formulation conditions, lisinopril ion-pair gel not only improved permeation (1.7 folds) of lisinopril through excised rabbit's skin but also the bioavailability (2.4 folds) compared to plain lisinopril gel. Hence, ion-pair based gel formulation can potentially be used as a carrier system to improve the skin permeability of lisinopril.

5. MATERIALS AND METHODS

5.1. Materials

Lisinopril was obtained as a generous gift sample by Micro Labs Limited, Mumbai. Carbopol 934 was obtained from Molychem, Mumbai. Propylene Glycol and Triethylamine were purchased from CDH Laboratory reagent, New Delhi. All other chemicals used were of analytical grade.

5.2. Methods

5.2.1. Preparation of lisinopril ion-pair

An equivalent amount of lisinopril in increments of 1 mg was separately added to the 5 mL each of ethanolic HCl (0.01N) and ethanolic NaOH (0.01N) until one of them was saturated. Further, the saturated solution was continuously added in small proportions to the unsaturated solution with continuous stirring at 500 rpm. The resultant solution was subjected to heating at 80°C and continuously stirred (500rpm) for removal of ethanol till seeding occurs. The resultant solution was kept aside for approximately 24 h to remove traces of ethanol [22, 23].

5.3. Characterization of lisinopril ion-pair

5.3.1. Fourier transform infrared (FTIR) spectroscopy

The FTIR spectra of lisinopril and lisinopril ion-pair were recorded using the potassium bromide (KBr) pellet pressing technique. Approximately, 3.0-mg samples were taken and separately mixed with KBr. The milled samples were scanned over the range 4000 to 400 cm^{-1} using FTIR spectrophotometer (Bruker) [23].

5.3.2. Differential scanning calorimetry (DSC)

The thermal behavior of lisinopril and lisinopril ion-pair was investigated using DSC (DSC System; Hitachi High-Tech Science Corp.) equipped with an intracooler and refrigerated cooling system. The samples were analyzed under nitrogen purging (20 mL/min) over the temperature range of 30 to 300 °C at a heating rate of 10 °C/min [24, 25].

5.3.3. X-ray diffraction (XRD) analysis

P–XRD spectra of lisinopril and lisinopril ion-pair were recorded over a diffraction angle (2θ) of 3° to 60° on an X-ray diffractometer (Brucker, Model D2 Phaser), using Cu Kα radiation (λ = 1.5405 A°). The voltage and current used were 40 kV and 35 mA, respectively [24, 25].

5.3.4 Zeta potential and particle size analysis

Prepared lisinopril ion-pair was evaluated for particle size and zeta potential. The system was diluted with water before the commencement of the study & analyzed in triplicates using Zetasizer (HORIBA Scientific SZ-100) [26].

5.3.5 Oil/ water partition coefficient study

The oil-water partition coefficient was determined using the shake-flask method. Briefly, 25 mL each of N-octanol and water were taken and saturated for 24 h. Further lisinopril (10 mg) and lisinopril ion-pair (10 mg) were dispersed in N-octanol followed by shaking of two-phase solution at 32. ± 0.5 °C for 72 h. The resultant solution was kept aside for 24 h devoid of any disturbances. Eventually, the two phases were separated using a separating funnel at room temperature and the concentrations of lisinopril within two phases were estimated using HPLC [23].

5.4. Preparation of lisinopril and lisinopril ion-pair gel formulation

Preliminary studies were performed to compare the effectiveness of plain lisinopril-based gel and lisinopril ion-pair-based gel. Brifely, carbopol 940 (0.5%) was dispersed in the distilled water allowed to soak for 24 h and stirred to form gel. Further 10 mg of lisinopril and lisinopril ion-pair (equivalent to 10 mg of lisinopril) were separately dissolved in in 400 mL of propylene glycol and added to separate gel base to produce lisinopril and lisinopril ion-pair gels. Glycerine was added to adjust mass to 1 gm and subsequently, triethylamine was gradually added to achieve the desired pH of 6.5. Resultant gel formulations were kept aside for 1 h and then used for further studies [9, 27].

5.5. Characterization of lisinopril and lisinopril ion-pair gel formulation

5.5.1. Physical examination

The prepared lisinopril and lisinopril ion-pair gel formulations were inspected visually for their color, homogeneity, consistency, and phase separation [28].

5.5.2. Determination of pH

The pH of each gel formulation was measured using a pH meter. The pH meter was calibrated with a standard buffer solution at pH 4, 7, and 9 before use. Ahead of estimation of pH, the electrode was inserted in the sample for 10 min at room temperature and then pH readings were noted.

5.5.3. Drug content

The drug content of each gel formulation was estimated to corroborate the uniform distribution of the drug. Briefly, an accurately weighed quantity of gel formulation (1 gm) was dissolved in 100 mL of methanol. Further, these stock solutions were quantitatively transferred to different volumetric flasks and suitably diluted with methanol. Resultant solutions were filtered by 0.45 mm membrane filters. Lisinopril concentrations were estimated by HPLC at 215 nm using standard calibration curve equation Y= 35880x-4096 (R^2=0.998) [29].

5.5.4. Viscosity measurement

The viscosities of lisinopril and lisinopril ion-pair gel (0.5g each) were assessed for viscosity using Brookfield's Rheometer (R/S-CPS+ Rheometer with C75-2 measuring system) at 30 rpm and room temperature (25 ± 2°C) [30].

5.5.5. Spreadability

The apparatus consisting of a wooden block with a pulley at one end was used for the estimation of spreadability. The weighted quantity of Gel (about 2 gm) was placed on a rectangular glass plate (10 × 5 cm) fixed on the block. Further, another glass plate of the same dimensions was placed over the gel. Then, 50 gm weight was placed on the top of the two plates for 5 min to expel air and to achieve uniform gel film followed by scraping off excess gel. Afterward, a weight of 50 gm was attached to the upper plate and a time (Sec) required for the upper plate to cover a distance of 10 cm was noted. The spreadability was calculated using the following equation, [31].

$$S = M \times L/T \quad \text{Eq. 3}$$

where, S= Spreadability, M=Weight tied to upper slide, L=Length of the glass slide, T=Time taken for plates to slide the entire length (sec).

5.5.6. Ex-vivo skin permeation test

Ex-vivo permeation studies were performed using excised abdominal skin of rabbits. Skin preparation and barrier integrity examination were performed as described in previous reports [32]. Briefly, modified two-chamber diffusion cells with a cell capacity of 20 mL and an effective diffusion area of 3.14 cm^2 were used during the investigation. The skin was mounted cautiously on the half-cell such that gel was adhering to the *stratum corneum* side of the skin while the dermal side contacted the receptor solution (phosphate buffer pH 7.4). The receptor solution was maintained at 32°C using water bath and stirred at 600 rpm. Aliquots (2 mL) of the sample were collected from each receptor compartment at the predetermined time intervals (i.e., 0.25, 0.50, 0.75, 1, 1.50, 2, 3, 4, 5, 6, 7, and 8 h) and replenished with an equal volume of fresh PBS to maintain the sink condition. Lisinopril concentration in the samples was estimated using HPLC. The study was performed in triplicates [32, 33].

5.6. Formulation and optimization of lisinopril ion pair topical gel

Formulation of lisinopril ion pair gel was carried out adopting the same procedure as mentioned earlier. Lisinopril ion-pair gel formulation was optimized using 3^2 factorial design. A 2-factor, 9 run design was generated using Design-Expert® (Version 13, Stat-Ease Inc., Minneapolis, USA). Generated 9 experimental runs (Table 2) were conducted randomly and the effect of two variables i.e. propylene glycol (X_1) and carbopol 934 (X_2) at three different levels were investigated systematically on viscosity (Y_1) and Permeability across the skin after 8h (Y_2) [19, 34].

The effect of independent variables on the responses was studied using ANOVA (F Value). The P-value < 0.05 was considered to be statistically significant. The relationship and interaction between the variables and responses were examined using 3D Response surface plots and Contour plots [19].

5.7. Evaluation of optimized lisinopril ion pair topical gel

Developed nine formulations (F1- F9) of lisinopril ion pair topical gel were evaluated for physical examination, drug content, viscosity measurement, spreadability, and *Ex-vivo* permeability study as mentioned earlier.

5.7.1. Lisinopril ion-pair and excipients interaction study

Drug Excipient interaction in the formulation was studied using FTIR Spectrophotometer. The FTIR spectra of lisinopril ion-pair and excipients were recorded over a range of 4000 to 400 cm^{-1} by the potassium bromide (KBr) pellet pressing method. Approximately 3 mg samples were taken and mixed with KBr before scanning [35, 36].

5.7.2. Thixotropy analysis

The rheological study of optimized lisinopril ion-pair gel formulation (F4) and plain lisinopril gel (0.5g each) was done using Brookfield's Rheometer (R/S-CPS+ Rheometer with C75-2 measuring system) at 30 rpm and room temperature (25 ± 2°C).

5.7.3. Stability study

The stability of the optimized formulation was assessed as per ICH guidelines for 6 months duration to ensure the drug product quality at specified conditions of temperature and humidity. Optimized lisinopril ion-Pair Gel (Batch F4) was kept in a humidity chamber at 40°C ± 2°C/75% RH ± 5% RH. Samples were evaluated for change in drug content, spreadability, pH, and viscosity before and after stability [37].

5.7.4. In-vivo study

Skin ırritation test

Skin irritation potential of optimized formulation was tested using rabbits and compared with plain drug-based gel. The protocol for animal studies was approved by the Institutional Animal Ethical Committee (IAEC) of Biocyte Institute of Research and Development, Sangli, Maharashtra, India (No. BIRD/IAEC/Sangli/2020/18). Briefly, 6 white New Zealand rabbits, each weighing 2.5–3 kg were taken and randomly divided into two groups namely test (group I) and reference (group II). About 24 h before the experimentation, the fur was shaved from the dorsal area and gel (0.5 g) was applied on the test area and uniformly spread within 4 cm^2 area. The sites were observed for any visible changes such as erythema (redness) or edema (swelling) at 24 h, 48h, and 72 h of application. The evaluation was done based on the scale given by Draize [37].

In-vivo bioavailability study

Nine New Zealand rabbits weighing 2.5–3 kg was taken and randomly divided into three groups containing 3 animals each. The blank gel was applied to the rabbits belonging to the control group. Test group, I received lisinopril ion pair gel (batch F4) while lisinopril gel was applied topically to the Test group II animals. Aliquots of blood samples (0.5 mL) were withdrawn from the retro-orbital plexus at intervals of 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 h after application. Withdrawn samples were separately centrifuged at 3000 rpm for 15 min to separate plasma. Further, 4.8 mL methanol was added to 200 µL plasma samples for deproteination and extraction of the drug. The resultant mixture was then vortexed for 2 min, followed by centrifugation for 5 min at 3000 rpm. The organic layer was separated and filtered using a 0.2-µm membrane syringe filter. About 20 µL of the filtrate was injected into the HPLC for estimation of lisinopril concentrations [38,39].

Pharmacokinetic parameter estimation

The pharmacokinetic parameters such as maximum concentration of drug in plasma C_{max}, the time for the drug to reach maximum concentration in plasma after administration t_{max}, were computed directly from measured plasma concentration data. The elimination rate constant (K) was estimated from the terminal slope of the plasma concentration-time curve values.

$$K = C9 - C8/T9 - T8 \qquad \text{Eq. 4}$$

Elimination half-life $t_{1/2}$ was calculated from quotient $0.693/K$

$$t_{1/2} = 0.693/K \qquad \text{Eq. 5}$$

The area under the plasma drug concentration-time curves up to measurable concentration, $AUC_{(0-t)}$ was calculated using the trapezoidal rule. To assess the degree of retardation of drug release, mean residence time (MRT) was calculated.

$$MRT = 1/K \qquad \text{Eq. 6}$$

Relative bioavailability was calculated by using the following equation:

$$F_{rel} = \frac{AUC_{test}/Dose_{test}}{AUC_{ref}/Dose_{ref}} \times 100 \qquad \text{Eq. 7}$$

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