RESEARCH PAPER

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# Studies in Transdermal Drug Delivery Systems for Estradiol

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**Adhesive matrix patches containing estradiol and oleic acid (as skin permeation enhancer); and Eudragit matrix patches containing triacetin and citric acid were prepared and evaluated in terms of their uniformity, skin irritation and *in vitro* skin permeation. The adhesive matrix patches caused only "very slight reddening" of rabbit skin which was less as compared to marketed control, Adhesive Tape, U.S.P., which caused moderate to severe reddening. The Eudragit matrix patches caused "very slight to well defined erythema". The *in vitro* skin permeation rates of estradiol through guinea-pig skin from both adhesive matrix and Eudragit matrix patches were faster than from the reservoir type Estraderm® patch.**

TRANSDERMAL estradiol delivery, provides physiological levels of estradiol in postmenopausal women in a convenient low-dose form which may avoid some of the complications of higher dose oral therapy. Different types of transdermal systems have been designed and manufactured for delivery of potent skin permeable drugs[2]. We have been investigating adhesive matrix type transdermal drug delivery systems in our laboratory[3]. In this paper, we present some of the work done on estradiol adhesive matrix and Eudragit matrix type patches.

## EXPERIMENTAL

### Materials:

Estradiol was received as a gift sample from CIPLA Ltd. The sample had a m.p. of 174-175° and contained 98.84% w/w estradiol on an anhydrous basis. It's infra-red spectrum was concordant with that of estradiol hemihydrate. The backing membrane, release liner and pressure sensitive adhesive were received as gift samples from 3M Medica, GmbH, Healthcare Department, Germany. Eudragit RL 100 (an acrylate polymer synthesized from acrylic and methacrylic acid esters with a low content of quaternary ammonium groups) was received as a gift sample from Röhm Pharma, GmbH, Germany. Solvents used were A.R. grade and were obtained from S.D. Fine Chemicals. Other materials used included oleic acid (L.R. grade), triacetin (L.R.grade), and citric acid anhydrous (A.R. grade). Reagents for the estimation of estradiol by radioimmunoassay method were provided by the World Health Organisation, Geneva.

In skin permeation studies, a marketed reservior type patch, Estraderm® 0.05 (CIBA Pharmaceutical Co.) was used. It has an area of 10 sq.cm. and contains 4 mg of estradiol dissolved in 0.3 ml of alcohol. The release is controlled by an ethylene vinyl accetate copolymer membrane.

### Analytical methods

Estradiol content in the patches was estimated by extraction in methanol followed by measurement of the absorbance at 280 nm on a Spectronic 2000

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UV-spectrophotometer. A radioimmunoassay method was used for the analysis of estradiol in skin permeation studies[4].

### Preparation of transdermal patches

The adhesive matrix patches were prepared by delivering a solution containing 4 mg/ml estradiol, 9 mg/ml oleic acid and 45 mg/ml pressure sensitive adhesive in ethylacetate onto a backing membrane surface and allowing the solution to dry in air under ambient conditions. The Eudragit matrix patches were prepared by delivering a solution containing 4 mg/ml estradiol, 40 mg/ml Eudragit RL 100, 8 mg/ml triacetin and 2 mg/ml citric acid onto a release liner and allowing the solution to dry in air under ambient conditions. The volume of solution delivered was 0.1 ml/cm$^2$ for both the adhesive matrix and the Eudragit matrix patches.

### Evaluation of patch uniformity

The patches were evaluated for thickness (using a micrometer screw gauge), area (using cut and weigh graph paper method), weight of the matrix layer and drug content. In these studies, the adhesive matrix patches were made using moulds of approximately 1 sq.cm inner diameter, and the eudragit matrix patches were made using moulds approximately 5 sq. cm inner diameter.

### Skin irritation

The primary skin irritation test was performed on seven healthy male albino rabbits weighing between 2 to 3.5 Kg. Adhesive tape, USP, (Johnson Plast, M/s. Johnson and Johnson Ltd., Bombay) was used as a control patch. Adhesive matrix and Eudragit matrix transdermal patches, 5 sq.cm in area, and containing 2 mg of estradiol were used as test patches. The test was conducted on unabraided skin of the rabbits. The control patch was placed on the left dorsal surface of each rabbit whereas the test patch was placed on the identical site on the right dorsal surface of the rabbit. The patches were removed after a period of 24 hrs with the help of an alcohol swab. The skin was examined for erythema/edema and scores were assigned as detailed elsewhere[5].

### *In-vitro* skin permeation studies:

A piece of full thickness abdominal skin freshly excised from female guinea pigs (Hindustan Lever Research Division, Bombay) was used for study of skin permeation using Erweka skin permeation system. The study was carried out in two sets. In Set I, skin permeation of estradiol from Estraderm® and adhesive matrix patches was compared and in Set II, the Eudragit matrix patches were compared to Estraderm®. All patches used had an area of 10 sq.cm. The diffusion cells were fabricated from borosilicate glass, had a height of 47 mm and an opening to the skin/membrane surface area of 10.17 sq.cm. The fluid volume was approximately 13.5 ml.

A freshly prepared aqueous solution of 40% v/v PEG 400 in the receptor compartement was maintained at 37 ± 1° with the help of a constant temperature circulating water bath (Siskin Julabo V). The receptor compartment was emptied of the entire sink solution at 24, 48, 72, 96 and 120 hrs and replaced by fresh sink solution. The amount of estradiol in each sample was determined by radioimmunoassay.

## RESULTS AND DISCUSSIONS

### a) Evaluation of patch uniformity

The thickness, area, weight of matrix layer and drug content are given in **Table I**. A two-way ANOVA on thickness measurement showed no significant differences within and between patches at the 5% level of significance. The weight of the matrix layer and drug content were in agreement with the theoretically expected values showing good mass balance. The low coefficient of variation in the estimated values indicated good patch uniformity and reproducibility.

**Table 1: Data obtained from evalulation of patches**

| Type of Patch | Thickness microns | Area sq.cm | Weight of matrix layer mg | Drug content mg/sq.cm |
|---|---|---|---|---|
| Adhesive matrix | 82.8 ± 2.485 (3.0%)* | 0.89 ± 0.017 (1.91%) | 5.7 ± 0.22 (3.86%) | 0.40 ± 0.003 (0.77%) |
| Eudragit matrix | 97.3 ± 3.70 (3.8%) | 5.09 ± 0.108 (2.12%) | 25.6 ± 0.64 (2.5%) | 0.42 ± 0.013 (3.1%) |

*Figures in parenthesis indicate percent coefficient of variation.

**Table 2: Data from primary skin irritation test**

| Rabbit | Adhesive matrix patch | | Eudragit matrix patch | |
|---|---|---|---|---|
| | Control | Test | Control | Test |
| 1 | 4 | 1 | 1 | 1 |
| 2 | 3 | 1 | 3 | 2 |
| 3 | 4 | 1 | 4 | 1 |
| 4 | 4 | 1 | 2 | 1 |
| 5 | 4 | 1 | 1 | 2 |
| 6 | 3 | 1 | 2 | 1 |
| 7 | 4 | 1 | 2 | 1 |

**b) Skin irritation**

The results of the primary skin irritation test performed on the adhesive matrix patch and the Eudragit matrix patch are given in **Table 2.** Only erythema was produced by the control and test patches on rabbit skin whereas no evidence of edema were observed. Grading system used was as follows[5].

| Erythema | Score |
|---|---|
| No erythema | 0 |
| Very slight erythema | 1 |
| Well defined erythema | 2 |
| Moderate to severe erythema | 3 |
| Severe erythema (beet redness) | 4 |

The data was analysed using the sign test. The control patches produced greater levels of redness than that produced by the adhesive matrix patches, whereas the level of redness produced by the control was comparable to that produced by the Eudragit matrix patches, at the 5% level of significance.

### c) *In-vitro* skin permeation

**Figure 1** gives the **in-vitro** skin permeation profiles of estradiol from Estraderm® and the adhesive matrix patches (Set I), and **Figure 2** gives the skin permeation profiles of estradiol from Estraderm® and Eudragit matrix patches (Set II). A steady state was obtained after about 48 hrs.

In these studies, the whole of the sink solution in the diffusion cell was collected quantitatively at each sampling time and then the amount of estradiol permeated through 10 sq. cm of skin in the 24 hrs interval estimated. A value of skin permeation rate (μg/sq cm/hr) was calculated from each of these measurements made after steady state had been achieved. A mean steady state skin permeation rate was calculated and the values are given in **Table 3**. This method was adopted because it defines the sample size (N) accurately for the purpose of constructing a confidence intervals for the difference in skin permeation rates between Estraderm® and the matrix patches. For Set I, sampling during steady state was at 72, 96 and 120 hrs in studies done in triplicate and thus sample size (N) was 9. For Set II sampling during steady state was at 72 and 96 hrs and thus sample size (N) was 6. the confidence interval for the difference in skin permeation rates was constructed using the formula.

$$(\bar{X}_1 - \bar{X}_2) \pm ts_p \sqrt{\frac{1}{N_1} + \frac{1}{N_2}}$$

where Sp is the pooled standard deviation[6]. The results are given in **Table 3**. The confidence interval for the difference did not cover zero and thus it may be concluded that the skin permeation rates from adhesive matrix patch and the Eudragit matrix patch were significantly faster than that from Estraderm®.

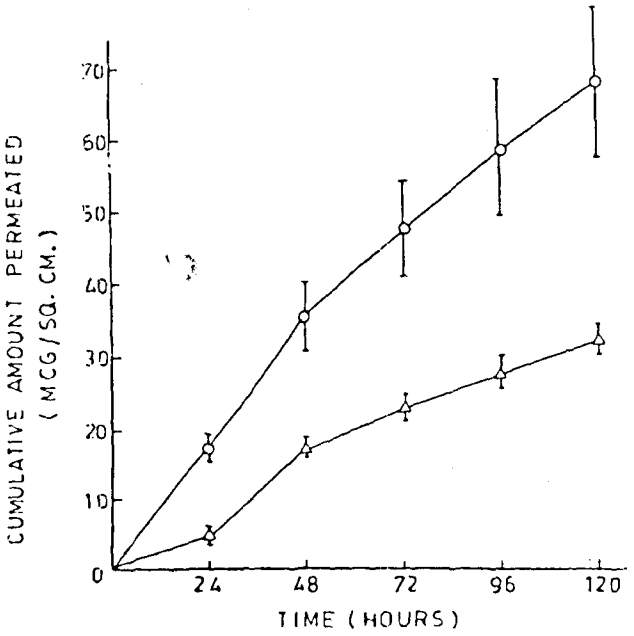


**Figure 1 : In-vitro skin permeation profile of estradiol into 40% v/v PEG 400 at 37 ± 1° using guinea pig skin (Set I). Key: Δ Estraderm®; O' Adhesive matrix patch.**

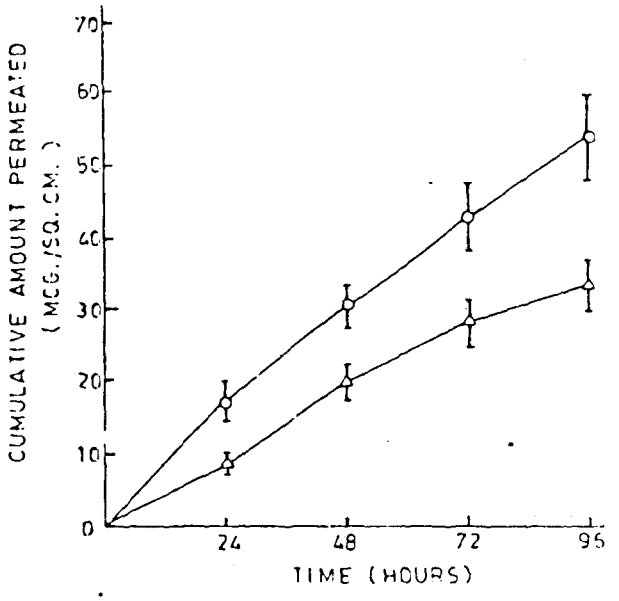


**Figure 2 : In-vitro skin permeation profile of estradiol into 40% v/v PEG 400 at 37 ± 1° using guinea pig skin Set II. Key: Δ Estraderm®; O Eudragit matrix patch.**
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**Table 3: Skin permeation of estradiol from matrix patches compared to Estraderm®**

| Patches | Steady state skin permeation rate (Mean ± S.D.) mcg/sq.cm/hr | |
|---|---|---|
| | Set I | Set II |
| Estraderm® | 0.2476 ± 0.09 | — |
| Adhesive matrix patches | 0.4474 ± 0.17 | — |
| Estraderm® | — | 0.2693 ± 0.1096 |
| Eudragit matrix patches | — | 0.4487 ± 0.1122 |
| 95% confidence interval for the difference | (0.133-0.405) | (0.095-0.264) |

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