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THE EFFECT OF PENETRATION ENHANCERS ON THE PERMEATION OF CAFFEINE

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ABSTRACT

Background: Caffeine is a chemical and edible substance found in various plants including coffee, cocoa, cola and tea. Caffeine is an alkaloid compound that is highly hydrophilic and has limited skin permeability. The lipophilic nature of the stratum corneum is a major barrier to the passage of this substance through the skin, therefore, the development of topical drug delivery systems that can effectively transfer caffeine to the skin, so in this study Appropriate of caffeine we investigated the effect of adsorption of surfactant additives on the skin absorption of caffeine. **Methods:** First, the skin was exposed to the adsorption of additives such as sodium lauryl sulfate, sodium lauryl ethyl sulfate, Tynoline, Texapon, Nanoxinol and Lecithin for 5, 15 and 30 minutes, and then the parameters of caffeine permeability and structural changes in the skin in the face of Adsorption uptake was investigated by FT-IR technique. **Result and Conclusion**: The results show that the adsorption of the additives used increases the permeability of the caffeine drug to water. There are several mechanisms including lipid liquefaction, degradation of fat structure, as well as irreversible denaturation of intracellular creatine due to the adsorption of additives. The main mechanisms are to increase the penetration of the drug. Based on the results, it was found that among the adsorption additives studied, sodium lauryl sulfate had the highest and Texapon had the lowest adsorption effect.

KEYWORDS: *Enhancing absorption, skin permeability, Caffeine and FT-IR.*

1. INTRODUCTION

Caffeine is an edible chemical found in a variety of herbs, including coffee, cocoa, cola, and tea, and is a stimulant that can prevent sleep deprivation. Caffeine is an alkaloid of the methyl xanthine family that has similar properties to theophylline and theobromine.[1] Pure caffeine is a soft, odorless, white, shiny and bitter powder. It is composed of a combination of carbon, hydrogen, nitrogen and oxygen. Caffeine is the most widely used drug among humans, with almost 90% of humans using it daily. Increasing the body's metabolism, stimulating the central nervous system and increasing awareness and environmental awareness are the most important effects of caffeine.[2]

Most drugs pass through the human skin so slowly that they cannot exhibit adequate systemic effects.[3] When a topical drug is placed on the skin, the active drug must pass through the stratum corneum and reach the living tissue. The limiting factor in this process is the slow penetration of the stratum corneum. Under normal circumstances, the main pathway is intercellular or lipid bilayers. The main limitation of dermal application is the ophthalmic impermeability of the human body to foreign substances. Therefore, the most important challenge for topical formulation today is to provide an appropriate increase in drug penetration into the skin.[4] So far, many studies have been done to identify and study the mechanism of various factors that affect the increase of permeability and absorption through the skin. These agents are used in the design of different drug formulations under the name of absorption and in different natural, synthetic forms. Adsorbents are factors that increase the absorption of drugs from the skin layers and speed up the absorption.[5]

Caffeine is an alkaloid compound that is highly hydrophilic and has limited skin permeability. The lipophilic nature of the stratum corneum is a major barrier to the passage of this substance through the skin, so it is essential to develop topical drug delivery systems that can effectively transport caffeine to the skin.[1]

In view of all the above, in this study, the effect of several chemical adsorbents on the skin absorption of caffeine was investigated and it is expected that the obtained results can be effective on the optimal skin permeability of this drug and in designing a new topical formulation, especially The shampoo product is efficient.

2. MATERIALS AND METHODS

2.1. Materials

Caffeine powder, SLS, SLES, Tynolin, Texapon, Nonoxinol and Lecithinwere prepared from Behsan Daroo Pharmaceutical Company. Sodium dihydrogen phosphate, disodium hydrogen phosphate from Merck company.

2.2. Animals

In this study, male Wistar rats were used. The mice used weighed about 150 to 170 grams and were about 10 to 12 weeks old. Mice were facilitated under the supervision and approval of the ethics committee of Jundishapur University of Medical Sciences. Mice were anesthetized and relieved with ketamine/xylazine (50 mg/kg). After killing the rats, the hairs of the abdomen were cut, and then the entire skin of this area was removed and the subcutaneous fat on the inner surface of the skin was removed with chilled pure acetone. Skin thickness was measured with a digital micrometer. It was stored in the freezer at minus 20° C until permeability tests. Before use, the skin samples were taken out of the freezer and kept at room temperature until they melted and reached room temperature.[6]

2.3. Caffeine Determination

To determine the amount of drug and the amount of drug that passes through the skin, it is necessary to use a valid method to measure the drug. In this study, UV spectroscopy with a wavelength of 273 nm was used. The selection of the mentioned wavelength is based on the spectral absorption spectrum of caffeine in phosphate buffer solution pH = 7, which has been considered for permeability and release studies. At this wavelength, Caffeine had maximum light absorption. First, to prepare the caffeine wavelength, a concentration of five milligrams per 10 milliliters was made and its wavelength was obtained in a spectrophotometer, which showed the desired wavelength of 273 nm.

The linearity of the relationship between the concentration and the response of the device in this study, the UV spectrophotometry technique was used to measure Caffeine. The response produced in UV spectroscopy is light absorption. To investigate the relationship between concentration and response, at least six standard concentrations of 0.0025, 0.005, 0.015, and 0.05 mg/mL of drug in phosphate buffer pH = 7 were prepared and then the relationship between the two was investigated.

To draw a standard curve of stock solution with a concentration of 5 mg / 10 ml of Caffeine in phosphate buffer pH = 7, six concentrations of 0.0025, 0.005, 0.015 and 0.05 mg / ml were prepared. The absorbance of the samples was measured by a UV device at 273 nm. This process was repeated for three consecutive days. The calibration curve is then plotted with the help of the concentration and the enhancers obtained.[6]

2.4. Investigation of Caffeine permeability

To evaluate the permeability of Caffeine to rat skin after skin preparation, it was placed on Franz diffusion cell. Initially, the skin was exposed to 1 gram of each of the various enhancers for 5, 15 and 30 minutes. After removing the adsorbent from the skin surface, the receptor phase was filled with a volume of 35 ml of phosphate buffer solution pH = 7 and the donor phase was filled with 5 ml of 0.05% drug solution.

The prepared cells are placed on a heater stereo at 37 ° C and 200 rpm. At the half, one, two, three, four, five, six, seven, eight, and twenty-four hours, two cells are removed from the receiving phase inside the cell each time. Two milliliters of phosphate buffer were replaced by pH = 7, and the sample absorbance in the UV device was read at 273 nm, with different absorptions. There is a need for a negative control in permeability. For this purpose, the rat skin was placed on the tuber and in the donor-phase only distilled water was placed, and in the receptor chamber, phosphate buffer pH = 7 to zero the device was used.[7]

2.5. Statistics and calculations of permeability parameters

All studies were repeated three times and the values were expressed as mean standard deviation. A two-way t-test and analysis of variance were used to statistically evaluate the results. Minitab 17 software was used to design the Full-Factorial test.

In this study, the permeability of Tadalafil from drug microemulsions to the whole skin of rats was investigated and permeability parameters such as equilibrium velocity (J_{ss}), permeability coefficient (p), incubation time (T_{lag}), and apparent diffusion coefficient (D_{app}) was calculated. Also, the results for ER_{flux}, ERD, and ER_p of drug-containing microemulsions compared to drug saturation control are given in Table 3-7. To calculate the permeability parameters, the cumulative value of the drug passing through the surface unit against time was plotted.

The permeability coefficient (p) was calculated from Equation 1.[7]

$$J_{SS} = P.C \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Eq. 1}$$

C = drug concentration in the donor phase

(T_{lag}) = The amount of commune time was obtained from the skin along the equilibrium line to the time axis in the cumulative drug curve:

The value of D was calculated from Equation 2.[7]

$$D = \frac{h^2}{6Tlag} \quad \text{.....Eq.2}$$

Because h does not represent the actual length of the drug passage, the D calculated from this formula is also the D apparent. Because all calculations are based on the Steady State area of the cumulative drug permeability diagram, it is necessary to establish the synchronous conditions for these parameters to be valid. In this study, the maximum concentration created in the receptor phase was less than 10% of the drug saturation solution in the receptor phase.

3. RESULTS

To evaluate the effect of increasing absorption on the skin permeability, the permeability of caffeine after skin pre-contact with the absorption of additives for five, fifteen and thirty minutes was examined. The parameters of caffeine permeability for each component are given in Tables 1 and 2. Has been. The effect of adsorption of different additives in contact of skin for five, fifteen and thirty minutes on caffeine permeability is shown in Tables 2 as ER_{flux}, ER_D and ER_P. The cumulative graph of caffeine passing through the surface unit before contact for five, fifteen and thirty minutes is shown in Figures 1 to 3. Phosphate buffer is used as a control in the phase chamber.

Table 1: Results of different enhancer adsorption on different parameters of Caffeine permeability of whole rat skin (n = 3, Mean ± SD).

Parameter	Flux ($mg.cm^{-2}.h^{-1}$)	T_{lag}(h)	D ($cm^2.h^{-1}$)	P(cm/h)
After 5 minutes' skin contact				
Control (Water)	0.001±0.00015	5.71±0.07	0.01865±0.0002	0.00018±0.00002
SLS	0.007±0.00060	4.35±0.09	0.02450±0.0005	0.00076±0.00006
SLES	0.001±0.00006	3.03±0.03	0.03514±0.001	0.00017±0.00001
Tynolin	0.018±0.00046	5.71±0.01	0.01868±0.0004	0.00181±0.00005
Texapon	0.018±0.00020	5.70±0.005	0.01871±0.00007	0.00185±0.00002
Nanoxinol	0.010±0.00040	5.94±0.11	0.01796±0.00028	0.00103±0.00004
Lecithin	0.006±0.00021	1.57±0.001	0.06796±0.003	0.00065±0.00002
After 15 minutes' skin contact				
Control (Water)	0.001±0.00015	5.71±0.07	0.01865±0.0002	0.00018±0.00002
SLS	0.007±0.00060	4.35±0.09	0.02450±0.0005	0.00076±0.00006
SLES	0.001±0.00006	3.03±0.03	0.03514±0.001	0.00017±0.00001
Tynolin	0.018±0.00046	5.71±0.01	0.01868±0.0004	0.00181±0.00005
Texapon	0.018±0.00020	5.70±0.005	0.01871±0.00007	0.00185±0.00002
Nanoxinol	0.010±0.00040	5.94±0.11	0.01796±0.00028	0.00103±0.00004
Lecithin	0.006±0.00021	1.57±0.001	0.06796±0.003	0.00065±0.00002
After 30 minutes' skin contact				
Control (Water)	0.001±0.0001	5.71±0.07	0.018±0.0002	0.00018±0.00002
SLS	0.009±0.0006	2.005±0.14	0.053±0.0033	0.0009±0.00006
SLES	0.003±0.0002	0.05±0.0008	1.843±0.0849	0.0003±0.00002
Tynolin	0.017±0.0004	5.44±0.05	0.019±0.0002	0.0017±0.00005
Texapon	0.014±0.0002	4.97±0.04	0.021±0.0001	0.0014±0.00003
Nanoxinol	0.014±0.001	4.70±0.20	0.022±0.001	0.0014±0.00011
Lecithin	0.003±0.0003	1.588±0.19	0.068±0.009	0.0003±0.00003

Table 2: Results for ER_{flux}, ER_D and ER_P, uptake (n = 3), Mean ± SD).

Parameter	ER_{flux}	ER_D	ER_P
After 5 minutes' skin contact			
SLS	4.18±0.18	1.24±0.02	4.18±0.18
SLES	1.52±0.28	1.35±0.02	1.52±0.28
Tynolin	5.16±0.27	2.86±0.21	5.16±0.27
Texapon	3.87±0.45	1.07±0.01	3.87±0.45
Nanoxinol	5.48±0.56	0.82±0.007	5.48±0.56
Lecithin	1.30±0.03	1.07±0.05	1.30±0.03
After 15 minutes' skin contact			
SLS	4.31±0.43	1.31±0.04	4.31±0.43
SLES	0.98±0.08	1.88±0.06	0.98±0.08
Tynolin	10.31±1.18	1.001±0.01	10.31±1.18
Texapon	10.52±0.85	1.003±0.01	10.52±0.85
Nanoxinol	5.85±0.70	0.964±0.01	5.85±0.70
Lecithin	3.67±0.34	3.64±0.18	3.67±0.34
After 30 minutes' skin contact			
SLS	5.16±0.27	2.86±0.21	5.16±0.27
SLES	1.78±0.17	98.87±5.37	1.78±0.17
Tynolin	9.82±0.89	1.05±0.008	9.82±0.89
Texapon	8.20±0.68	1.14±0.02	8.20±0.68
Nanoxinol	8.24±1.30	1.21±0.08	8.24±1.30
Lecithin	2.15±0.06	3.65±0.54	2.15±0.06

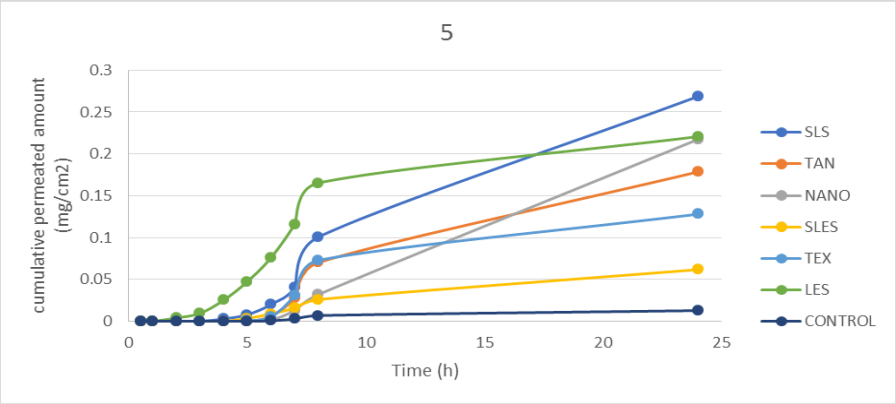

Figure 1: Caffeine cumulative diagram crossing the surface unit after 5 skin contact with enhancers from whole rat skin.

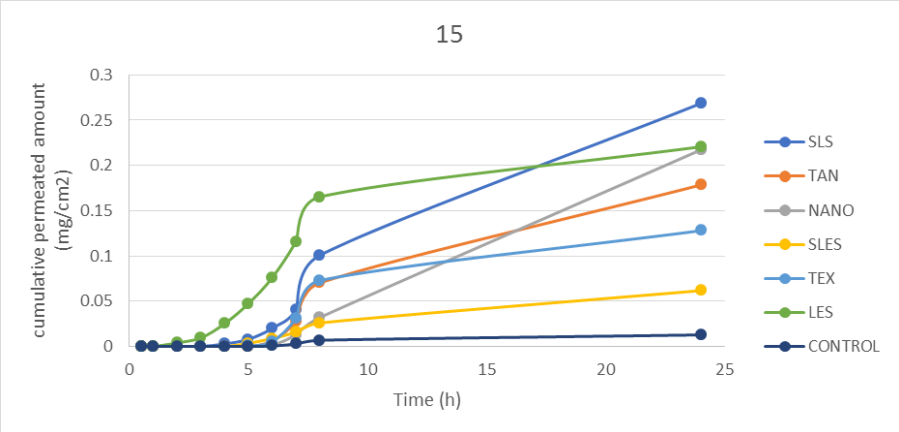

Figure 2: Caffeine cumulative diagram crossing the surface unit after 15 skin contact with enhancers from whole rat skin.

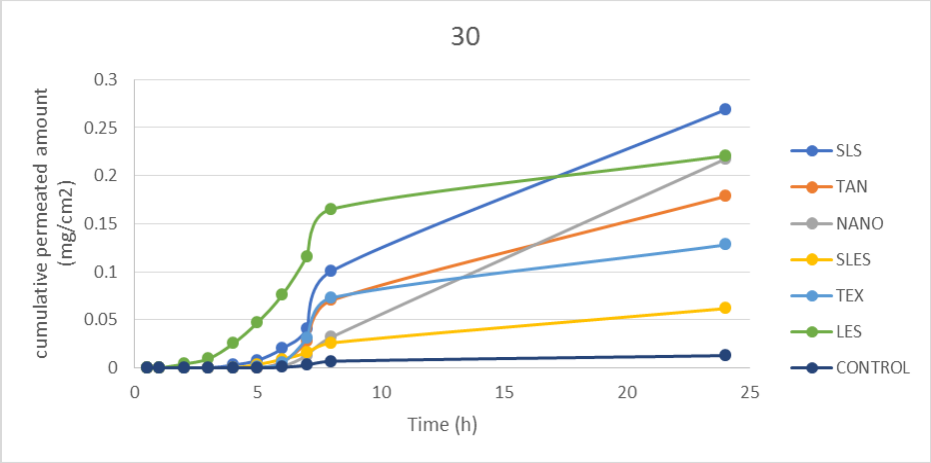

Figure 3: Caffeine cumulative diagram crossing the surface unit after 30 skin contact with enhancers from whole rat skin.

Analysis of the FT-IR spectrum of pre-contact skin with adsorption is a practical way to study the interactions between chemical adsorbents and the stratum corneum, which are revealed by the formation of absorption bands at different wavelengths. The FT-IR absorption spectrum bands represent the vibrations of lipid and protein molecules in the stratum corneum.[8] The FT-IR table obtained from the effect of adsorption on the whole skin of rats is shown in Tables 3 to 6.

Table 3: Peak height and percentage reduction of peak height -CH symmetric and asymmetric and C = O tensile group due to component effect (n = 3, mean ± SD).

Enhancer	Asymmetric C-H stretching		Symmetric C-H stretching		C=O stretching of lipid ester	
	Peak height	D%	Peak height	D%	Peak height	D%
Control (Water)	0.395	-	0.242	-	0.015	-
SLS	2.916	N.S	0.807	N.S	1.164	N.S
SLES	1.531	N.S	1.121	N.S	3.747	N.S
Tynolin	1.717	N.S	0.989	N.S	1.280	N.S
Texapon	1.857	N.S	0.990	N.S	1.543	N.S
Nanoxinol	0.745	N.S	0.538	N.S	0.850	N.S
Lecithin	0.450	N.S	0.590	N.S	1.450	N.S
N.S: Not Seen						

Table 4: Peak height and percentage reduction of the peak height of C = O tensile and C-N tensile groups due to component effect (n = 3, mean ± SD).

Enhancer	C=O stretching	Of keratin	C-N stretching	Of keratin
	Peak height	% D	Peak height	% D
Control (Water)	0.012	-	0.414	-
SLS	2.490	N.S	0.708	N.S
SLES	1.444	N.S	1.438	N.S
Tynolin	1.555	N.S	1.096	N.S
Texapon	1.860	N.S	2.121	N.S
Nanoxinol	1.610	N.S	1.118	N.S
Lecithin	0.650	N.S	0.770	N.S
N.S: Not Seen				

Table 5: The wavelengths related to –CH are symmetric and asymmetric and the C = O group stretches amid 1 and 2 in hydrated skin and skin pre-contacted with components (n = 3, mean ± SD).

Enhancer	C-H stretching asy	C-H stretching sym	C=O stretching Of lipid ester	Amid I	Amid II
Control (Water)	2915.13±0.2	2840.74±0.4	1690.2±1	1642.58±0.9	1535.9±0.2
SLS	2999.98±0.15	2863.51±0.11	1760.89±0.24	1640.87±0.21	1580.65±0.17
SLES	2946.49±0.1	2845.88±0.1	1725.45±0.1	1676.76±0.9	1537.27±0.2
Tynolin	2960.66±0.15	2880.40±0.11	1769.663±0.24	1599.42±0.21	1502.55±0.17
Texapon	2918.80 ± 0.224	2805.93 ± 0.13	1735.21 ± 0.212	1628.56 ± 0.96	1566.317 ± 0.141
Nanoxinol	2917 ± 0.382	2855.32 ± 0.229	1760.89 ± 0.19	1640.87 ± 0.404	1629.58 ± 0.258
Lecithin	2916.92±0.15	2808.49±0.11	1758.58±0.24	1672.06±0.21	1672.06±0.17

4. DISCUSSION

The effect of additive uptake on drug passage through rat skin was obtained in comparison with control by calculating ERFlux, ERP and ERD. The results showed that all the adsorption of sodium lauryl sulfate, sodium lauryl ethyl sulfate, Tynolin, Texapon, Nanoxinol and Lecithin in contact with the skin for five, fifteen and thirty minutes caused a significant increase in ERFlux, ERP and ERD. While Lecithin was not significantly different from the control in the five-minute pre-contact mode compared to the control, the effect of the 15-minute and 30-minute pre-exposure of this combination significantly increased the rate of caffeine skin passage.

The results also show a significant relationship between Jss and the adsorption sample used in all three cases of five, fifteen and thirty minutes compared to the control sample, which indicates an increase in permeability using the adsorption under study. According to a study by Mr. Tabosa et al., High Jss can cause higher concentrations of the drug in the upper layers of the skin. It can also be used to enhance the penetration of the components of the formulation into the bloodstream through the skin[9,10] because in each formulation all the components can act as penetration components with different mechanisms.[11]

The results showed that all adsorption of additives had a greater increase in drug delivery rate (flux) than drug release (Dapp) (except for Nanoxinol, which at times five and fifteen had this amount of control under control, indicating that with Increasing the call time in this case is more than controlled in thirty minutes). Among them, Tynolin and Lecithin showed the greatest effect in increasing the permeability of rat skin in five and fifteen minutes, respectively.

The results also show a significant Jss adsorption relationship of all compounds used in a 30-minute contact with the control sample, which indicates an increase in permeability using these adsorbents.

As it was found from the present study, the highest and lowest absorption efficiencies of the drug were related to the increase of dermal absorption of the drug, respectively. Ethyl sulfate, Tynolin, Texapon, Nanoxinol and Lecithin.
Five minutes:
Lecithin> Sodium lauryl ethyl sulfate> Texapon> Sodium lauryl sulfate> Nanoxinol> Tynolin

Fifteen minutes:
Sodium Lauryl Ethyl Sulfate> Texapon> Tynolin> Nanoxinol> Lecithin> Sodium Lauryl Sulfate

Thirty minutes:
Texapon> Sodium lauryl ethyl sulfate> Lecithin> Tynolin> Nanoxinol> Sodium lauryl sulfate

The results of FT-IR from pre-contact skin with sodium lauryl sulfate show that it has increased the absorption wavelength in the asymmetric CH region and increased the position of the absorption wavelength in the symmetric CH, indicating liquefaction in the stratum corneum membrane followed by impairment of barrier properties. And it is possible that increasing the passage of the drug through the stratum corneum of this uptake increases the C = O wavelength of stress, which indicates the weakening of hydrogen bonds between lipid molecules. It also reduces the wavelength number of amide I and increases the wavelength number of amide II, which increases and decreases the dam effects, respectively; These results indicate that this compound has an effect on lipid bilayers of horny tissue and protein parts of this tissue, but the effect of this adsorption on the lipid parts of the stratum corneum is more than the protein part and it seems that this compound is disturbed in the group. Horny lipid has increased the permeability of the drug through the skin.

The results of FT-IR pre-contact skin adsorption of sodium lauryl ethyl sulfate show that it increases the absorption wavelength in the asymmetric and symmetrical CH region, indicating a blue shift of liquefaction of bilayers in the stratum corneum and disturbance of the dam properties and possibly increase. The drug passes through the stratum corneum. This adsorption increases the C = O stress wavelength, indicating a weakening of the hydrogen bonds between the lipid molecules. Sodium lauryl ethyl sulfate also increases the wavelength number of amide I and the wavelength number of amide II, which reduces the effects of the barrier.

The results of FT-IR of pre-contact skin with Tynolin show that C-H is asymmetric and C-H is more symmetrical towards the absorption band; Indicating an aqueous shift, Tynolin indicates the orientation of lipid bilayer groups, impairs barrier properties, and possibly increases drug passage through the stratum corneum. The results also showed that Tynolin shifted the absorption band in the amide region I and II to a lower wavelength; Such a state indicates the orientation of protein groups in the bilayer of the stratum corneum.

The results of FT-IR of skin in contact with Texapon show that asymmetric CH did not change to control, but CH asymmetrically shifted towards the absorption band position, indicating that Texapon oriented lipid bilayer groups and ultimately increased the effect is to prevent the drug from entering. Also, the FT-IR results of this peak show that Texas has caused the red shift of the absorption band in the region of amide 1 and the blue shift of amide 2, which indicates the orientation of protein groups in the bilayer of horny tissue. The results of FT-IR pre-contact skin with Lecithin are similar to the results of Texapon.

The FT-IR results from the skin of pre-contact rats with Nanoxinol show that this compound caused a very small increase in the wavelength of asymmetric CH peak absorption, an increase in the absorption wavelength in the CH symmetric region, indicating liquefaction of bilayers in the stratum corneum membrane. And there is a disruption in the skin's barrier properties to this drug. This attraction decreased the absorption wavelength in the amide region 1 and increased the absorption wavelength in the amide region 2, which indicate the increase and decrease of the skin barrier effects, respectively.

5. CONCLUSION

Transdermal drug delivery always has several problems. Overcoming the barrier of the cutaneous stratum corneum has challenges that can be overcome by helping the transcutaneous drug delivery. All the absorption of chemical and herbal

additives used in this study led to changes in the structure of the skin and increased therapeutic drug delivery. The use of adsorbents used in this study can greatly aid in the dermal delivery of Caffeine.

6. ETHICAL ISSUES

Not applicable.

7. CONFLICT OF INTEREST

The publication has been approved by all co-authors and the responsible authorities at the institute where the work has been carried out.

8. ACKNOWLEDGMENTS

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