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NaF Patch Formulation and Transport Test to Determine Fluoride Diffusion via Mouse Skin as Membrane (Transdermal in Vitro Test)

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Abstract: ***Background: Transdermal route as an innovation of fluoride delivery for increasing enamel resistance has been developed. Based on transport test of NaF solution, fluoride can be absorbed into the skin. Further research on NaF patch formulation need to be developed. Aims of this research are to formulate NaF patch with good physical properties and find out fluoride weight after using transport test of NaF patch via mouse skin. Methodology: The design of the research is quasy experimental with post test only control group design and use Franz Like Diffusion cell used as the instrument. Compartment cell was NaF patch fixed on mouse skin that had been prepared before. The compartment cell was Phosphate Buffer Saline solution 0,1 M pH 7,4. Several NaF patch based on NaF concentration was determined based on physical appearance. Sample was taken for time interval of 8, 24 and 48 hours and fluoride content was measured by Potensiometer Spesific Ion Fluoride. Result shows that NaF patch can be formulated with maximum NaF concentration around 1.000 ppm. After transport test of NaF patch 1.000; 750 and 500 ppm and 0 ppm as control, fluoride can penetrate via mouse skin. Fluoride weight was 0,08-1,73 µg; 0,72-1,23 µg; 0,33-0,94 µg and 0,06-0,1 µg respectifully. Based on repeated measured anova there is an influence of transport time and NaF concetration toward fluoride weight.***

Keywords: fluoride weight , NaF patch, transport test

1. Introduction

Recently, transdermal drug delivery system (TDSS) has potential route for delivering drugs locally and systemically.[1] Transdermal drug delivery is the non-invasive delivery of medications from the surface of the skin - the largest and most accessible organ of the human body - through its layers, to the circulatory system. This route has several advantages including reduced side effects and improved therapy due to maintenance of plasma levels up to the end of the dosing; avoid hepatic first past effect; improved bioavaibility; increased convenience to administer drugs which would otherwise require frequent dosing and more uniform plasma level of drugs. [2] Medication delivery is carried out by a patch that is attached to the body surface. Transdermal patch is a medicated adhesive pad that is designed to release the active ingredient at a constant rate over a period of several hours to days after application to the skin.

Transdermal drug delivery is theoretically ideal for many injected and orally delivered drugs, but many drugs cannot pass through the skin because of skin's low permeability. The foremost requirement of TDDS is that the drug has the right mix of physicochemical and biological properties for transdermal drug delivery. It is generally accepted that the best drug candidates for passive adhesive transdermal patches must be non ionic, low molecular weight (less than 500 Daltons), have adequate solubility in oil and water (log P in the range of 1-3), low melting point (less than 200°C) and potent (dose in mg per day).[1] Based on preliminary research about transport test of NaF solution it was found that fluoride can diffuse via stratum corneum skin mouse dan fluoride weight increased from time to time.[3] Oleic acid as chemical enhancer also proven increased fluoride weight on transport test for 8, 24 and 48 hours. [4] Based on several advantages of transdermally drug delivery and also trends of increasing caries prevalence, an innovation of patch containing Sodium Fluoride (NaF) need to be developed.

The patch were prepared by solvent casting method with polimer *polyvinylalcohol* (PVA) and *polyvinylpyrolidone* (PVP), oleic acid and isopropyl alkohol as enhancer and propilenglikol as plasticizer[1]. Natrium fluoride as drugs was added with variation of weight to provide patch with good physical appearance.

The purpose of this study is to develop formulation and to evaluate in vitro permeation of NaF patch systematically. Transport test of NaF patch is needed to determine whether fluoride can be absorbed into mouse skin as membrane and the influence of transport time and NaF patch concentration towards fluoride weight transported.

2. Material and Methods

Materials

Calcium and magnesium free phosphate buffer saline (CMF PBS 0,1 M pH7,4); Aquabides; Total ionic strength adjusment buffer (TISAB II); Natrium fluoride; White mouse (Rattus novergicus); Polimer poly vinyl pyrolidone (PVP) and poly vinyl alcohol (PVA); oleic acid; Iso propyl alcohol (IPA); Prophylene glycol (PG)

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Instruments

Glass Franz-like diffusion cell; potensiometer spesifik ion fluoride (ISE/Ion Selective Electrode); hot plate electric; stirer magnetic; volume pipe; micro pipe; shaver electric; petry disc ; metal mal punch; digital balance

Preparation of NaF weight[5]

Determining weight of NaF, calculation based on diameter of petry disc and cell diffusion on Frans-like diffusion cell with sliding calipers. Variation of fluoride weight resulted in fluoride concentration of the patch. Patch concentration 10.000 ppm equal to 10.000 mg/10.000 ml

Preparation of NaF patch[6]

NaF patch are prepared by dissolving 333 mg PVA into 2 ml aquabidest; then dissolving 167 mg PVP into 2 ml aquabidest boiled in water bath until polimer disolved; dissolving NaF into 2 ml aquabides added by 0,1 ml oleic acid and 0,1 ml IPA mixed properly in glass ring. The medicated polymer formed is then molded into rings with defined surface area and controlled thickness over the mercury on horizontal surface followed by solvent evaporation at an elevated temperature. Variation of NaF patch concentration range from 10.000 ppm; 5.000 ppm; 3.000 ppm; 2.000 ppm; 1.000 ppm; 750 ppm; 500 ppm and 0 ppm. Formulation based on NaF concentration with good physical appearance.

The weight NaF is adjusted by diameter petry disc. All material was mixed into a glass tube and stirred until dissolved. The mixture solution then is poured in a petri disc and allowed to stand for ± 3 days. When it is dry, the matrix is taken with a special knife and stored in aluminum foil until used. As control we use patch without NaF which has the same preparation as NaF patch.

In vitro skin permeation study using skin mice

In vitro skin permeation study was carried out by using Franz-like diffusion cell and mice skin as membrane. Wistar mouse weighed between (200 gm - 250 gm) are taken and sacrificed by excessive chloroform inhalation. The abdominal hairs were removed with marketed hair removers. The abdominal skin was carefully separated from the body, with the dermis part remaining intact. Subcutaneous tissues were surgically removed. The inner part of the skin was washed with distilled water thoroughly to separate the adhering fat. The full thickness skin thus obtained was kept in normal saline solution and stored at 4 ± 1°C until used for the experiment. The contents of the donor and receptor compartments were separated by placing the excised skin in between two compartments. The skin was mounted in such a way that the stratum corneum side of the skin continuously remained in an intimate contact with the transdermal patch in the donor compartment. The receptor compartment contained 100 ml phosphate buffer *(pH 7.4)* at 37 ± 2°C. The content of the diffusion cell was stirred using a teflon coated bead at a constant speed (100 rpm). Samples were withdrawn (5 ml) at predetermined time intervals (8, 24 and 48 hours) and replaced with same amount of phosphate buffer *(pH 7.4)* to maintain the sink condition. After suitable dilution the samples were analyzed for fluoride content using Potensiometer specific ion fluoride.



Figure 1: permeation skin test using Franz like diffusion cell

Data Analysis

Repeated ANOVA test is used to determine the influence of time transport test for 8, 24 and 48 hours and the content of fluoride released from the patch in solution then it is followed by using LSD test.

3. Result and Discussion

After testing preparation of NaF patch with a broad variety of petri disc with concentration of 10,000 ppm, 3,000 ppm, 2,000 ppm and 1,000 ppm, it showed that concentration of 1,000 ppm resulted the most homogeneous patch. Higher concentrations shows crystallized patch. Patches image can be seen as below.

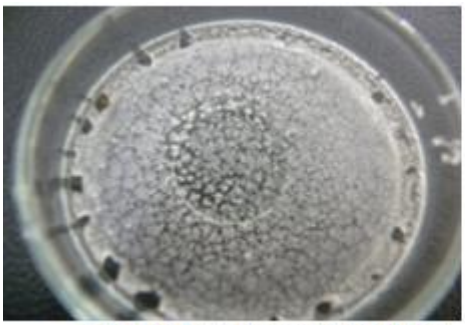


Figure 2: NaF patch 3.000 ppm

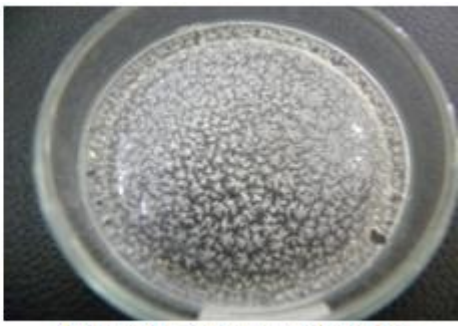


Figure 3: NaF patch 2.000 ppm

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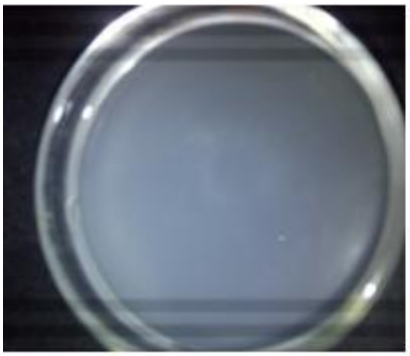


Figure 4: NaF patch 1.000 ppm

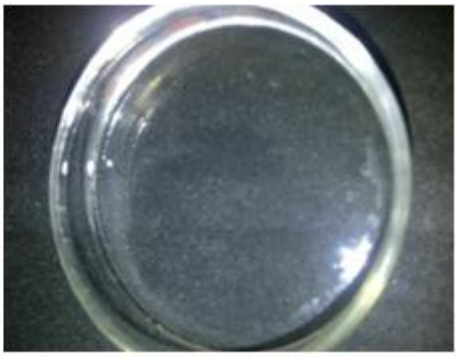


Figure 5: patch without NaF (0 ppm)

In vitro transport tests were conducted for 8, 24 and 48 hours using NaF patch concentration of 1,000 ppm, 750 ppm, 500 ppm and 0 ppm. It is used to calculate F ions content transported in solution recipient.

The result shown as in table 1.

Table 1: Mean ± standard deviation fluoride concentration and content after transport test for 8, 24 dan 48 hours in NaF patch concentration 1.000 ppm, 750 ppm, 500 ppm dan 0 ppm in vitro

Time in hours	Mean ± SD a. concentration (µg/ml) ion F b. content (µg) ion F			
	NaF patch 1.000 ppm (n=3)	NaF patch 750 ppm (n=3)	NaF patch 500 ppm (n=3)	NaF patch 0 ppm (n=3)
8	a. 0,04 ± 0,02 b. 0,88 ± 0,05	a. 0,04 ± 0,05 b. 0,72 ± 0,1	a. 0,02 ± 0,05 b. 0,33 ± 0,1	a. 0,003 ± 0,005 b. 0,06 ± 0,01
24	a. 0,06 ± 0,03 b. 1,15 ± 0,07	a. 0,05 ± 0,02 b. 0,98 ± 0,02	a. 0,03 ± 0,01 b. 0,63 ± 0,03	a. 0,01 ± 0,002 b. 0,11 ± 0,04
48	a. 0,09 ± 0,06 b. 1,73 ± 0,09	a. 0,07 ± 0,02 b. 1,23 ± 0,4	a. 0,05 ± 0,09 b. 0,94 ± 0,2	a. 0,01 ± 0,01 b. 0,18 ± 0,02

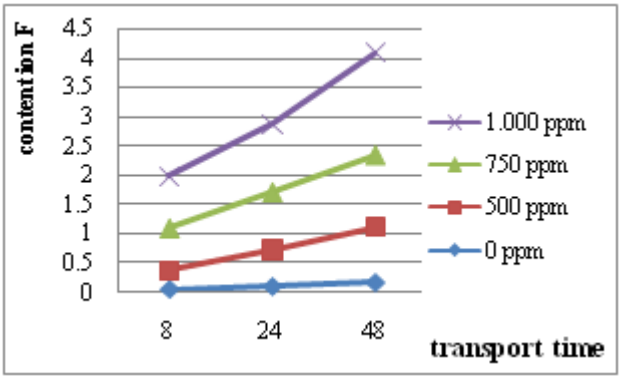


Figure 6: Line graph shown increase of F ions weight after transport test on 4 types of patch

In vitro transport test with variation of NaF concentration determine the fluoride permeability through mice skin. The first discussion is the presence of F ions which penetrate into mice skin. The ion F is released from patch then attached to mice skin as membrane and diffused into recipient compartement.

The second discussion is the longer test to measure the weight of ion F diffused to receptor compartment. Two-way repeated ANOVA obtained significant differences among the average weight of F transported on all types of patches in 8, 24 and 48 hours ($p < 0.05$). This condition due to the use of hydrophilic polymer in the patch formulation. The polymer (PVP and PVA) will attract water molecules which cause increasing patch permeability.[6]

These results are consistent with studies which examine propranolol.[7,8] Hydrochloride and repaglinide mixed in the form of a patch. Both of these drugs have similar properties to the NaF which is hydrophilic. Both studies reported a linear correlation profile which increases the permeation test of 0-30 hours against drug concentration.

Polymers in the formulation of patch are similar with spine in a transdermal system. Preparation of NaF patch is using two polymers, PVA and PVP. Two polymers work together. One polymer acts to prevent drugs get out from reservoir while other acts as an adhesive. [9] PVA is soluble in water and will experience the dissolution in the moist environment when it is mixed with water. This polymer has a molecular weight of 20.000 Dalton. PVP has the properties soluble in water and organic solvents such as alcohols with molecular weights between 10.000 -50.000. Both of these polymers are cross -linked that can bind to each other.[10]

Both polymers play a role in controlling the release of drugs (NaF) as well as protect it from patch preparation.[9] NaF in the form of molecules have the nature of water-soluble polymers which can bind to both after mixed in preparation. Polymer protection mechanism is against drug with slow dissolve NaF, and inhibit drugs out of the matrix. The mechanism of drug release from the patch is PVA and PVP. They will react to form tiny pores that become a way for the drug to slowly break away from the polymer bonds.[11] Fluoride ions as its nature can freely move and move separated from the polymer bond.[10]

Another study using a hydrophilic polymer PVA and PVP are the manufacture of papaverine HCl patch. The results showed combination of two hydrophilic polymers have a faster release when compared with the two hydrophobic polymers or a combination of hydrophilic and hydrophobic polymer.[6] Further results in this trial based on a repeated two-way ANOVA is the concentration of NaF in patch significantly affect the weight of the F ion transpoted in PBS solution. Theoretically penetrating power will increase when the drug dose in patch reach saturated condition. [12] Drug permeability is affected by the drug concentration and variations of skin surface.

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4. Conclusion

Preparation of NaF patch can be done with maximal concentration of 1.000 ppm.

Fluoride in the form of patch can penetrate to mouse skin as membrane.

Length of transport time and concentration of NaF in patch, influence the ion transported to receptor compartment.

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