*Jordan Journal of Pharmaceutical Sciences, Volume 15, No. 4, 2022*

# Preparation, Characterization and Transdermal Permeation of Losartan-Amlodipine Molecular Salt

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## ABSTRACT

Drug molecular salt composed of the antihypertensive compound losartan (LOS) as the anion and the antihypertensive drug amlodipine (AMLO) was prepared. The prepared salt (LOS-AMLO) was characterized by measurement of purity, water content, solubility, partition coefficient, and melting behavior in addition to common spectroscopic techniques (UV, FTIR and NMR). NMR spectral shifts of particular protons of LOS in particular were quite useful in explaining the points of interaction and association between the two ionic species so that a 3D structure could be proposed. LOS-AMLO exhibited a significantly lower melting point than its parent compounds (65 $^{o}$C) which places the salt within the ionic liquids category, in a broad sense of the definition. LOS-AMLO was found to have much lower solubility than LOS with a substantially higher apparent partition coefficient. The high partition coefficient together with lower melting temperature is favorable properties for the transdermal permeation of pharmaceuticals. However, diffusion studies through the human stratum corneum, from an aqueous solution based on propylene glycol revealed a vast decrease in the permeation of both drugs from the molecular (ionic liquid) salt form. Interestingly the experiment demonstrated that the salt structure might be maintained during permeation but with indications of strong chemical interaction between the salt and the constituents of the barrier.

**Keywords:** Amlodipine, losartan, transdermal, delivery, ion-pair.

## INTRODUCTION

Transdermal drug delivery (TDD) is an attractive route of administration of drugs due to several advantages. Advantages of TDD include convenience, non-invasiveness, avoidance of variables that affect drug absorption in the GIT, and overall potential improvement of bioavailability. The very low permeability of stratum corneum (SC) to most foreign compounds is the main obstacle facing TDD, which explains why were not many drugs made commercially available in that format. For a drug to be a good candidate for TDD, it has to have suitable physicochemical properties, such as solubility in oils and water of more than 1mg/ mL at pH 6 to 7.4 and an optimum partition coefficient, i.e., logP lies in the range 1-3 [1]. High partition coefficients help dissolve the drug in the fatty constituents of the SC, while adequate hydrophilicity allows partitioning into the viable tissues of the epidermis. Therefore, for very hydrophilic drugs, skin penetration is generally poor [2]. Over the past few decades, there have been efforts to investigate and develop novel strategies for enhancing the transdermal permeation of drugs including use of permeation enhancers [3]. The formation of lipophilic ion pairs has been investigated to increase the SC penetration of charged hydrophilic compounds, and promising enhancements were demonstrated [4-5].

In this study, we investigated the effect of ion pair

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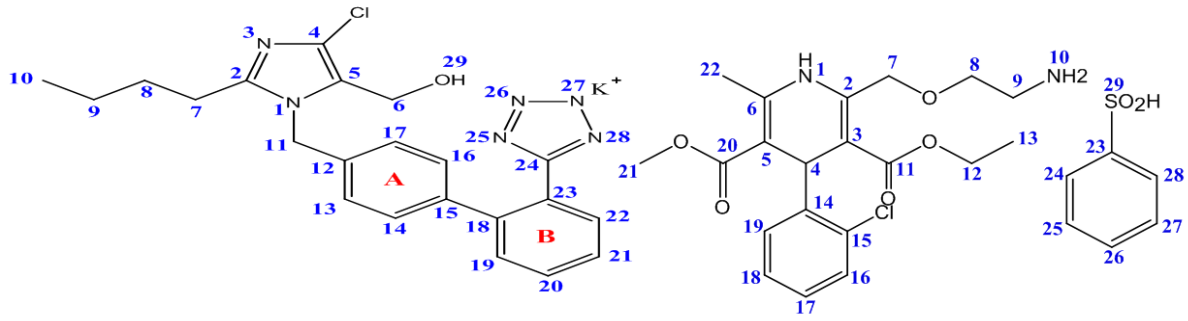
Received: 17/3/2021 Accepted:19/3/2022.
DOI: https://doi.org/10.35516/jjps.v15i4.677

formation between the acidic antihypertensive drug losartan (LOS) and the basic drug amlodipine (AMLO). Losartan is the prototype of angiotensin II receptor blockers that are used for treatment of hypertension. LOS is a non-carboxylate weak acidic compound with a pKa value of 5.55 [6] and LogP = 4.5 [7].

The bioavailability of LOS, which is marketed as potassium salt in tablet preparations (LOS-K, Fig. 1), is variable and low mounting to about 30% due in a good part to extensive liver metabolism, but to some extent, due to hindered absorption [8]. Therefore, transdermal route might present a good alternative to the oral one for LOS-K. However, at physiological pH, LOS-K is almost fully ionized and thus highly hydrophilic (water solubility > 600 mg/ mL) with a low apparent partition coefficient (log Papp = 1.01; [9]). Therefore, transdermal diffusion of the unmodified LOS-K through skin might not be optimum. Our choice of Amlodipine (AMLO) as the cationic counter ion, was based on its substantial lipophilicity, in addition to being an antihypertensive drug by itself that has been marketed in combination dosage forms with other angiotensin II receptor blockers. Amlodipine besylate (AMLO-BES, Fig. 1) is a widely used antihypertensive that belongs to calcium channel blockers group of drugs. Amlodipine is a basic compound (pka = 8.60) with substantial lipophilicity (log Papp = 3) [10]. Amlodipine besylate is almost completely ionized at physiological pH with a reported water solubility of ~ 1.59 mg/ mL[11].Thus, we aimed at preparing a drug-drug salt comprising both antihypertensive drugs that might offer favorable physicochemical properties; namely enhanced lipophilicity. Drug-drug salts are gaining much attention as they potentially offer advantages related to physicochemical and pharmacological properties of pharmaceutical compounds [12].

Few studies utilizing penetration enhancers have been reported on the enhancement of LOS-K permeation through skin. One of the earliest studies on transdermal permeation of LOS-K has shown improvement of permeation (48.94%) using capsaicin aspermeability enhancer [13]. Other studies were also reported with variable levels of success [14-15]. However, none of the reported studies examined the effect of ion-pair (molecular salt) of LOS-K on its permeation through skin.




**A** **B**

**Figure1. Chemical structures of LOS-K and AMLO-BES**

## Experimental

### Materials

LOS-K and AMLO-BES were kind gifts from The Jordanian Pharmaceutical Manufacturing Group (JPMG, Amman, Jordan), and Al Hayat Pharmaceutical Industries Company (HPIC, Amman, Jordan) respectively. HPLC grade methanol (Fisher chemicals, Loughborough, UK) and tetrabutylammonium bromide (TBAB) (ICN

Biomedicals Inc, Caliofornia, USA) were employed. Human female skin sheets obtained from a hospital in Amman, following a plastic surgery to the abdominal area of a healthy female volunteer (the approval of donation was granted after the research objectives were explained to her). Trypsin as lyophilized powder from porcine pancreas, 1,000-2,000 units/mg was obtained from (Sigma Aldrich®, **Missouri,** USA) and stored at -20ºC.

**Equipment**

Two HPLC units were employed; the first was utilized for the various characterization work of the prepared salt while the second was only used for determination of the amount of drugs diffused through SC. The first unit was composed of a pump (Sykam® S1125, GmbH, Germany) with a Sykam®UV/VIS detector (3245, GmbH, Germany). Clarity® Chromatography Software (developed by DataApex) was used for monitoring chromatograms **(**Madison, USA). The second unit was composed of Shimadzu LC-2010A HT HPLC system (Kyoto, Japan) equipped with an autosampler, degasser, column temperature controller, UV-VIS detector and LC solution software. HPLC column was

BDS Hypersil® phenyl column (150*4.6mm I.D., 5 µm particle size) equipped with a proper guard column. Water content in the prepared samples was carried out using **Mettler-Toledo** Karl Fischer (KF) Titrator (Ontario,Canada). UV spectra were obtained using UV-Vis spectrophotometer (Cecil Instruments Ltd, Cambridge, UK). Thermal analysis was carried out using NETZSCH DSC (NETZSCH-Gerätebau GmbH, Bavaria, Germany). Fourier Transform Infrared spectrum was obtained using Nexus 670 Thermo Nicolet FTIR (Spectra-Tech Inc, Tennessee, USA).NMR measurements where carried out using NMR Bruker 500 MHz-Avance III instrument (Bruker, Massachusetts, USA).*In-vitro* diffusion studies were performed using PermeGear jacketed Franz diffusion cells (Hellertown, PA, USA).Volume of receptor and donor compartments were 8 and 1.8 mL respectively; with receptor opening surface area (effective diffusion area) of 1 $cm^2$.

**Methods**

**Preparation of losartan -amlodipine salt (LOS-AMLO)**

The proposed salt of LOS and AMLO (LOS-AMLO) was prepared by reacting LOS-K, at **equimolar amount** with AMLO-BES. An accurately weighed 9 g ($19.53*10^{-3}$ mole) of LOS-K were transferred to a beaker contained10mL DW and dissolved with the aid of heating (50ºC) and stirring. The equimolar amount of the cation (AMLO-BES) was dissolved in 10 mL of methanol, which was added, while stirring, to the aqueous LOS-K solution. Distilled water was then added drop wise, which allowed immediate precipitation of the product out of solution. The formed salts were transferred onto filter paper (by filtration) and dried properly inside a desiccator filled with fresh $CaCl_2$granules. The dried salt was then grinded using a dry mortar and pestle and filled inside a tightly closed 10 mL plastic tubes.

**Characterization of LOS-AMLO salt**

**HPLC methods**

Two HPLC analytical methods were developed and employed in this study. Both methods were capable of separating and quantifying LOS and AMLO. The first one is an isocratic elution system that was employed for determination of drugs in all pertinent characterization. In the course of method development various mobile phase compositions, pH, mobile phase additives e.g. t**etrabutylammonium bromide (TBAB),** were tested. The finally recommended mobile phase consisted of 0.063% w/v TBAB, 35% v/v acetonitrile: 65% v/v phosphate buffer (50mM, pH 3.5), and a detection wavelength of 210nm with a flow rate of 2 mL/min and an injection volume of 100 µl. The method was validated for linearity, selectivity and precision with satisfactory results. All RSD values (n>3) were less than 2%, and typical

calibration equations for LOS and AMLO were: y=89.451x+95.878
($R^2$ =0.9999) and y=35.041x + 54.151 ($R^2$ =0.9997) respectively.

For diffusion studies, the employed chromatographic conditions comprised a gradient mobile phase based on acetonitrile (solvent B) and phosphate buffer pH 3.5 (solvent A) as follows: 15% B to 30% B within 3min, 30%B to 50% B within 2min, 50% B to 10% B within 1min, 10% B to 80% B within 2min. The temperature of the column was maintained at 25 ˚C, and the flow rate was kept at 1mL/min, with a detection wavelength set at 230 nm and an injection volume of 100µL. The method was fully validated for selectivity, linearity, precision and accuracy; a summary of the validation data is presented in Table 1. Representative chromatograms showing the separation of the two analyses and lack of interference (selectivity) are presented in supplementary material (Fig. S1).

**Table 1: Summary of the validation data for the quantitative determination of LOS and AMLO in diffusion experiments by HPLC.**

| | Linearity range (µg/mL) | Equation | $r^2$ | Highest RSD in recovery |
|---|---|---|---|---|
| **LOS** | 0.8-50 | y = 617070x -78289 | 0.9997 | 0.331 |
| **AML** | 1.2-144 | y = 32866x + 189563 | 0.9997 | 1.292 |

**Water content**

The potentially formed salt in addition to the two parent compounds (LOS-K and AMLO- BES) were analyzed for their water content, i.e., traces of water using KF titrator. The analyzed sample weight in each case was around 0.2 g and the results of water content were expressed as mean % w/w (n=3).

**Determination of molar ratio of association**

The molar ratio of association between LOS and AMLO was estimated by completely dissolving a 0.05 g sample of LOS-AMLO in methanol up to100 mL (n=3). Solutions were then diluted ten times in phosphate buffer (pH 6.8) before being injected onto HPLC. Relevant calibration equations were employed to determine the average concentrations of LOS and AMLO (µg/mL) in the sample. Thus, the number of moles of each component, and eventually the molar ratio between LOS and AMLO could be estimated.

**Saturation Solubility**

Saturation solubility studies were determined in two media: phosphate buffer (50mM, pH 6.8) and 60% propylene glycol (PG) in phosphate buffer (pH 6.8). Solubility values were determined in triplicate by placing excess amounts of the solid product.

**Apparent Partition Coefficient (Log $P_{app}$)**

From supernatants of saturation solubility experiments, 300 µl were transferred to a new properly labeled tube containing 300 µl of 1-octanol. Tubes were incubated in the shaking water bath, which was set at 37ºC and 200 opm for 24 hr. Aliquots from the aqueous layers were carefully withdrawn and suitably diluted with phosphate buffer (pH6.8) and injected onto HPLC. Concentration of each of LOS and AMLO in aqueous layer could be determined using the relevant calibration equations, while their concentrations in octanol were determined by difference.

**UV Spectroscopy**

UV spectra were recorded in the range 200 – 350nm for separate solutions of each of LOS, AMLO and their salt product at concentrations of 5 µg /mL. Moreover, a series of suitably diluted solutions of each compound were prepared (1-200 µg/mL), and their UV absorbance values at 250 nm were recorded.

**Differential Scanning Calorimetry**

Differential scanning calorimetric scans were obtained for the parent compounds and the salt product in the range 0 – 300 ºC with a heating rate of 10 ºC /min. Two DSC thermograms were also obtained for samples of LOS-AMLO after being heated on a hot plate with controlled temperature to either 80 or 190 ºC.

**FTIR and NMR**

Fourrier–transform infrared (FTIR) and proton nuclear magnetic resonance ($^1$H-NMR) spectra were obtained for LOS-AMLO and its parent compounds. Bruker NMR spectrophotometer (500 MHz) was employed using deuterated dimethylsulfoxide (DMSO) as a sample solvent.

***In vitro* diffusion studies**

*In-vitro* diffusion studies were performed using stirred Franz diffusion cells of 1cm$^2$ diffusion area. The cells were jacketed with water at 32 °C. The drugs in the donor compartment were prepared as solutions using a suitable solvent system that was found to provide sufficient solubility for both of LOS and its AMLO salt, which is 60%PGin phosphate buffer (50mM, pH=6.8).

Preparation of skin was made according to previously published techniques [16] The intact excised human skin sheets were immediately transferred after the surgery to the lab where it was defatted, cleaned by tapping with dry wipes, neatly placed on a paperboard that was wrapped with aluminum foil so that the skin surface is facing upward, and then kept in a plastic bag stored at -20°C until use. 3 days from the diffusion study time, the frozen skin sheets were thawed and 10 circular discs (more than six/every single diffusion study) of 25 mm diameter, were punched out using an iron metal puncher. SC isolation was done using trypsin [17].The punched whole thickness skin discs were placed in a covered Petri dish and soaked in 1%w/v trypsin solution in DW with SC side up and incubated for 24 hr in an incubator at 32ºC. The SCs were then transferred into another Petri dish to be washed from trypsin by soaking for 2 hr in two consecutive 20 mL portions of DW. Before use, the SCs were visually inspected to ensure the absence of any damages or scratches.

The prepared SC sheets were soaked in60% PG/phosphate buffer, pH 6.8 for 30 min, placed on a piece of cellulose membrane previously soaked in the same solvent system then mounted on the diffusion cells. The receiver compartment (8 mL) was filled with 60% PG/phosphate buffer 6.8 previously filtered by 0.45µm nylon filter and equilibrated at 32°C. The donor compartment was then placed and fastened by a clamp. Adjustment of the receiver volume was made followed by placing 1mL of the corresponding drug-containing solution having a concentration equivalent to 80% of the drug solubility in 60% PG/phosphate buffer, pH 6.8. The donor was covered by parafilm, and the cells were stirred at 32°C for 24hr. At specified time intervals (0.25, 0.5, 1, 2, 3, 5, 7, 12 and 24hr), 500µL of the receiver medium was withdrawn and immediately replaced with an equal volume of fresh medium previously filtered by 0.45µm nylon filter and equilibrated at 32°C. The experiments were performed as 3-5 replicates. The obtained samples were analyzed using the HPLC method described above. The diffusion profiles were constructed by plotting the average cumulative diffused amount (µg) versus time (h).

**Results and Discussion**

**Characterization**

Color change was perhaps the first obvious indication that a new product was obtained since the obtained material exhibited a yellowish color that was unlike either of LOS-K or AMLO-BES indicating salt formation. Appearance of two peaks in the HPLC chromatograms obtained for LOS-AMLO that corresponds to retention times of the parents compounds represented a strong evidence on that the product was indeed a salt. Because on HPLC, the two ions of the salt (ion pair) are expected to be separated apart according to their characteristic retention behavior with a net result that each would elute at the characteristic retention time of the parent compound [18-19]. As regards the counterions of the parent

compounds i.e. potassium and besylate, they seemed to form a separate ion pair from the product (LOS-AMLO). The best evidence for this conclusion was obtained from NMR spectra which showed loss of the characteristic signals for the aromatic protons of besylate (at 7.57 ppm) as the product was formed (detailed discussion in NMR section).

**Water content and binding ratio**

The obtained average water contents for LOS-AMLO as determined by Karl Fisher titration was 2.1%. Analysis of a sample of LOS-AMLO by HPLC revealed it contained 48.2% and 49.8% of AMLO and LOS respectively. Thus, the percentage of total weight of LOS and AMLO in the sample was 97.9% of sample weight, which reflects high purity of the sample given that the percentage of water was 2.1%. Moreover, these values support the existence of the two drugs (LOS and AMLO) within the prepared salt in a 1:1 ratio.

**Saturation solubility and apparent logarithm partition coefficient**

Saturation solubility was determined in two media: phosphate buffer (50mM, & pH = 6.8), and 60% propylene glycol in phosphate buffer (50mM, & pH = 6.8). Similarly, the apparent octanol/aqueous partition coefficients were determined using the two aqueous phases mentioned above. For the product LOS-AMLO, since it appeared to dissociate to two species, LOS and AMLO, and these were separable and quantifiable on HPLC, solubility and partition coefficients were determined in terms of each of LOS and AMLO. A summary of the results is presented in Table 2. Our solubility data agreed reasonably with those reported previously for the parent compounds [9, 20-22].

In phosphate buffer (pH 6.8), the measured solubility for LOS-AMLO (in terms of LOS) was 1000 times less than that of the parent compound LOS-K (0.4 versus > 684.2). In terms of AMLO, which is intrinsically much more lipophilic than LOS at the studied pH, the solubility of LOS-AMLO showed only about 5 times decrease in comparison to AMLO (0.23 versus 1.29 mg/mL).

**Table 2: Saturation solubility (mg/ml and mM shown in bold line between sequared brackets), and apparent Log P in octanol/ buffer. Two buffer systems were employed; (50mM, pH=6.8), and the same but containing 60% PG; at 37 ± 1 ºC, (n=3; RSD is shown between brackets).**

| Studied compound/ Salt | Phosphate buffer pH 6.8 | | Buffer containing 60%PG | |
|---|---|---|---|---|
| | Solubility (mg/mL) | Log $P_{App}$ | Solubility (mg/ml) | Log $P_{App}$ |
| LOS-AMLO (as LOS) | 0.40 (0.11) **[0.95]** | 1.68 (0.15) | 158.78 (0.03) **[375.5]** | 0.91 (0.42) |
| LOS-AMLO (as AMLO) | 0.23 (0.18) **[0.54]** | 1.95 (0.41) | 137.21 (0.05) **[324.5]** | 1.28 (0.34) |
| LOS-K | > 684.21 (0.06) **>[1618]** | 1.23 ( 0.09) | 275.90 (0.11) **[652.4]** | -0.21 (0.34) |
| AMLO-BES | 1.29 (0.26) **[3.1]** | 1.48 ( 0.87) | -- | -- |

The observed huge decrease in solubility was a clear evidence of a lipophilic ion pair (salt formation), which is understandably having lower aqueous solubility. In accordance with expectations, partition coefficients increased for LOS-AMLO in comparison to parent compounds. However, the increase in partition coefficients did not seem to parallel that of solubility i.e. while the solubility of LOS-AMLO decreased 1000 times (measured as LOS), there was

only 3-4 orders increase in partition coefficient.

In fact, the choice of solvent systems containing propylene glycol (PG) incorporated in phosphate buffer was based on the preliminary data that showed vastly reduced solubility of LOS-AMLO in simple phosphate buffer solutions. Thus, in order to have LOS-AMLO dissolved at sufficiently high concentrations that might enable reasonable permeation through SC in diffusion studies; PG was incorporated in phosphate buffer. In the solvent systems where PG was employed, differences in solubility of the various salts were minimized so that the system appeared to encourage the dissolution of the more lipophilic species e.g. LOS-AMLO while it minimizes the solubility of the extremely hydrophilic parent LOS-K. On the other hand, more obvious differences in log p were revealed using systems containing PG; so that the partition coefficients of LOS-AMLO was about 15 times higher in comparison to LOS-K. These findings (making LOS more lipophilic) were encouraging to undertake diffusion studies.

**UV spectroscopy**

UV spectra were obtained for LOS, AMLO and the product salt LOS-AMLO (supplementary material, Fig. S2). The obtained spectrum for the product exhibited a different shape than either of the parent compounds suggesting molecular interactions that are maintained in solution state (Phosphate buffer pH 6.8).Solutions having increasing concentrations of either LOS-AMLO or its parent compounds were prepared and their absorbance measured and plotted against concentration (Fig. 2).Although this experiment represents obtaining a calibration curve for each compound, the purpose was in fact to firstly test if the absorptivity of the product differ from that of the parent compounds. Secondly, it was performed in order to look for possible breaks (more than one slope) in the obtained absorbance versus concentration plot of the product as compared to parent compounds. Presence of breaks in the plot could reflect dissociation or aggregation of the salt in aqueous solution (Phosphate buffer pH 6.8).

While the plot for LOS-K was linear, that for LOS-AMLO exhibited breaks in linearity (i.e two linear parts). That might be explained as a result of changes between dissociated and un-dissociated forms of the salt as concentration was changed, where the two species having different absorptivity values.

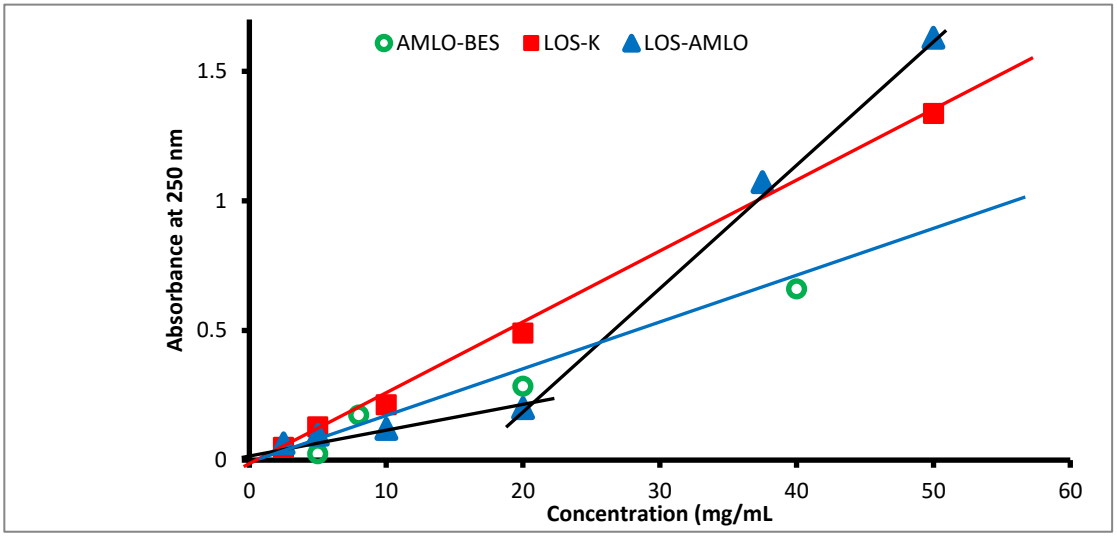



**Fig. 2. Plot of measured absorbance values for LOS –AMLO and its parent compounds against concentration. Note the break in slopes for the lines belonging to LOS-AMLO in particular.**

**Differential scanning calorimetry**

The obtained DSC thermograms for LOS-K, AMLO-BES and their proposed salt product (LOS-AMLO) are presented in Fig.3.

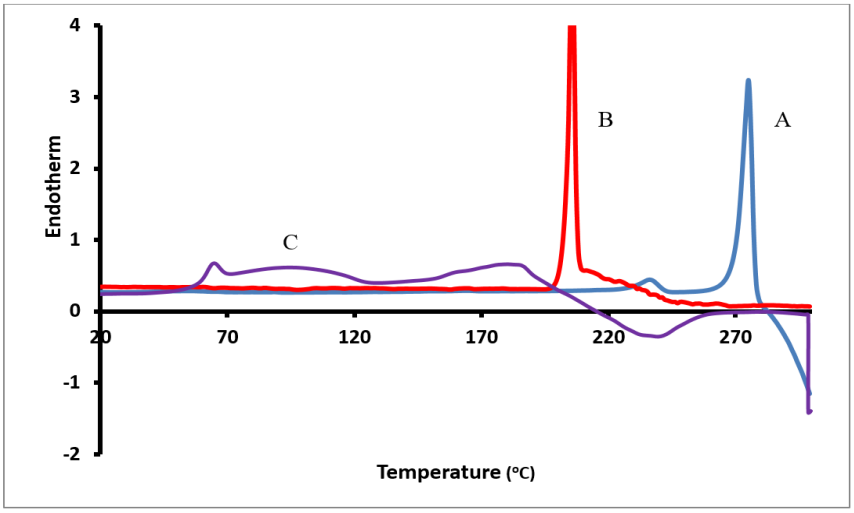



**Figure3. DSC thermograms for:LOS-K (A), AMLO-BES (B)and LOS-AMLO (C).**

For LOS-K and AMLO-BES the obtained DSC thermograms were identical to the previously reported profiles [23-24] showing sharp and symmetric endothermic melting peaks at ~270 ºC and ~ 200ºC respectively. The thermogram of LOS-AMLO was obviously different from that of LOS-K and AMLO-BES as their characteristic melting peaks at ~ 270 and 200ºC disappeared, and new three endothermic peaks emerged at lower temperatures. The first endothermic peak at ~ 64.8ºC represents melting of the salt (with potential dissociation), the broad endothermic peak centered at about 100 reflects loss of water or evaporation process (or potential breakdown accompanied by evaporation e.g. methanol from the ester group, see later discussion), while the third one at about 190 ºC might due to chemical modifications (break down reactions) of the compounds. The exothermic peak towards the end of the thermogram (>220 ºC) indicates an apparent burning (decomposition).

In order to better understand the multiple endothermic transitions that occurred in the thermogram of LOS-AMLO, a sample was heated on a hot plate under controlled temperature and actual melting was observed at about 65 ºC. Samples were taken at 85 as well as at 190 ºC. These samples were further investigated using either DSC or NMR spectroscopy. The obtained DSC thermograms for the samples, after being heated, was different than that obtained for the original ones which supports chemical conversion of the prepared salt at temperatures >85ºC. $^{1}$H-NMR spectra for the samples heated at 85 and 190 ºC were obtained, and compared to that of LOS-AMLO, and showed significant changes that further supported the chemical conversion of the prepared LOS-AMLO salt (Supplementary material, Fig. S3). Almost all proton resonances in NMR spectrum of AMLO exhibited significant decrease in intensity or splitting pattern or both while those for LOS exhibited minimal changes. This observation suggests that the chemical change (break down) involved mainly AMLO, and accords very well with a previously published report [24] regarding thermal degradation of AMLO –BES. AMLO-BES was shown to break down when heated up to 196 ºC most probably accompanied with the release of ammonia and

methanol [24]. That accords very well with our NMR results which show an almost complete loss of the resonance corresponding to the aromatic amine proton (at about 8.5 ppm) of AMLO i.e. lost as ammonia. Visual inspection of the sample during melting confirmed chemical reactions occurring at those particular temperatures as vapor was seen coming out of the sample at 85ºC (mostly methanol from hydrolysis of the methyl ester group) and at 190ºC(most likely ammonia). However, presence of AMLO as a counter ion for LOS within the ion pair salt (LOS-AMLO) seemed to accelerate its thermal degradation as at least one of the degradation reactions started at about 85ºC.

In terms of physicochemical and pharmaceutical properties, the observed significant decrease in melting point for the salt (~65 ºC) in comparison to parent compounds (200 and 270 ºC) is expected to be favorable for transdermal permeation of the drugs through skin. Previous studies on ibuprofen, revealed a positive effect of decreasing drug melting point on its transdermal permeation [25].In strict sense, the obtained salt satisfy the definition of ionic liquids [26]. Ionic liquids have been shown to offer potential solutions to limitations of transdermal delivery of pharmaceuticals either as enhancers or as being active by themselves [27].

**Fourier-transform infrared spectroscopy**

The obtained FTIR spectra for LOS-K, AMLO-BES and their proposed salt product (LOS-AMLO) are presented in Fig. 4 and in Fig. S4 (supplementary material). FTIR spectra for the two parent compounds agreed with those previously reported [28-29]. The FTIR spectrum of LOS-AMLO differed from that of the two parent compounds mostly within the frequency range of 2800-3500 $cm^{-1}$, where the distinctive peaks of hydroxyl groups of LOS-K exist. In addition, obvious changes in the region of the carbonyl functionweres also evident in the FTIR spectra of the LOS-AMLO salt. All of theses changes to accord with salt formation with significant changes in the hydrogen bonds that formed between molecules involving OH of LOS, NH and carbonyl groups of AMLO.

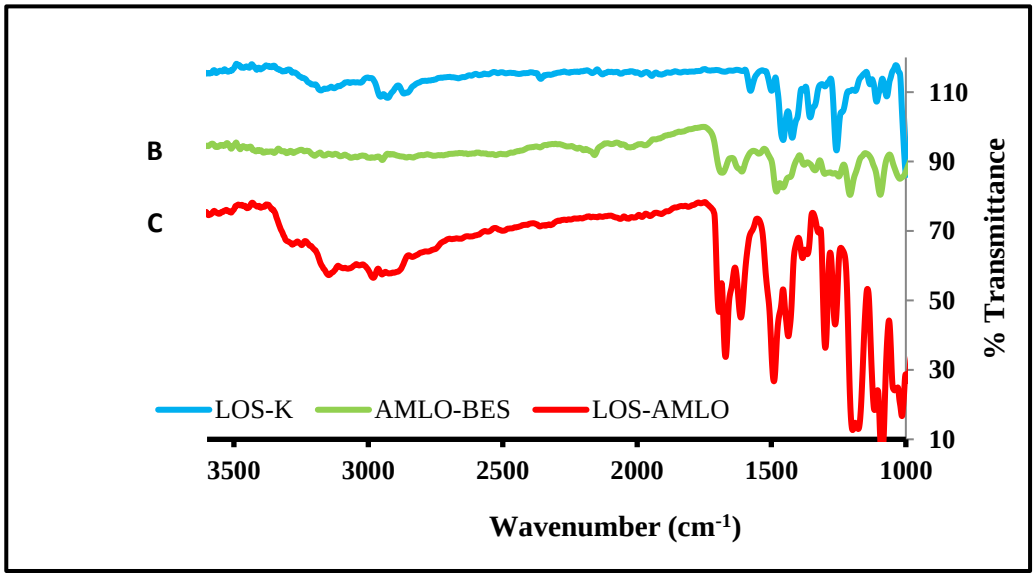



**Fig. 4: FTIR spectra for: LOS-K(A), AMLO-BES (B) and LOS-AMLO (C)**

**Proton nuclear magnetic resonance**

The aliphatic and the aromatic regions of the obtained $^{1}$H-NMR spectra for LOS-K, AMLO- BES, LOS-AMLO are shown in Fig. S5(supplementary material) and Fig.5 respectively. The spectra of LOS-K and AMLO-BES agreed well with the previously reported spectra [30-31]. Peak assignments for protons of the parent compounds together with the observed major changes in chemical shifts are shown inTableS1 (supplementary material).Accordingly, the most affected protons of LOS in LOS-AMLO were the aromatic protons (No. 14 and 16, shielded) and to a lesser extent proton 19, and the two aromatic protons at $C_{20}$ and $C_{21}$ (deshielded). The most shifted protons in AMLO were the aromatic amine proton (No.1) which became more deshielded with significant peak broadening. To a lesser extent, protons number 8 and 9 of AMLO became more shielded upon the formation of LOS-AMLO salt. That is consistent with the negatively charged tetrazole group of LOS being in close proximity of the two basic amine groups, with a twist in the aliphatic side chain pairing the tertiary amine, thus the aromatic amine proton (No. 1) would then become less accommodated (exchangeable) and resonate at higher frequency (Fig.6). That is also consistent with the observation that there was almost no shifts in the frequencies of the aromatic protons of AMLO, and that is because the only aromatic protons in AMLO (chlorinated phenyl ring) would be nearly perpendicular to the aromatic ring plane of LOS according to the proposed structural arrangement (Fig.6). On the other hand the amino proton of pyridine is likely to be close enough to the tetrazole ring of LOS, and perhaps contributing through H-pi interactions [32], and thus becoming more electron deficient and acidic (Fig.6). Another distinct change was the disappearance of the signal of the OH group in LOS (at ~5.3), which reflects changes in the pattern of hydrogen bonding system that were formed within the salt molecules, and obviously readily exchangeable with residual water molecules. Therefore, an approximate three dimensional structure could be proposed based on the observed NMR shifts and using a specialized software that calculates atomic distances and account for potential spatial clashes between the two molecules (Fig. 6). The proposed three dimensional structures suggests the existence of the aliphatic side chain of LOS in close proximity to the plane of the chlorinated aromatic ring of AMLO, thus allowing for aliphatic C-H/pi interactions[33] which help to explain the observed upfield shifts in the frequency of LOS protons (No. 8 and 9).

For AMLO- BES, other seriously affected protons were the proton at $N_{10}$and $S_{10}$ (besylate group) in addition to all aromatic protons of besylate moiety, which disappeared in the salt. The disappearance of the signal for besylate accords with the replacement of besylate anion by LOS as an anion. The disappearance of the signal for proton on No. 10 reflects its involvement in acid-base interaction with a high degree of hydrogen bonding involvement between LOS and AMLO moieties. That is also consistent with what one would expect, because the difference in pKa between LOS and AMLO is 3.05 [12].

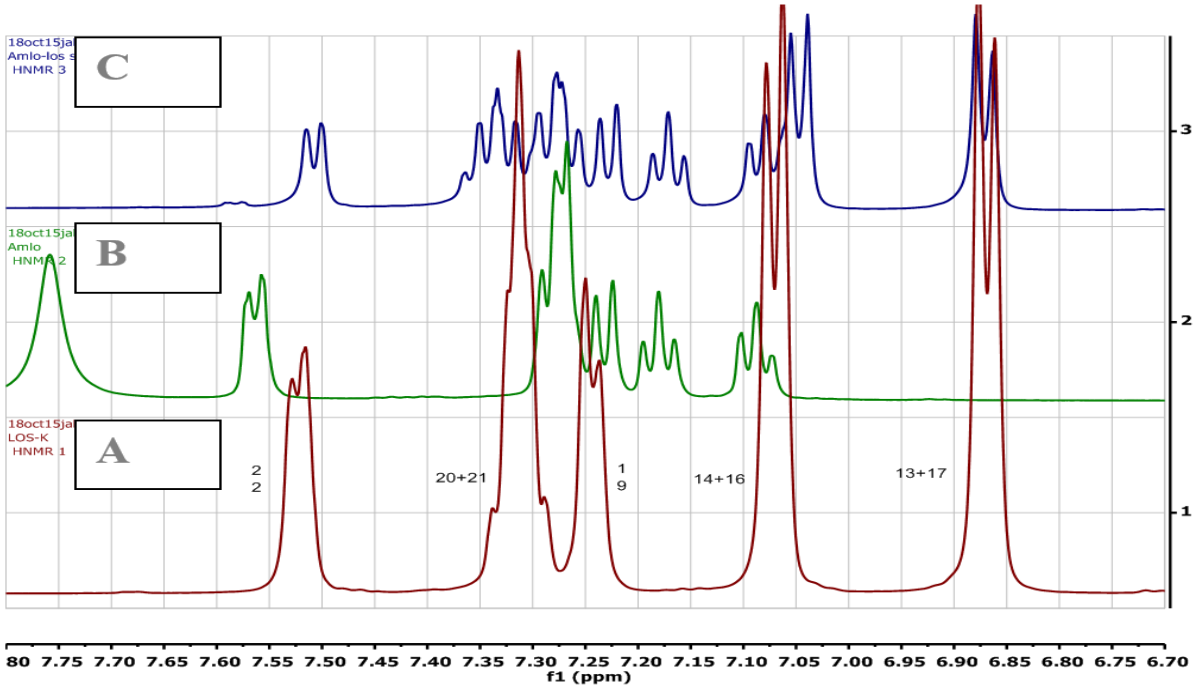



**Fig. 5: $^{1}$H-NMR spectra for the aromatic region for LOS-K (A), AMLO-BES (B), LOS –AMLO (C).**

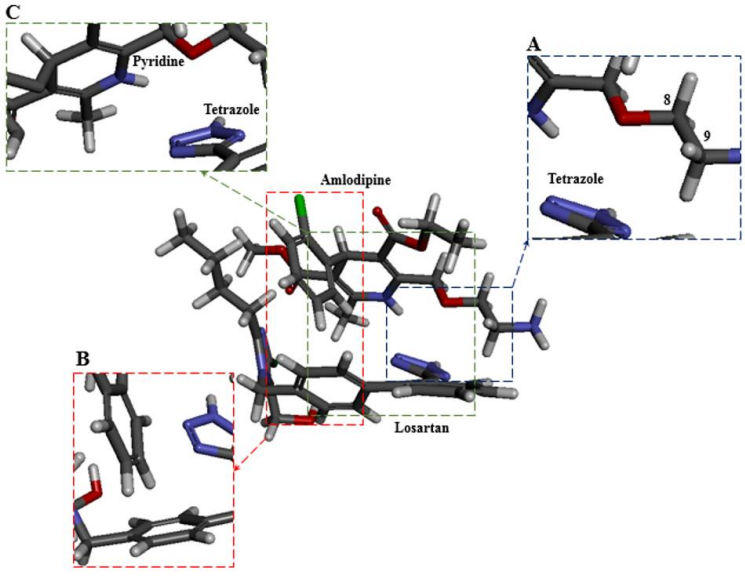



**Fig. 6:Proposed3D molecular structure showing points of associations between the two molecules of LOS and AMLO in the salt product; A, B and C are enlargements of the selected sections in the central drawings to provide closer look.**

The stoichiometry of association between LOS and AMLO could be determined by the ratio of integrated peaks areas for particular protons on the two compounds. The signals for the three protons of the methyl group ($C_{10,}$ at~ 0.78 ppm) for LOS and the signals for the protons of AMLO ($C_{21}$, at ~3.5 ppm) were employed for this comparison. Accordingly, an approximate ratio of association between LOS and AMLO of 1:1 was concluded, which was consistent with HPLC analysis.

**Diffusion Study**

Because only little amount of LOS could be dissolved from LOS-AMLO using simple buffer system, an aqueous system based on PG was employed in transdermal diffusion experiments. PG based solvent system would provide higher concentration gradient for LOS-AMLO, thus favoring higher efflux. Moreover, PG is known to act as a permeation enhancer by itself [34], and the system also revealed differences in partition coefficients of the prepared salts in comparison to parent compounds.

The cumulative diffused amounts of each of LOS and AMLO from their parent compounds (AMLO- BES and LOS-K) as well as from the prepared LOS-AMLO salt are presented inFig.7. It was evident that the permeation of LOS and AMLO from the prepared salt was significantly lower than that achieved from the parent salts. The diffused amount of AMLO-BES was intrinsically 10 times higher than that of LOS-K. However, it was interesting to observe that the permeated amounts of LOS or AMLO from the salt (LOS-AMLO) were almost equal (about 10 □g each, corresponding to 23.6 and 24.5 nanomole for LOS and AMLO respectively) inspite of that value being much lower than either of the parent compounds whose intrinsic permeateion values were vastly different from each other. This observation clearly suggests that both LOS and AMLO, in the prepared salt, permeates SC as one entity rathar than a dissociated individual species of LOS and AMLO.

Although the lipophilicity of LOS became significantly higher in its LOS- AMLO form (log P apparent 0.9 versus -0.21) which is a shift towards ideal values, and should favour its permeation, the experimental evidence revealed otherwise, suggesting the contribution of other factors. One possible explanation is that the larger size of the ion pair molecular salt as compared to individual compounds hinders its permeation.

Another important factor is the solubility of the salt form in the solvent environment of the SC. The hydrated SC was soaked in 60% PG/phosphate buffer, pH 6.8 for 30 min prior to starting the diffusion experiments; however, it is possible that the diffusion of PG into the hydrated SC may create a different PG/water ratio and thus altering the solubiltyof the salt. Lower PG/water ratio are associated with decreased salt solubility hence lowering partitioning of the salt to the SC. It is also possible that the salt enters the SC but crystallizes there as a result of the unfavored solvent environment. Finally, the potential chemical interaction of the salt with the SC constituents should also be taken into consideration. Previous studies have demonstrated chemical interactions of some pharmaceutical compounds with skin constituents and subsequent effect on skin permeability [35]. The possibility of the salt crystallizing inside the SC or to interact with its constituents highlights potential retention of the salt form and deposit formation in SC.

The permeation profiles for LOS (Fig. 7), shows the occurrence of physically untenable negative lag times in both cases of LOS-K and LOS-AMLO. Such an observation often indicates donor depletion [36]or insufficient sink conditions [37]. No indications to occurunce of any of these effects could be observed in the diffusion expirements. The total permeated amounts were much lower than those introduced in the donor compartment, thus donor depletion was not an issue. In addition the concentration of LOS in the recievor compartment at the end of the expirement was 52.3µg /8 ml and 9.4µg/8 ml for the parent compound and salt form respectively, which comprizes less than 10% of the corresponding solubility in 60% PG/buffer (Table 2), hence the sink conditions were met. So, it can be safely concluded that there is a rapid equilibration for LOS between the donor vehicle and the SC and there is no drastatic changes in the

initially permeated amount in both cases. This obsevation together with the observed vast reduction in permeation of LOS from LOS-AMLO support the possibilty that LOS is strongly retained inside the SC. The retention effect of LOS is attributed to its binding to spesific SC components rather than to its crystallization since the very slow LOS flux occurred in the case of the salt form only, wich also leads to the conclusion that AMLO is playing a role in this interaction.

In regrad to AMLO permeation, there was a drastic difference in the lag time between the parent compound and LOS-AMLO salt form as AMLO started to appear in the reciever after 30 min and 7 hr respectively. It seems that salt-SC interaction combined with decreased AMLO permeability take place and lead to decreased flux and increased lag time for the salt form.For the first glance the 7 hr lag time for AMLO might contradict the conclusion that both drugs travel together through SC, because LOS started to diffuse almost immediately. However, that could be explained as initially both drugs enter SC as one lipophillic salt (ion pair), but immediately they would be involved in interaction with the constituents of SC. Thus ther will be the equilibria for the dissociated species with their salt form in addition to equilibria between each of the involved species and the constituents of the SC. It appeared that AMLO in particular had higher affinity towards SC components, therefore it would be retained in the initial phase of diffusion until enough of it diffused to saturate the interaction sites. After that it copes up with the rate of diffusion of LOS so that the molar ratio between the diffused LOS and AMLO from the salt form approaches 1-2 at 12-24 hrs.

Although the cases of LOS-AMLO ion pair salt appeared to hinder the transdermal permeation of the individual drugs, in contrast to other cases with different drugs [38], still some benefits of the observed retardation could be exploited. The ineraction of LOS-AMLO salt with SC components and subsequent deposit formation can be the key for its potential employment in the development of prolonged release transdermal patch delivery systems, i.e, to accomplish the release over several days. Further studies wih proper techniques, including specialized spectroscopy, are needed and have been already started to reveal and further characterize the nature of this interaction. The role of PG in the occurrence of the salt-SC interaction and characterstics of the release needs also to be addressed.

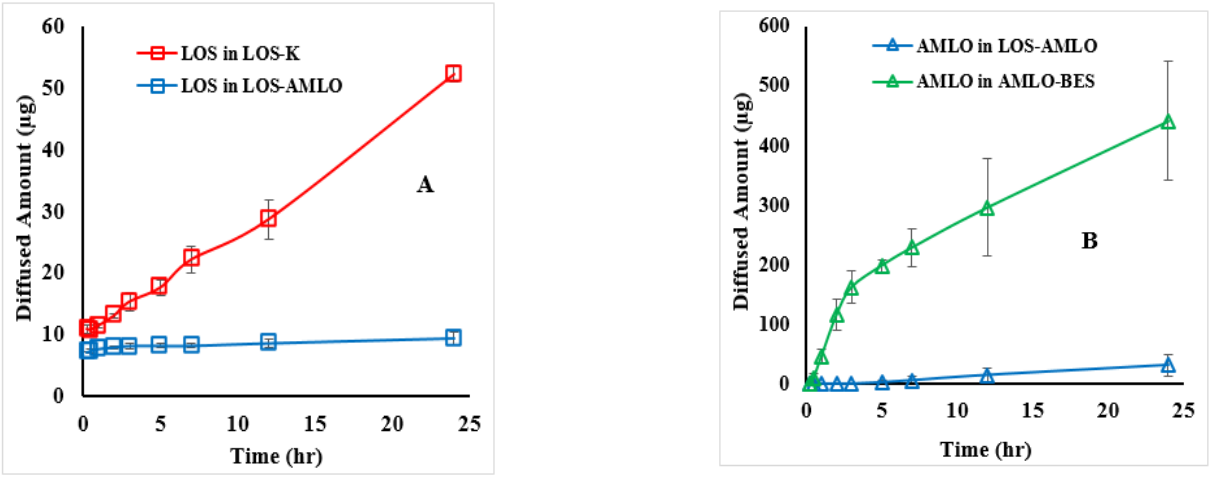



**Fig. 7: Cumulative diffused amount for LOS (A) and AMLO (B) from solutions of LOS-AMLO salt or the parent compound (AMLO-BESand LOS-K).**

**Conclusion**

An ion pair drug-drug salt (LOS-AMLO) could be prepared from LOS-K and AMLO-BES. Water solubility of LOS from the prepared salt vastly decreased in comparison to the LOS-K (>1000 times). LOS-AMLO is significantly more lipophilic than LOS (about 10 times in phosphate buffer/ PG system), with a significantly lower melting point (~65 ºC), thus placing the compound within the category of ionic liquids. Although the obtained physicochemical properties might suggest a favorable transdermal absorption profile, practical diffusion experiments revealed the complete opposite. Using human SC, diffusion experiments revealed that both of LOS and AMLO exhibited much lower permeability from the prepared LOS-AMLO salt compared to their diffusion from the parent compounds. The reason behind the substantial decrease in permeation most likely due to chemical interaction between the ion pair salt and constituents of SC. Interestingly the salt seemed to be maintained as intact ion pair without dissociation as it travelled through SC Accordingly, the drug-drug ion pair salt might be useful for very prolonged transdermal delivery of LOS and AMLO.

**Acknowledgment**

The authors would like to thank the deanship for scientific research (DSR) at the University of Jordan for continuing support.

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# حضير وتوصيف وتغلغل الملح الجزيئي لوسارتان – أملوديبين عبر الجلد

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## ملخص

تم تحضير الملح الجزيئي المكون من عقارين خافضين للضغط هما اللوسارتان والأملوديبين حيث يشكل اللوسارتان الطرف السالب من الملح. شملت عملية توصيف الملح المحضر ما يلي: النقاوة، محتوى الماء، الذائبية، معامل التوزع، سلوك الانصهار بالإضافة إلى تقنيات التحليل الطيفي الشائعة (UV، FTIR و NMR). كانت التحولات الطيفية بالرنين المغناطيسي النووي لبروتونات معينة من اللوسارتان على وجه الخصوص مفيدة جدًا في شرح نقاط التفاعل والارتباط بين النوعين الأيونيين بحيث يمكن اقتراح بنية ثلاثية الأبعاد. أظهر الملح المحضر درجة انصهار أقل بكثير من مركباته الأصلية (65 درجة مئوية) مما يجعل الملح ضمن فئة السوائل الأيونية، بالمعنى الواسع للتعريف. وجد أن قابلية ذوبان الملح أقل بكثير من اللوسارتان مع معامل توزع أعلى بكثير حيث تعتبر هذه الخصائص مواتية للتغلغل عبر الجلد للمستحضرات الصيدلانية. وبالرغم ذلك، كشفت دراسات الانتشار من خلال الطبقة القرنية البشرية، من محلول مائي للملح يعتمد على البروبيلين غليكول، عن انخفاض كبير في نفاذية كلا العقارين المكونين للملح. ومن المثير للاهتمام أن التجربة أظهرت أنه يمكن الحفاظ على بنية الملح أثناء عملية التغلغل ولكن مع وجود مؤشرات على وجود تفاعل كيميائي قوي بين الملح ومكونات الطبقة القرنية البشرية.

**الكلمات الدالة:** أملوديبين، لوسارتان، توصيل عبر الجلد، زوج أيوني، خلايا انتشار.

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تاريخ استلام البحث 2021/3/17 وتاريخ قبوله للنشر 2022/3/19.