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.

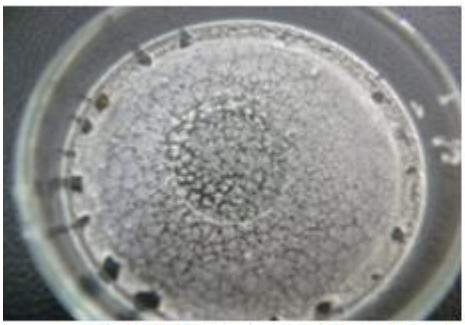

Figure 2: NaF patch 3.000 ppm

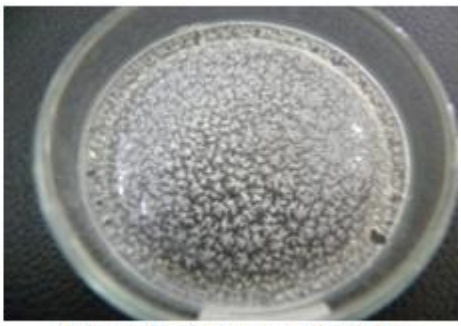

Figure 3: NaF patch 2.000 ppm

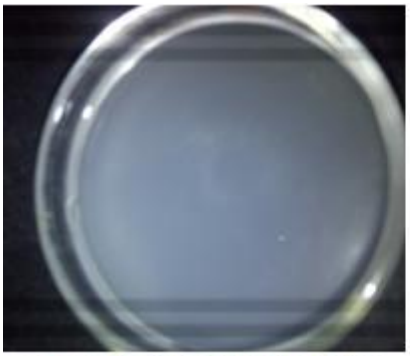

Figure 4: NaF patch 1.000 ppm

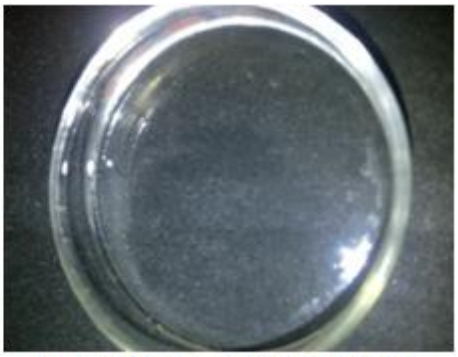

Figure 5: patch without NaF (0 ppm)

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

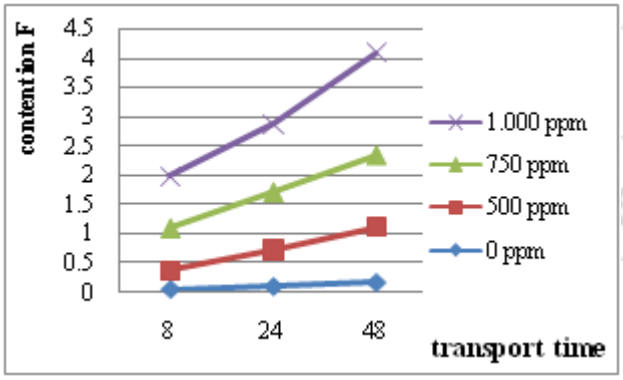

Figure 6: Line graph shown increase of F ions weight after transport test on 4 types of patch

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.

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.

References

[1] Aggarwal G, Development, fabrication and evaluation of transdermal drug delivery system : A Review, *Latest Reviews* 2009,7 (5).
[2] Patel,D., Patel,N., Parmar,M., dan Kaur,N., Transdermal Drug Delivery System : Review, *Int. J. Biopharm & Toxicol*., 2010,1,(6): 1.
[3] Fatmasari D, Prahasto ID, Nugroho AK, Widjijono, Can fluoride penetrate to the skin? (Preliminary study of fluoride transdermal drug delivery system, The 2nd International Joint Symposium on Oral and Dental Science, Proceeding Book, Gadjah Mada University, 2012[a]
[4] Fatmasari D, Prahasto ID, Nugroho AK, Widjijono, Additional of chemical enhancer for increasing the penetrational fluoride mouse skin, dentika Dental Journal, 2012[b],16 (2): 14-16.
[5] Wang J, Ruan J, Zhang C, Yujie YE, Cai Y, Wu Y, Development and Evaluation of the Sinomenine Transdermal Patch, *J Pharm Sci*. 2008, 21 (4): 407-10
[6] 6. Prabhakara,P., Koland, M.,Vijaynarayana K, Haris NM, Shankar G, Mohd GA, Narayana C, Satyanarayana, Preparation and Evaluation of Transdermal Patches of Papaverine Hydrochloride, *J. Res. Pharm* 2010,1(3) 259-266.
[7] Sharan G, Dey BK, Nagaraja K, das S, Kumar SU, Dinesh V, Effect of various permeation enhancers on propoanolol hydrochloride formulated patches, *International Journal of Pharmacy and Pharmaceutical Sciences*, 2010, 2 (2): 21-31.
[8] Prajapati ST, Patel CG, Patel CN, Formulation and evaluation of transdermal patch of repaglinide, *ISRN Pharmaceutics.* 2011
[9] Kandavill S, Nair V, Panchagnula R, Polymers in transdermal drug delivery system, *Pharmaceutical Technology*, *www.pharmtech.com*, 2002
[10] Rowe RC, Sheskey PJ, Owen SC, *Handbook of Pharmaceutical Excipient*, fifth edition, , Pharmaceutical Press, 2006
[11] Baviskar DT, Parik VB, Gupta HN, Maniyar AH, Jain DK., Design and evaluation of patches for transdermal delivery of losartan potassium, *J Pharm Sci Technol*. 2012, 66 (2) :126-35.
[12] Variankaval E.N., Jacob K.L., Polymorphism of 17-β estradiol in a transdermal drug delivery system, *Journal of Material Science:*, 2002, 13: 271-80.