Development and *in vitro* evaluation of a *Transdermal Hydrogel* Patch for *Ferulic* acid

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Abstract: Current work aimed to develop and evaluate a transdermal delivery system of hydrogel patch for ferulic acid to treat skin damage induced by UV radiation. VISCOMATE™ NP700, dihydroxy aluminium aminoacetate, glycerine, tartaric acid were used in combination in different ratios to design the hydrogel patch. *In vitro* release rate was selected as an index to optimize the formulation. The formulated hydrogel patch was evaluated by several parameters like tacking strength, cohesive strength, peeling strength, residuals after peeling and drug content determination. The *in vitro* penetration was determined by Franz diffusion technology with hairless mouse skin as permeability media. Different kinetics models were employed to simulate the release and penetrate patterns of ferulic acid from patches in order to investigate the drug transport mechanism. The residual drugs in the patch and skin were determined after the penetration experiment. The optimized preparation was dihydroxy aluminium aminoacetate: NP700: glycerine: ferulic acid as a ratio of 0.02:0.4:1.5:1.25:0.25. The cumulative percentage of release was 60.4465±1.7679% for 24h, which results from a combination of diffusion effect and polymer erosion effect. For the barrier of stratum corneum, the cumulative penetrate rate was only 1.3156±0.3588% and the release mechanism turn out to be the effect of erosion of polymer surface. The residual drugs in the patch were 97.5949±1.4932%. The *in vitro* data revealed that it was easy for ferulic acid to release from the paste while difficult to permeate through the skin barrier, which resulted in most of drugs residued in the paste. Hence, further experiments will be necessary for finding the penetration enhancer in ferulic acid transdermal delivery.

Keywords: Transdermal patch, Ferulic acid, VISCOMATE™ NP700, *in vitro* evaluation

INTRODUCTION

Ultraviolet (UV) radiation in sunlight generates oxidative stress in skin that can result in skin cancer and photoaging changes (Murray *et al.*, 2008). One way to protect skin from damage induced by UV is to apply light-absorbing substance on the skin surface. Since damage is the most direct response to oxidative, another effective way, which is especially useful for damage already induced in skin, is using antioxidant to eliminate reactive oxygen species(ROS) generated by oxidant (Oresajo *et al.*, 2012) . Among many available antioxidants, ferulic acid (FA) has aroused scientists' great interest. FA is one of the most abundant phenolic acids distributed in plants especially in cereals, fruits and vegetables (Zhao and Moghadasian, 2008). Well recognized as its antioxidant activity, a recent study shows that FA has protective effect to the skin against UV-induced erythema (Murray *et al.*, 2008) and can slow down the stand break rate of DNA (Ghatak and Panchal, 2010). But FA undergoes a marked first-pass effect, which limits its oral bioavailability. So delivery of FA via skin is an attractive alternative to oral dosing (Zhang *et al.*, 2010). Literature has reported FA can penetrate skin in aqueous solution (Saija *et al.*, 2000), but such form restricted its application in clinic. So this study aims to develop a novel hydrogel patch containing FA to treat skin damage induced by UV radiation. In such formulation, the release and penetrate characters need to be investigated.

There are many pharmaceutical excipients can be used to prepare hydrogel patches, such as methyl cellulose (MC), Hydroxy propyl methyl cellulose (HPMC), carbopol, alginate, collagen, chitosan and so on (Jayaprakash *et al.*, 2010; Barreira Joao *et al.*, 2013; Zheng *et al.*, 2012). Partially Neutralized Polyacrylate (NP700) is one of new pharmaceutical adjuvants which can be used to develop hydrogel patches with quantitatively administer medicaments and repetitious stick movement (Showa Denko K.K., n.d.). This formulation consists of three parts: a release liner, an adhesive layer and a backing cloth. The adhesive layer, containing matrix and medicaments, has great contribution to the pharmacological function. Besides NP700, dihydroxyaluminium aminoacetate, glycerine, tartaric acid and water are also used in combination to prepare the formulation (Kusunoki *et al.*, 2000). The role of dihydroxyaluminium aminoacetate is a cross-linking agent. Aluminum ions can dissociate from the compound in an acidic condition and cross link with NP 700, and then a gel with three-dimensional network structure and moderate strength is obtained. The role of tartaric acid is a regulator of cross link. In the existence of a certain amount of water, the tartaric acid will promote the dissociation of aluminum ions. The ratio of above five matrixes will affect the release rate of drug in hydrogel. So the present work optimized the formulation using *in vitro* release rate as index and evaluated the transdermal delivery system by physical testing and penetration characters.

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MATERIALS AND METHODS

Materials

Ferulic acid reference substance was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP), Beijing, China. Ferulic acid, purity>98%, was obtained from Shanghai Chemical Reagent (Shanghai, China). Release liner and backing laminate were supplied by Tianjin Ruikang Biomedical Technologies Inc. (Tianjin, China). NP 700 was denoted by ISP (New Jersey, USA). Dihydroxyaluminium aminoacetate was purchased from Guangzhou Biours Bioscience Co., Ltd. (Guangzhou, China). Glycerine, tartaric acid and Ethanol were obtained from Beijing Chemical Reagent (Beijing, China). All other chemicals used for this study were of analytical grade.

Instrument

Aglient HPLC 1260 system with Variable-wavelength UV detector (Agilent Technologies, Santa Clara, CA, USA). Franz diffusion cell (Tianjin pharmacopoeia standard Inc., Tianjin, China). Mechanical stirrer (IKA, Germany).

The transdermal patch preparation

Ferulic acid was weighed and dissolved in ethanol as A phase. NP700, dihydroxyaluminium aminoacetate, glycerine and tartaric acid were accurately weighed in pre-set ratios shown in table 1 and mixed as B phase. Pour A phase into B phase and mix thoroughly with the help of a magnetic stirrer. Additional water shown in table 1 was accurately added into the mixture. Stir the mixture until it became sticky, and then cast it on the backing cloth placed under a glass mould with a size of 5×3 cm^2. The mould containing polymeric solution of drug was kept for 12 hours at room temperature for drying. After drying, remove the mould and cover the patches with release liner. Three groups were divided to investigate the effect of the amount of tartaric acid, dihydroxyaluminium aminoacetate and water on the release rate.

Physical evaluation of transdermal patches

1. Tacking strength,

The test was performed with a tacking testing machine (Labthink, Jinan, China) according to the method mentioned in Chinese Pharmacopeia Volumn I Appendix XII E (National Pharmacopoeia Committee, 2010). A series of fitting friction of balls with different weight under the table roll pass the adhesive surface of the inclined plate separately. The tacking was evaluated by the biggest friction ball the adhesive surface could cling.

2. Cohesive strength

The test was performed by maintaining adhesive force testing machine (Labthink, Jinan, China) using the method in Chinese Pharmacopeia Volumn I Appendix XII E (National Pharmacopoeia Committee, 2010). Apply the patches to the plate surface, keep the plate vertically, hanging poise with a standard mass along the length direction of the patches, and record the time period the patches slip from starting to dropping out. Cohesive strength was the average of 5 replicates.

3. Peeling strength

The test was conducted by 180° peel strength test method using a peeling strength tester (Yuelian Testing Machines Co., Ltd., Guangdong, China). Peel off the release line and place the patch unloaded 2h under room temperature. Apply the patch to a stainless steel plate, smoothen three times with a 2.0 kg roller, maintained for 20 minutes at 25°C, and pull from the plate at a 180° angle at 300 mm/min rate. The force was expressed in N. Peel strength was the average of 5 replicates.

4. Residuals after peeling

Patches are smoothened with 2 kg roller three times and then peeled off the release liner. Residual paste on the release liner was accurately weighted and the proportion in the total paste weight was calculated.

Drug content of ferulic acid patches

FA was extracted by methanol to evaluate the amount of drug in the patch and calculate the release and penetration rate. Patch samples were accurately weighted and grinded with triple amount of diatomite (Liu *et al.,* 2012), then 30 ml methanol was added. The mixture was extracted by ultrasonic wave for 30min. Methanol solution was filtered through 0.45μm filter for further determination.

Determination of in vitro drug release from ferulic acid patches

In vitro drug release tests from patches were performed using a vertical Franz diffusion cell (Tianjin pharmacopoeia standard Inc., Tianjin, China) whose diffusion area was 3.14cm^2. Cellulose acetate membrane was used as the release media. Patches were carefully stuck on the membrane before mounting on a receptor cell. The receptor cells was 17ml in volume, and filled with phosphate buffer solution (PBS, pH 7.4) whose temperature was maintained at 32±0.2°C with an external circulating water bath. The receipt solution was continually stirred at 300 rpm. At each predetermined time intervals, aliquots of 400μl were taken from the receptor cell and the receipt solution was immediately refreshed by an equal volume of PBS. During the experiments, the donor compartments and sampling arms were occluded to prevent evaporation. The concentration of FA in each sample was determined by HPLC.

Drug release kinetics

The data obtained from *in vitro* release experiments was fitted to various mathematical models shown in table 2 to assess the drug release kinetics (Nasir *et al.,* 2012).

In vitro skin permeation test

Hairless mouse skin was excised and subcutaneous fat and fascia was moved with dry cotton. The patch was sticked on the surface of the stratum corneum facing upward to the donor cell. The receipt solution and experiment conditions were equal to the release experiment. The concentration of FA in each sample was also determined by HPLC.

Drug permeate kinetics

The *in vitro* penetration data were fitted to various mathematical models shown in table 2 to assess the drug permeate kinetics.

Drug residues in patch

After penetration experiment, peel off the patch from skin and determine the drug content residues in the patch using the method mentioned in "Drug content of ferulic acid patches".

Drug residues in skin

Peel off the patch and wash the skin surface with saline, then rub gently with dry cotton to remove the residuals in the folds and furrows of the stratum corneum surface. After washing, the skin was placed into a tube, with 1ml ethanol/ saline (1/1) added, then homogenized at a 10000 r/min rotary speed for 2min. Supernatant of 600μl was removed to another centrifuge tube. To precipitate proteins in the sample, 2ml methanol was added and vortexed for 60s, and then the sample was centrifuged at 10000r/min for 10 min. Supernatant of 20 μl was injected into the HPLC system for analysis.

Analytical methods

1. HPLC condition

Drug content in the patch was analyzed using Agilent 1260 HPLC system. The column for this analysis was Hibar C18 (250mm×4.6mm i.d. 5μm, Merck). The mobile phase was acetic acid / water (1/100) and methanol 40:60. The eluant was analyzed at 323 nm.

2. Validation of HPLC for drug content in patch

FA content in patch by HPLC resulted in good separation with no interfering peaks. The reference samples were prepared by spiking various quantities of FA into methanol. Linearity, recovery, and precision were studied to validate the analysis method.

3. Validation of HPLC analysis for drug content in receipt solution

Analyzing FA in receipt solution by HPLC resulted in good separation with no interfering peaks. The reference sample was prepared by spiking various quantities of FA into PBS. The linearity, limits of detection, intra-day precisions, and stability were also studied.

4. Validation of HPLC analysis for residual drug in receipt skin

Analyzing FA in skin homogenate by HPLC resulted in good separation with no interference from endogenous components. Skin standards covering the expected sample concentration range were prepared by spiking various quantities of FA into blank skin homogenate. These calibration standards were used to validate the linearity, recovery, and precision of the analytical method.

RESULTS

Patch preparation

24h release rate of FA from patch was selected as the index to optimize the preparation. fig. 1 showed that the amount of tartaric acid from zero to 0.5g has no significant effect on the release of FA.

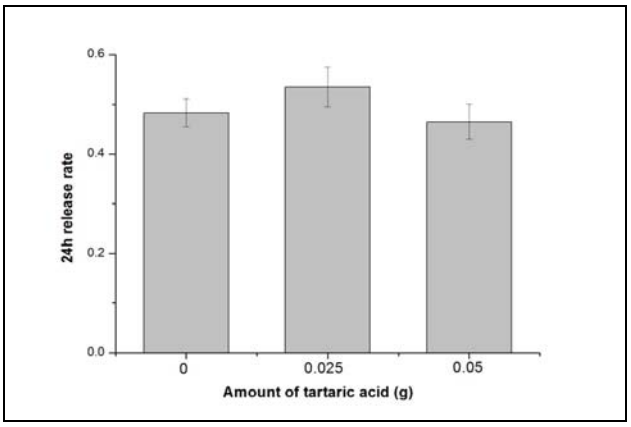

Fig. 1: Effect of tartaric acid amount on the 24 release rate of FA from patch (mean ±SD).

The effect of dihydroxyaluminium aminoacetate on 24h release rate can be found in fig. 2. There is statistically significant difference compared with 0.04g but no statistically significant difference between 0.01g and 0.02g.

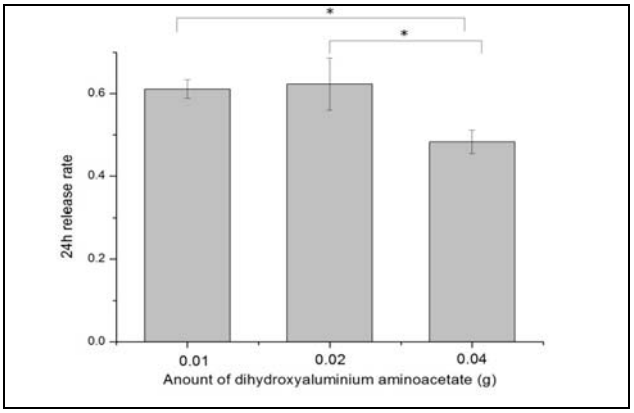

Fig. 2: Effect of dihydroxyaluminium amioacetate amount on the 24h release rate of FA (mean ±SD). $p<0.01$

The effect of amount of water on the 24h release rate of FA was shown in fig. 3. There wasn't statistically significant difference among the water amount.

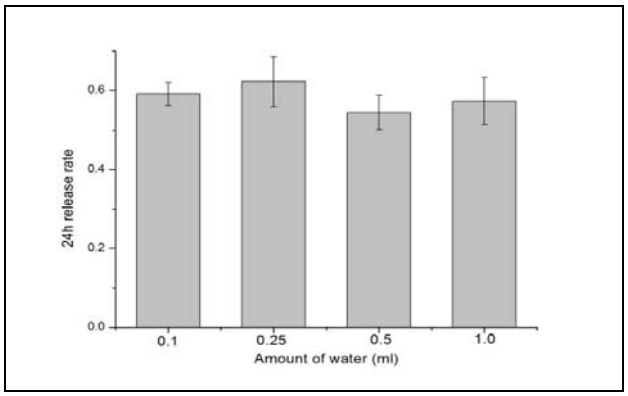

Fig. 3: Effect of water amount on the 24h release rate of FA (mean ±SD).

Physico evaluation of transdermal patches

The Physico evaluation data was shown in table 3, and compared with a commercial hydrogel patch. The FA patch could cling a ball of No.8, the cohesive strength was 15±1s, and the peeling strength was 1.17±0.3N. After peeling, there were about 0.08% paste residualed.

Drug content of FA patches

Drug content was determined for calculating the release and penetration rate. Three batches of samples were prepared for the drug content test. Drug content was from 8.5001 $mg \cdot g^{-1}$ to 8.8881 $mg \cdot g^{-1}$ with an average value of 8.851 $mg \cdot g^{-1}$ (table 4).

In vitro drug release from FA patches

The *in vitro* release profiles of FA from patches prepared according to the optimized prescription was shown in fig 4. It showed the formulation retarded the drug release about 12h and that the release rate reached 60.4465±1.7679% in 24h. The equation fitted by release data and regression coefficient (R^2) values obtained from the mathematical models were shown in table 4. The data followed first order kinetics with R^2 of 0.92. The coefficients obtained from Higuchi model and Hixson–Crowell model were 0.963, 0.9017 respectively. The slope obtained from Korsmeyer-Pappas was 0.5326.

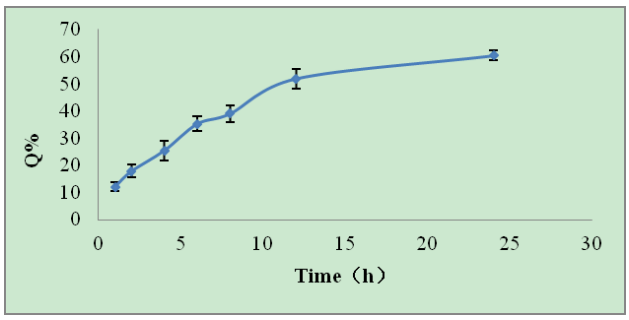

Fig. 4: The Qn-t Curve of the FA patches release (n=6).

In vitro skin permeation

The *in vitro* permeation profile of FA was shown in fig 5. The cumulative percentage of drug penetration for 24h was 1.3156±0.3588%. The equation fitted by permeation data and regression coefficient (R^2) values obtained from the mathematical models were shown in table 5. The formulation permeated through the skin followed zero order kinetics with R^2 values of 0.9855. The data obtained best fits Hixson–Crowell model as indicated by the correlation coefficient of 0.9853. The slop in Korsmeyer–Pappas model was 1.7121.

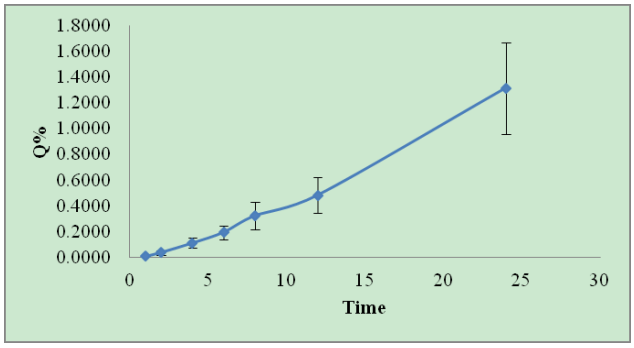

Fig. 5: The Qn-t Curve of the FA patches infiltration (n=6)

The residuals in patch and skin were determined and shown in table 7. It can be found that the majority of drug was residual in patches. Because of the thickness differences among skins, the percentage of residual drug in skin varied from 0.02% to 0.5468%, which was about a

Table 1: Composition of transdermal patches containing ferulic acid

	NP700 (g)	Dihydroxyaluminium aminoacetate (g)	Glycerine (mL)	Water (mL)	Tartaric acid (g)	Ferulic acid (mg)
Group 1	0.4	0.04	1.5	0.25	0.05	25
	0.4	0.04	1.5	0.25	0.025	25
	0.4	0.04	1.5	0.25	0	25
Group 2	0.4	0.04	1.5	0.25	0	25
	0.4	0.02	1.5	0.25	0	25
	0.4	0.01	1.5	0.25	0	25
Group 3	0.4	0.02	1.5	0.1	0	25
	0.4	0.02	1.5	0.25	0	25
	0.4	0.02	1.5	0.5	0	25
	0.4	0.02	1.5	1	0	25

25-fold difference. But comparing with the residuals in patch, the value was too small that it can be neglected.

Table 2: models used to assess the drug release/ penetration mechanism

Model	Equation
Zero order kinetic model	Q% = K t
First order kinetic model	Ln (1-Q %)= Kt
Higuchi model	$Q\% = Kt^{1/2}$
Hixson–Crowell model	$(1-Q\%)^{1/3}= Kt$
Ritger-Peppas	LnQ%=n Lnt

Q is the cumulative drug amount in receipt solution (μg/cm^2), K is the kinetic constant indicative of the release or penetration rate and t is time (h).

Validation of HPLC

Three HPLC methods have been established and validated. Table 7 showed the validation of each method. The validation of each HPLC method indicated these methods can be applied to the analysis of drug concentration in patches, receipt solution and skin respectively.

Table 3: Physico evaluation of transdermal patches containing FA

	FA hydrogel patch	Commercial hydrogel patch
Tacking strength	8	7
Cohesive strength	15±1s	14±1s
Peeling strength	1.17±0.3N	0.82±0.2N
Residuals after peeling	0.08±0.005%	0.006

Table 4: FA patches sample detection (in a humidity of 25%)

S. No	Weigh of paste (g)	Drug content (mg·g^{-1})	Average drug content (mg·g^{-1})
1	0.5422	8.5001	8.651
2	0.7312	8.5627	
3	0.5953	8.8881	

DISCUSSIONS

The aim of the present study was to develop a noval hydrogel patch for ferulic acid. For drug release kinetics has significant influence on the in vivo behaviour and tissue distribution of the drug loaded in the formulation (Mu *et al.*, 2004), the 24h release rate of FA from patch was selected as the index to optimize the preparation.

In the preparing, tartaric acid, dihydroxyaluminium aminoacetate and water, the three factors influence each other. Fig 1 showed that the amount of tartaric acid from zero to 0.5g had no significant effect on the release of FA. But with the increase of tartaric acid amount, a quicker cross linking speed would be obtained, which may result in difficult coating procedure. As literature suggested, at least 20min cross linking time was moderate (Showa Denko K.K., n.d.). Then tartaric acid was not added in the patch preparation in the situation of 25mg FA loaded in the formulation. To some extent, the existance of ferulic acid reduces the amout of adjuvant.

The presence of water will accelerate the dissociation of H^+ from organic acid and result in the dissociation of aluminum ion. But with the increase of water a faster cross-linking time will be obtained which will increase the difficulty of stirring and coating. When the added amount was 0.1ml, it needed to stir about 30min to obtain certain adhesiveness, but when the added amount was 0.5ml, the difficulty of mixing increased, with more bubbles in the paste. As a result, 0.25ml was added to prepare the FA patch.

Tacking strength, cohesive strength and peeling strength are comprehensive reflection of the patch viscosity. Chinese Pharmacopeia only provides the testing procedure with no evaluation criterion, so we compared the test data with some commercial hydrogel patches. The results indicated the preparing patch had moderate viscosity with good spreadability. There was less than 0.1% paste residual in the release liner which indicated that the patch could be repeatedly used.

The drug content tested was beyond the theoretic value, which was the proportion accounted for all the compounds added. For hydrogel patches made from NP 700 would absorb or lose water during the drying procedure, water content in the patch finally reached equilibrium to the environmental humidity, which resulted in different drug content. The observed concentration was obtained in a humidity of 25%, and all the samples used for drug content test, release and penetrate experiment were weighted in the same condition. So the absolute drug content expressed in g/piece was constant.

The drug release from the hydrogels patches are governed by various parameters such as solubility of the drug in the polymer and water, water diffusion rate into the polymer gel (Nasir *et al.*, 2012). In this study, different kinetic models were employed to study the penetration mechanism of feruclic acid from patches. The equation fitted by release data and regression coefficient (R^2) values obtained from the mathematical models were shown in table 4. The data followed first order kinetics with R^2 of 0.92, indicating the drug release was dependent of drug concentration within the system. The coefficients obtained from Higuchi model and Hixson–Crowell model were 0.963, 0.9017 respectively, indicating Fickian diffusion played a predominant role in the drug release procedure, though there was a polymer erosion effect. The

Table 5: Fitted equation by release data

Model	Fitted equation	R^2
Zero order kinetic model	$Q\%=0.0206\ t$+0.1783	0.8596
First order kinetic model	Ln(1-Q%)=-0.035t-0.1738	0.92
Higuchi model	$Q\%$=13.98$t^{1/2}$+0.8122	0.963
Hixson–Crowell model	$(1-Q\%)^{1/3}$= -0.0097t+0.9408	0.9017
Korsmeyer–Pappas model	LnQ%=0.5326lnt-2.0751	0.9833

Table 6: fitted equation by penetration data

Model	Fitted equation	R^2
Zero order kinetic model	Q%= 0.0006t - 0.0011	0.9855
First order kinetic model	Ln(1-Q%)=0.0006t-0.0012	0.9551
Higuchi model	Q=0.328$t^{1/2}$-0.4941	0.8972
Hixson–Crowell model	$(1-Q\%)^{1/3}$= -0.0002t+1.0004	0.9853
Korsmeyer–Pappas model	LnQ%=1.7121lnt-9.4688	0.9694

Table 7: Drug distributions after penetration experiment

Code	Cumulative penetration (%)	Residuals in skin (%)	Residuals in patch (%)	Total (%)
1	1.1367	0.0200	99.6265	100.7832
2	0.9293	0.5468	99.0141	100.4902
3	1.7167	0.1595	97.1559	99.0321
4	1.7814	0.2617	96.0854	98.1285
5	1.0248	0.0713	97.6827	98.7789
6	2.7255	0.0768	96.0048	98.8072

Table 8: Validation of HPLC methods for drug contents in patch, receiptor and residuals in skin

Validation item	Method for drug content in patch	Method for drug content in receptor	Method for drug residuals in skin	
Linearity	A =92.68C–1.4123，r = 0.9999	A=92.334C– 1.7245，r = 0.9999	A=2.1432C+ 1.2296，r = 0.9997	
Liner range	0.016 ~ 440.8μg·mL^{-1}	0.016 ~ 0.8μg·mL^{-1}	1.625 ~ 162.5ng·g^{-1}	
Intra-day precision (RSD)	0.18%	1.31%	3.25 μg·g^{-1}	2.23%
			32.5 μg·g^{-1}	1.21%
			162.5 μg·g^{-1}	0.84%
LOQ	-	15ng/ml	0.42μg·g^{-1}	
Stability (RSD)	0.79%	1.64%	3.25μg·g^{-1}	2.92%
			32.5 μg·g^{-1}	1.02%
			162.5 μg·g^{-1}	0.40%
Method recovery	98.87±2.19%	-	3.25 μg·g^{-1}	93.23±2.72%
			32.5 μg·g^{-1}	97.32±1.01%
			162.5 μg·g^{-1}	99.89±0.40%
Extract recovery	-	-	3.25 μg·g^{-1}	91.30±2.83%
			32.5 μg·g^{-1}	94.78±5.16%
			162.5 μg·g^{-1}	86.62±4.88%

slope obtained from Korsmeyer-Pappas equation between 0.45 and 0.89 indicated that FA's release was results from a combination of diffusion effect and polymer erosion effect.

From the *in vitro* permeation data it can be found that the formulation permeated through the skin followed zero order kinetics with R^2 values of 0.9855, suggesting that the formulation could provide a sustained and controlled permeation of the drug. The data best fitted Hixson–Crowell model, indicating the drug releasing from formulation followed polymer erosion. The slop in Korsmeyer–Pappas model was much higher than 0.89, which also demonstrates FA releasing from patches was

the result of polymer surface erosion. Comparing with the release data, it can be found that the mechanism has changed. Because of the water resistance of stratum corneum, only a little water can penetrate to the donor compact. Polymer swells water rapidly, which retards the diffusion of FA. While in the release experiment, there is sufficient water in the donor compact, thus, the diffusion is easier to occur.

After permeation experiment, the residuals in patch were tested. It can be found the data was more than 90%. Correlating with the penetration data, it may illustrate that stratum corneum is the most important barrier retarding the drugs permeate through skin. To obtain better penetration, further experiments are needed to study the effect of penetration enhancers.

In conclusion, formulation FA patch (dihydroxyaluminium aminoacetate: NP700: Glycerine: Ferulic acid was 0.02:0.4:1.5:1.25:0.25) has shown moderate viscosity with good spreadability. The patch achieved extended release, but because of the barrier of stratum corneum, some penetration enforcer would be added to obtain better penetration.

ACKNOWLEDGEMENTS

This work was supported in part by Team Development Program of Beijing University of Chinese Medicine (No.2011-CXTD-13).

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