DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 19(11), 1343-1356 (1993)

CONTROLLED TRANSDERMAL OCCLUSIVE DELIVERY DEVICE OF PRIMAQUINE

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ABSTRACT

Primaquine an antimalarial drug was studied for its permeation behavior across the human cadaver skin. Ethylene vinyl acetate copolymer (E.V.A. cop) was used for the preparation of drug reservoir. To optimize the drug delivery from the drug reservoir E.V.A. cop of different vinyl acetate mole content (40%, 25%, 18%) was used. To achieve an enhanced skin permeation an occlusive face adhesive type delivery system was fabricated. The prepared systems were characterized for in-vitro studies. The system that delivered the drug in accordance with the theortically calculated required delivery rate was selected for in-vivo performance evaluation . The prepared system functions over an predicted definite time period in an uniform and defined fashion. The drug transdermal application has therapeutic potential.

INTRODUCTION

Primaquine is an exceptionally important antimalarial drug active against several life cycle stages of parasites (1). Its mode of action is exoerythocytic (2). It is active against the

primary tissue schizonts thus can function as prophylactic agent. Primaquine has low therapeutic index, low clearance and extensive bio - distribution. It is subjected to first pass metabolism and does not accumulate into blood cells unlike other anti-malarials (3).

Of numerous methods that have been employed to enhance the percutaneous absorption of drugs, the complete impairment of passive trans-epidermal water loss (occlusion) at the application site is the simplest method. The increased clinical efficacy of topical drugs caused by covering the site of application was first documented by Garb (4). Occlusion has also been reported to increase the percutaneous absorption of various other topically applied compounds (5,6).

Ethylene vinyl acetate co-polymer is being used for memberane controlled diffusion devices. The polymer has been found biocompatible, stable and possesses optimal diffusion properties for such application. The vinyl acetate content in the polymeric memberane regulates release kinetics and other physico-chemical properties of the memberane. The greater vinyl acetate content in the polymer resulted in higher memberane permeation of nicardpine hydrochloride (7).

The present study is aimed at exploring and utilizing the drugs permeation behavior to achieve transdermal delivery. Finally an attempt was made to prepare transdermal therapeutic system of primaquine. The systems were studied for *in-vitro* characterization and the performance was evaluted using human cadavers as subjects.

MATERIALS AND METHODS

Primaquine diphosphate was obtained from IPCA Laboratories Bombay India. The polymers used were ethylene vinyl acetate co-polymer of 40%, 25%, 18% mole content. Aldrich Chemical Company, Wisconsin USA and Polystyrene, Sigma Chemical Company, St. Louis, USA, N-octanol from Fluka Chemical Company. All the solvents

Table - I. *Characterization of Primaquine Diphosphate.*

Partition coefficient (km)	*Drug permeation rate ($mg/cm^2/hr$)*
1.49 ± 0.08	*0.055 ± 0.019*

and other chemicals used were of AnalaR grade and were used as supplied by E. Merck India Limited.

Characterization of Drug

(a) *Partition Coefficient : The partition co-efficient (km) of drug was determined using octanol-phosphate saline buffer (pH 7.4). The drug was equilibrated in both the phases at 37±1°C for 36 hours, subsequently the drug concentration in both the phases was determined spectrophotometrically.*

(b) *Drug Permeation : Continuously perfused Franz diffusion cell was used for skin permeation studies. The saturated solution of primaquine diphosphate was prepared by suspending an excess amount of drug in 40% v/v aqueous PEG-400 solution. The permeation studies were carried out on human cadaver skin (Medical College Jabalpur, India). The same solution except drug was used in the receptor compartment. The studies were carried out for 24 hrs. The temperature of diffusion cell was maintained at 37±1°C (Table-I).*

(c) *Drug Analysis : The drug content in each aliquot was determined spectrophotometrically by measuring the absorbance at 254 nm using Shimadzu UV-150-02 double beam spectrophotometer. Beer's law was obeyed in the concentration range of 1-14 mcg/ml.*

Preparation of Delivery Device

(a) *Film Casting (Preparation of Drug Reservoir) : The 10% w/v polymeric solution of ethylene vinyl acetate copolymer was*

Table-II. *Formulation description and specifications of the components of Occlusive Transdermal device (OTDS).*

DESCRIPTION			SPECIFICATIONS				
Device	*Polymeric description*	*Solvent system used*	*TDDS components*	*Polymers used for preparation*	*Area (cm^2)*	*Thickness (mcm)*	*Drug content (mg/cm^2)*
$OTDS_1$	*E.V.A. cop (40% mole vinyl acetate)*	*Methylene chloride*	*Backing memberane*	*Polystyrene*	20.0±0.5	60.5±1.0	-
$OTDS_2$	*E.V.A. cop (25% mole vinyl acetate)*	*Methylene chloride + Methyl ethyl ketone (2:1)*	*Drug reservoir*	*E.V.A. cop*	20.0±0.5	40.5±0.8	1.50
$OTDS_3$	*E.V.A. cop (18% mole vinyl acetate)*	*Methyl ethyl ketone + Ethyl alcohol + Water (1:2:1)*	*Adhesive layer*	*Pressure sensitive adhesive*	20.0±0.5	25.5±0.4	0.05

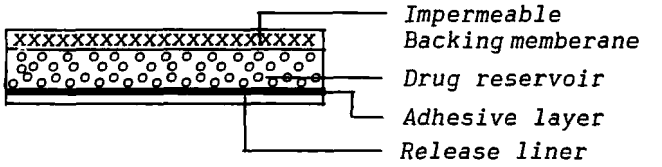

Fig. 1- *Occlusive Transdermal Delivery System (OTDS).*

used in the preparation of matrix in order to establish the effect of vinyl acetate content on drug release modulation, ethylene vinyl acetate copolymer of varied vinyl acetate content i.e, 18, 25, 40 mole percent were used for the preparation of drug reservoir matrix. The different solvent systems were used for the preparation of polymeric solution (Table-II). Dibutyl pthalate 5% w/w was incorporated as a plasticizer and propylene glycol 10% w/w as a cosolvent. The drug reservoir films were prepared using the method developed by Iyer and Vasavada (8). The dimensions of the films were controlled by pouring constant volume of polymer solution of known concentration in the glass rings of the same diameter and dimensions. The prepared films were stored at controlled humidity at Room temperature 30±1°C.

(b) *Drug Loading : The required release rate was calculated on the basis of pharmacokinetic parameters using the formulae* $C_p \times Vd \times K_e$ *(9,10). The polymeric films were loaded with drug 1.5 mg/cm*^{2} *and the adhesive layer contained 50 mcg/cm*^{2} *drug as priming dose.*

(c) *Preparation of Occlusive Transdermal System : The OTDS was prepared using impermeable backing memberane of polystyrene that checks the transepidermal water loss. The adhesive layer of the system was of pressure sensitive polymer loaded with priming dose. The delivery device was protected using a release liner (Fig. 1). The specifications of the components of the delivery device are shown in Table II.*

Table-III. *In-vitro Characterization of the Delivery Devices.*

Delivery systems	Cumulative percent drug released in 24 hrs	Release flux ($mg/cm^2/day$)	Transdermal flux ($mg/cm^2/day$)
$OTDS_1$	58.89 ± 3.77	0.883 ± 0.076	0.766 ± 0.046
$OTDS_2$	41.88 ± 2.76	0.628 ± 0.056	0.498 ± 0.031
$OTDS_3$	33.17 ± 2.11	0.497 ± 0.033	0.316 ± 0.029

In-Vitro Release Profile

The intrinsic in-vitro release of drug from transdermal systems was measured following the conventional method (continuously perfused Franz diffusion cell). The formulations were placed between two halves of the diffusion cell. For each polymeric system four replicates were run and the data provides cumulative amount of drug released per unit time (Table-III).

Transdermal Delivery

Validation studies have been conducted with human cadaver skin. The full thickness human cadaver skin (procured within 48 hours of death, stored in deep freezer) was mounted on donor compartment of the Franz diffusion cell and the patches were placed on stratum corneum site of the skin surface. The primaquine content in the receptor compartment was estimated spectrophotometrically. The experiments were run in triplicate (Table III).

In-Vivo Studies

(a) Specifications of Human Volunteers : Male human volunteers of age group (21-29 years) weighing 60 to 72 kg with no premedication history were choosen.

(b) Oral Administration : Two tablets of primaquine diphosphate 7.5 mg each (IDPL Hyderabad) were administered to 10 healthy

human volunteers. The drug plasma concentration was monitered periodically. The withdrawn blood samples were estimated for primaquine using HPLC method (11).

(c) Transdermal Application : Occlusive transdermal delivery device (20 cm^2) was applied for 30 hours on forearm region. A washing period of 7 days after oral administration was allowed prior to application of transdermal system.

(d) Drug Estimation : The blood samples (5 ml) were taken from median cubital vein, centrifuged for 15 min at 5000 rpm, after addition of 0.03 ml heparin solution (5000 unit/ml in 0.4% physiological saline). The drug content in supernatent (serum) was determined following (sensitive with UV detection) HPLC method described by Park hurst et al. (11) using LKB high pressure liquid chromatogram (model 2158 UVI Cord SD). The estimation was performed in triplicate and mean of values was considered.

RESULTS AND DISCUSSION

A saturated solution of primaquine diphosphate in 40% PEG phosphate buffer saline of pH 7.4 was applied to human cadaver skin, the drug permeation rate determined was 0.55 ± 0.019 mg/cm^2/hr. Drug partitioning in octanol/PBS (pH 7.4) system was found to be 1.49 ± 0.08. The values indicate the amphilic nature of the drug. The possible drug diffusion pathway shall be through both aqueous and lipophilic layers existing in the skin. Although because of relatively less lipophilic nature of the drug, the stratum carneum can be the rate limiting.

The ethylene vinyl acetate copolymer with different vinyl acetate content has been used in the preparation of memberane permeation controlled transdermal therapeutic system. Previous reports have shown that EVA copolymer memberane permeabilities to various types of drugs increased with decreasing ethylene content (7). The EVA copolymer was used to prepare the drug

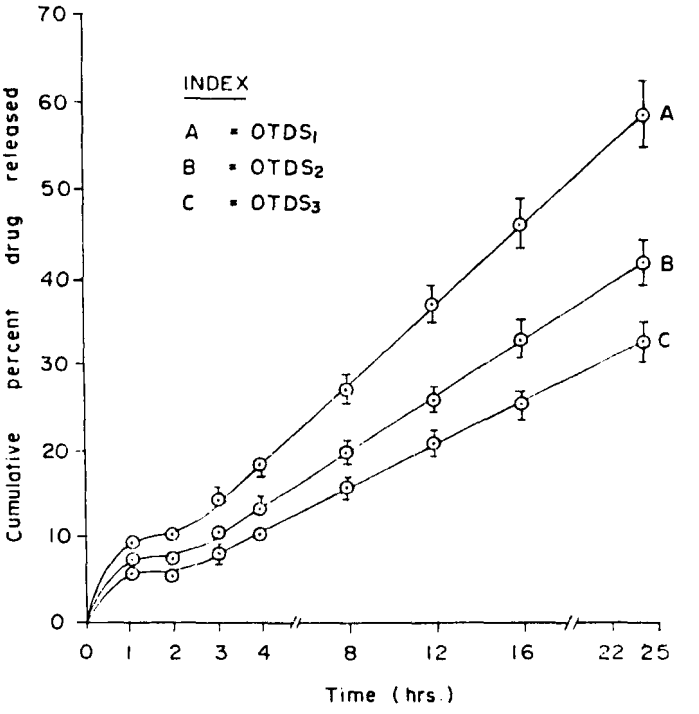

FIG 2. Comparative release profiles of primaquine from various transdermal delivery devices ($OTDS_1$, $OTDS_2$, $OTDS_3$) (n = 3; 37 ± 1°C).

reservoir with 40%, 25%, 18% mole vinyl acetate content, to optimize and to achieve desired release kinetics.

Occlusive transdermal delivery systems were designed and fabricated as occlusion induced hydration not only reduces the lag phase but also gives the enhanced skin permeation effect. Polystyrene with almost negligible to nil water permeability was used to prepare backing memberane of the device. The backing memberane checks transepidermal water loss (TEWL) and eventually induces the hydration effect.

The required theoritical release rate to achieve therapeutic effect was calculated considering pharmacokinetic parameters (3,12). The effective plasma concentration to be achieved was taken 30 ng/ml. The prepared drug reservoirs were loaded with 1.5 mg/cm^2

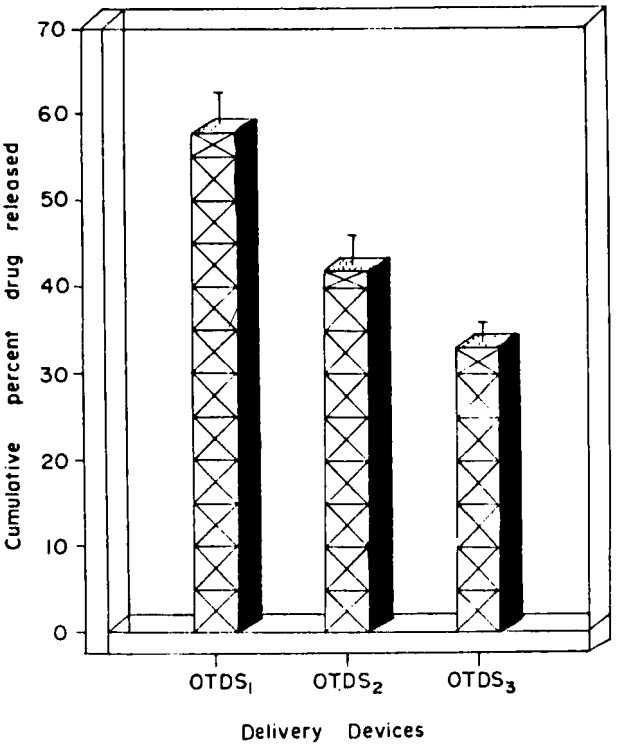

FIG 3. Cumulative percent drug released (± S.D.) in 24 hours from different delivery devices at 37 ± 1°C.

drug for drug delivery at desired rate for 24 hours. The adhesive layer was loaded with 50 mcg/cm^2 of drug as priming dose (13). During the release rate studies higher burst release was observed invariably with each product ($OTDS_1$, $OTDS_2$, $OTDS_3$) in first hour reason likely attributed to the priming dose contained in the adhesive layer. Almost near zero order release kinetics was observed for all the products upto 24 hrs (Fig. 2). The delivery device $OTDS_1$ with 40% vinyl acetate content showed the highest cumulative percent drug released 58.89 ± 3.77. The release rate studies revealed that as the vinyl acetate content in co-polymer decreases the cumulative percent drug release decreases (Fig. 3). Ethylene portion in the co-polymer restricts the diffusion of the drug hence checks the permeation characteristics of the memberane.

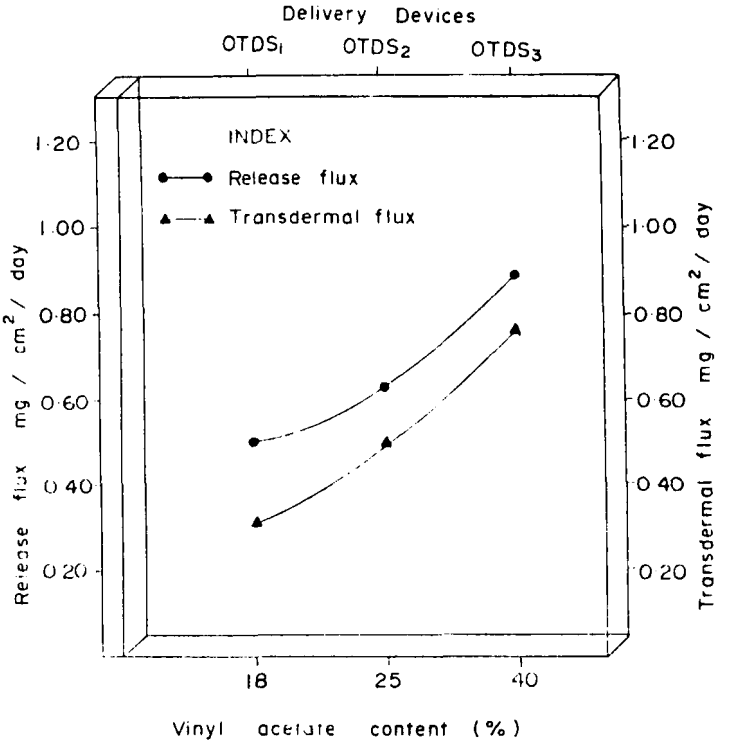

FIG 4- Correlation of transdermal flux of primaquine with different vinylacetate content in the delivery devices ($OTDS_1$, $OTDS_2$, $OTDS_3$) (n = 3; 37±1°C). Dependency of the enhancement factor (vinylacetate content) for the skin permeation (n = 6; 37±1°C) and release (n = 3; 37±1°C) of primaquine is shown.

To characterize the prepared products the transdermal permeation studies were carried out using human cadaver skin. The transdermal flux was noted to be related with vinylacetate content in co-polymer, which affects drug permeation/diffusion. The corelation between variation in vinylacetate content and release flux and transdermal flux was established (Fig. 4). The vinyl acetate content governs both the release as well as transdermal flux. The increase in vinyl acetate content increases the release flux thus eventually the transdermal flux also.

The occlusive delivery device $OTDS_1$ delivers the drug across the skin 0.766 ± 0.48 mg/cm^2/day was chosen for in-vivo studies

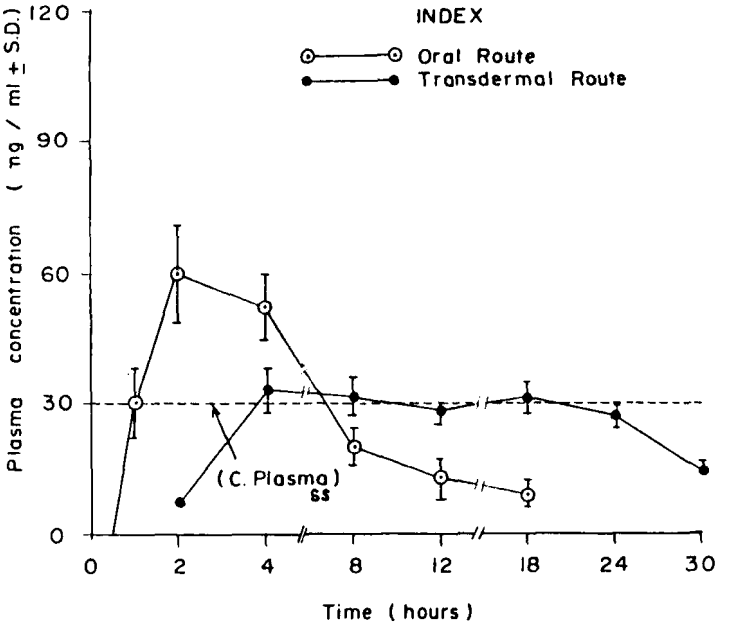

FIG 5. Plasma concentration time profile during oral treatment (two tablets 7.5 mg each) and transdermal once a day application (20 cm^2) of $OTDS_1$ system. The data were collected at short time interval during the first 4 hours (n = 10).

as the delivery device can deliver the drug at the calculated theoritical release rate (0.759 mg/cm^2/day).

The two primaquine diphosphate tablets weighing 7.5 mg (IDPL, Hyderabad) each were given to six human volunteers. Periodically collected blood samples were estimated for drug content. The delivery devices were applied on the forearm region (inner portion) of another six human volunteers for 30 hrs. Comparison of generated plasma profile (Fig. 5) reveals that in the case of oral treatment 0.63 ± 0.11 hrs lag time was observed while 1.5 ± 0.41 hrs lag time was observed with transdermal treatment. Although lag time observed in case of transdermal treatment was higher than the oral treatment but significantly lower as compared to the lag time normally recorded in the case of non occlusive transdermal

Table-IV. *Pharmacokinetic parameters of primaquine after oral (15 mg) and transdermal application (20 cm^2).*

Parameters	Oral	Transdermal
C_{max} (ng/ml)	60.00 ± 11.30	33.29 ± 4.89
T_{max} (hr)	2.11 ± 0.53	4.11 ± 0.39
Aue (mcg.h/ml)	4.23 ± 0.79	7.07 ± 1.86
Duration[a] (hr)	9.00 ± 1.80	21.00 ± 2.60
Lag time (hr)	0.63 ± 0.11	1.50 ± 0.41

(a) Time for which effective plasma concentration (30 ng) was maintained.

systems. Occlusive systems because of induced hydration effect on the corneocytes of stratum corneum reduces the lag time considerably.

In case of oral treatment C_{max} 60 ± 11.30 ng/ml was achieved in 2.11 ± 0.53 hrs while the C_{max} after transdermal treatment (33.29 ± 4.89) was achieved in 4.14 ± 0.39 hrs. Oral treatment shows rapid fall in plasma concentration as the time passed at 8 hrs post administration 9.0 ng/ml was estimated in the plasma which is significantly low when compared with plasma concentration achieved through transdermal route.

However in case of transdermal treatment, once the C_{max} was achieved it was almost maintained for 21.0 ± 2.6 hrs at or around 30 ng/ml concentration level. The 15 mg oral dose only maintained 30 ng concentration for 9.0 ± 1.8 hrs. Whilst approximately the same quantity when delivers by the device OTDS, the blood level was maintained at or around 30 ng/ml concentration for around 21 hours. The in-vivo datas are discussed in the table IV.

The system was tested for occlusion induced irritation. It was observed that out of six human volunteers only one volunteer showed a slight redness of the skin after 30 hrs applica-

tion. This suggest that an elaborated skin toxicological study followed by clinical trial should be conducted.

It is concluded from this study that primaquine holds promise for transdermal application. The composition of polymer modifies the release kinetics of the drug hence programed release of the drug can be achieved. The occlusive device reduces the lag phase and helps in achieving C_{max} earliar hence should be prefered over non occlusive system. The scope and limitation of this approach should be further defined.

ACKNOWLEDGEMENTS

The authors are grateful to Head, Department of Paediatrics for providing human cadaver skin and Dr. P.S. Thakur, Department of Paediatrics Jabalpur Medical College, Jabalpur (M.P.) for his help.

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