Egyptian J. Pharm. Sci., June 1998
Vol. 3, No. 1, pp. 105 - 110

ISSN 1110 -5089

IN-VITRO AND *IN-VIVO* AVAILABILITY OF NAPROXEN FROM TRANSPARENT OIL / WATER GELS IN COMPARISON TO SOME CONVENTIONAL TOPICAL BASES

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ABSTRACT

Within the scope of evaluating transparent oil/water (TOW) gels as dermatological vehicles, the anti-inflammatory and analgesic agent Naproxen (NA) was incorporated in a transparent oil-water gel containing two emulsifying agents (Emulgin B3 and Cetiol HE), an oily liquid (isopropylpalmitate) and water. The release rate of NA from the TOW gel formulation through a cellophane membrane into phosphate buffer (pH 6) at 37°C was studied. The percutaneous absorption in rabbits was evaluated and compared with the absorption from a hydrogel and from a hydrophilic base formulation. The area under the curve and the C_{max} values in plasma were significantly higher for the TOW gels in comparison with other formulations after single application. A good correlation was observed between the in-vitro and *in-vivo* data.

INTRODUCTION

In the past two decades the research concerning transdermal drug delivery has expanded greatly. The skin has long been looked on favorably as a route of drug administration. Much effort has been spent to develop transdermal dosage forms, which create a systemic effect. Drug therapy via percutaneous absorption can be carried out in a simple way and has various advantages with respect to sustained drug release over a fairly long time, and avoiding gastrointestinal side effects as well as a first-pass effect of the drug.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used as analgesics, and in the treatment of locomotor pathologies and of local inflammation [1]. Naproxen (NA) is a member of this group and unfortunately, like other (NSAIDs), it carries the risk of gastrointestinal irritations and a number of other side effects such as nausea, vomiting, headache, etc. To overcome the disadvantages of NA, several attempts have been made to develop a topical dosage form having local activity without systemic toxicity. Percutaneous absorption of NA and the resulting anti-inflammatory activity using animal and human models have been reviewed [2-5]. Gels are widely used as dermatological vehicles. Besides oleogels and hydrogels, transparent gels containing water, oil and one or more surfactants have been described and used extensively in cosmetics and pharmaceuticals. The terminology "transparent oil-water (TOW) gels" has been proposed earlier by **Provost**[6] for clear, homogeneous, optically isotropic, thermodynamically stable, ringing semisolid systems.

The purpose of the present study was to formulate NA in a topically applied TOW gels, in order to overcome the side effects shown on oral administration of the drug and to evaluate and compare the topical bioavailability of NA from TOW gels with that from conventional topical bases.

EXPERIMENTAL

Materials :

Naproxen (NA) was of pharmacopoeial (B.P) grade (Synopharm, Hamburg, Germany). The surfactants were commercial lots; polyoxyethylene (30) cetosteryl alcohol (Eumulgin B3; Henkel, Dusseldorf, Germany) and polyoxyethylene (7) glycerol stearic acid ester (Cetiol HE; Henkel, Dusseldorf, Germany); Sorbitan mono- oleate (Span 80, Atlas, D-Essen, Germany); Sorbitan sequioleate (Arlacel C, Atlas, D-Essen, Germany); Isopropylpalmitate (S.C. Federa, Brussels, Belgium) ; Oleyl oleate (Cetiol, Henkel, D-Dusseldorf), white soft paraffin; white wax; spermaceti (B. P. grade) as the lipophilic component, and distilled water. All the other chemicals were either of analytical or pharmaceutical grades and were used without further purification.

Methods :

Composition and preparation of TOW gels:

The composition of different formulations is shown in Table 1. The TOW gels were prepared by heating a mixture of Cetiol HE, Emulgin B3 and isopropylpalmitate in a water bath at 75°C. (NA) was solubilized in the melt. Water (75°C) was added and the preparation was stirred and allowed to cool to room temperature.

In vitro release studies :

An accurately weighed one-gram of the sample, was placed on a piece of standard cellophane membrane (30/32 Fischer Sci., GB-London) which was previously soaked in water overnight and then dried. The loaded membrane was fastened on the open end of a glass tube having a surface area of 4.45 cm^2, using a silk thread. The tube was then immersed into 400 ml of phosphate buffer (pH = 6), contained in a 600 ml beaker maintained at 37 ± 0.5°C in a constant temperature water bath. The whole assembly was stirred at a rate of 30 rpm. 2 ml of the buffer was placed directly over the sample inside the glass tube. At each sampling interval (5, 10, 15, 30, 45, 60, 75, 90, and 120 minutes), 3 ml of

the diffusion medium was withdrawn and was replaced with an equal volume of phosphate buffer solution. The samples were appropriately diluted and were evaluated spectrophotometrically for their NA content at 331 nm. Interference experiments showed that the components of the different systems did not interfere with the spectrophotometric measurements of the drug at the specified wavelength. Each release experiment was performed in triplicate and the mean of the absorbance readings for the three sets was used for calculation.

Percutaneous Absorption in Rabbits :

White male New Zealand rabbits, weighing 2-4 kg, were used for percutaneous absorption release studies. Four rabbits were utilized for each sample. The animals were fasted for 24 h and their back skin was mechanically shaved 12 h before the experiment. The site of application was visually inspected to ensure that no dermatological irregularities existed or a dry skin conditions are present. Then exposed area was cleaned using detergents and thorough washing with water and perfectly dried. The formulations were applied (1 g either of the TOW gel, the hydrogel or the hydrophilic base containing 5% NA) on the rabbits dorsal skin over an 8 x 12 cm area after liberation of the animals from their boxes. Following application, blood samples, each equal to (3 ml) were collected from the ear vein of the rabbit 5 min. before and every hour up to 8 h after dosing. During the application, the rabbits were housed in individual boxes where only restricted movements were allowed for a 8 h period. The contact of the rabbits back skin with the upper part of the boxes was avoided by choosing suitable boxes. The samples were allowed to clot at room temperature for 15 minutes and then centrifuged for 3000 rpm. The serum was separated and kept in a freezer until analyzed.

Extraction of Naproxen from Serum :

Naproxen was extracted from the serum using the method developed by **Broquaire et al.** [7]. One-half ml of the serum was taken into a test tube and 1.0 ml of 1 M potassium chloride buffer (pH = 2) was added to it. The mixture was shaken on a Vortex mixer and extracted with 6 ml of chloroform for 20 minutes. The two phases were then separated by centrifugation at 300 rpm for 10 minutes and the aqueous phase was discarded. The chloroform extract was transferred into a test tube and evaporated to dryness at 60 °C under a gentle stream of nitrogen.

HPLC Analysis :

The samples were chromatographed on a cyanide bonded column using a mobile phase of acetonitrile 0.005M/dibutylamine phosphate (20 :80 v/v). The flow rate was adjusted to 2 ml per minute with an inlet pressure of 2000 psig. The recorder chart speed was adjusted to 0.1 inches per minute. The ultraviolet detector set at 254 nm, with the sensitivity set at 0.02 continuously monitored the column effluent. The ratio of the peak area of (NA) to that of the internal standard indomethacin was used to calculate the concentration of (NA) in the sample, using the calibration curve constructed previously from known concentrations of (NA) and fixed concentrations of internal standard.

Internal Standard :

Indomethacin was used as the internal standard. It was used at a concentration of 20.0 mcg/ml. Under the conditions of chromatography, it had a retention time of 4.2 minutes, while (NA) had the retention time of 2.8 minutes.

Assay Procedure :

The dried extract previously obtained was dissolved in 0.25 ml of methanolic solution of NA (having a concentration of 2.0 mcg/ml of NA). One ml of the internal standard solution was added to it and mixed thoroughly. A 25 ul portion of this solution was injected into the column through a stop flow injector port for the determination of (NA) content according to the previously described conditions.

RESULTS AND DISCUSSION

In-Vitro Studies

In a previous study , **Provost & Kinget** [8] defined a concentration range for the semi-solid formulations of drug-free TOW gels consisting of isopropylpalmitate, Emulgin B3, Cetiol HE and water. The concentration limits specified for TOW gel formulation were followed to exclude formation of other structures as micellar solutions, emulsions or creams.In a first approach, the influence of (NA) on the consistency and appearance of the TOW gels was investigated and only the formulation which showed an acceptable semisolid consistency was chosen for further experimental work. The in-vitro release of (NA) from the TOW gel was carried out over a period of 2 hr for different (NA) concentrations. From the results shown in (Fig.1), it appears that the drug release is proportional to its concentration within the concentration limits under investigation i.e. 5% NA showed the highest release rate within the 15 min. The efficiency of the TOW gel for delivery of (NA) at the 5% concentration level was compared with three other dermatological preparations, namely : a hydrophilic base, a hydrogel and a w/o emulsion gel (Table 1, Fig. 2). The rate of release decreases in the following order: TOW gel > MC gel > hydrophilic base > w/o emulsion base. After 2 h, the amount released of (NA) from TOW gel is about 2.9, 1.7 and 1.3 times greater than that from the w/o emulsion base, the hydrophilic base and the hydrogel, respectively. The fast release of (NA) from the TOW gel is due to the fact that the incorporation of the non-polar (NA) in the TOW gel, which is a

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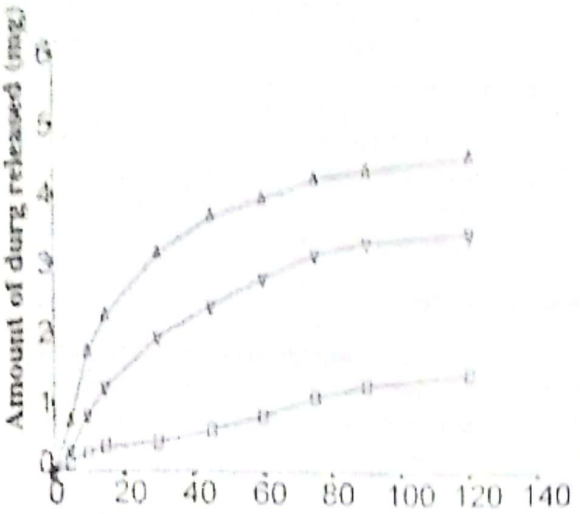

Fig. (1): *In vitro* release of Naproxen from TOW gel base containing different durg concentrations of 1% (▯), 2% ( ) and 5% (Δ).

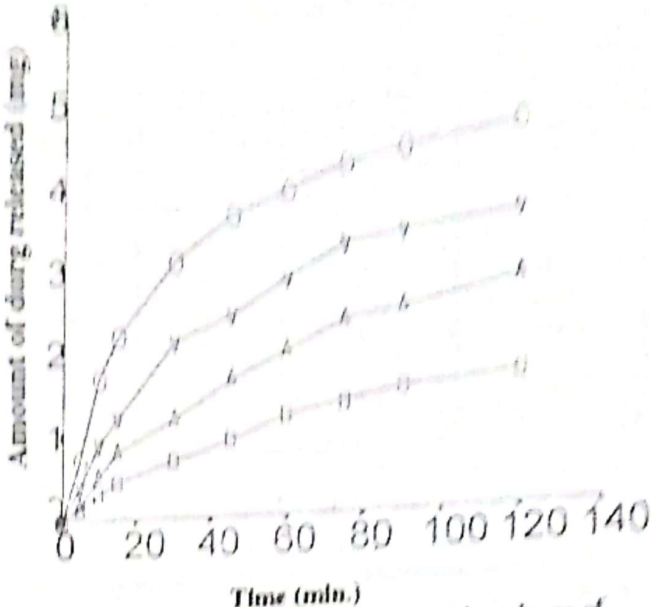

Fig. (2): Influence of base composition on the release of Naproxen from different bases containing (5% w/w) drug. (0) TOW gel ( ) MC gel, (Δ) Hydrophilic base and (▯) w/o emulsion base.

Table (1) : Composition of the different bases.

A TOW gel		B Hydrogel (MC GEL)		C W/O emulsoid gel		D Hydrophilic base	
Isopropylpalmitate	8.0 g	Methylcellulose	5.0 g	White wax	18.8 g	White Petrolatum	25.0 g
Emulgin B3	18.0 g	Water to	100.0 g	Spermaceti	9.4 g	Stearyl Alcohol	10.0 g
Cetiol HE	15.0 g			Oleyl oleate	28.1 g	Propylene glycol	12.0 g
Water to	100.0 g			Sorbitan mono-oleate	2.7 g	Sodium lauryl sulphate	1.0 g
				Sorbitan Sesquioleate	8.0 g	Water to	100.0 g
				Water to	100.0 g		

monophasic system, resulted in maximum solubilization and thermodynamic stability of the poorly soluble drug. The release of the drug from the MC gel is less than that from TOW gel and this may be due to interaction of the drug with the gel network and the high viscosity of the fluid phase filling the pores of the network . As for the release from the hydrophilic base, which contains petrolatum, appears to be better suited for (NA) than the aqueous phase. The slow release of the drug from w/o emulsion gel may be due to the fact that, (NA) exists in this base as bound and dissolved in the external phase of the vehicle. Because of the very poor water solubility of (NA), partitioning between the oil and the water phase of the w/o emulsion gel is negligible.

The analysis of the release data of (NA) from different bases were carried out using the Higuchi equation [9] which is valid when the release of drug from the base is less than 30%. The diffusion coefficient of (NA) from different bases was calculated using Eq. (1)

$$Q = 2\,C_o \cdot A \cdot (Dt/\pi)^{1/2} \qquad (1)$$

Where, Q is the amount of drug released [mg]; A is the area of the diffusion membrane [cm^2]; Co is the initial concentration of drug in the bas e [mg/cm^3]; D is the diffusion coefficient [cm^2/s]; and t is the time after the application (seconds) and π = constant.

These results indicate a direct dependence of the release rate on the diffusion coefficient, which in turn is dependent among other factors such as the solubility of the drug in the base. Therefore, a greater release of drug is expected when there is less affinity of the drug for the base as in the case of the TOW gel formulation, which gave the highest diffusion coefficient value of 4.59 x 10^{-8} (Table 2).

Table (2) : The calculated diffusion, Permeability and Partition coefficients for tested bases using the in vitro release data

Base	Diffusion Coefficient [D. 10^{-8} cm^2/sec.]	Permeability Coefficient [P] [P. 10^{-6} cm/ sec.]	Partition Coefficient [Kp]
TOW Gel	4.59	6.5	4.48
Emulsoid Gel	0.57	0.99	6.14
Hydrophilic Base	1.59	2.72	5.62
Hydrogel	2.70	4.41	5.06

The permeability coefficient values of (NA) for each base were calculated according to Fick, s Law of diffusion;

$$Q = P.\ A.\ C_o.\ t \qquad (2)$$

Where P is the permeability coefficient [cm/sec.] and the other paramters are the same as for Eq. (1). The highest value of 6.5 x 10^{-6} was obtained for the TOW gel formulation (Table 2).

The partition coefficients were calculated by utilizing the Eq. (3);

$$Kp = p.\ h/D \qquad (3)$$

Where Kp is the partition coefficient, P is the permeability coefficient [cm/s]; h is thickness of the membrane [cm]; and D is the diffusion coefficient [cm^2 /sec.].

The partition coefficient factor is considered as one of the important parameters for the estimation of the interaction of the drug with the vehicle and the receiving medium. The partition coefficient values of the various formulations are given also in Table 2. It was observed that (NA) had a lower partition coefficient in TOW gel (4.48) or less affinity for the base. Therefore, the drug had a faster release from this particular base.

Kinetic estimation of the release data was performed adopting zero, first order kinetics together with Higuchi equation.

The values of the correlation coefficient (r) are cited in Table (3). From the table and (Fig. 3 & 4) it is obvious that the release of (NA) from all bases is in agreement with the well known Higuchi model.

Bioavailability study

The percutaneous absorption of (NA) from either the TOW gel, methylcellulose (MC) gel or the hydrophillic base was studied in rabbits. The mean serum concentration of the drug versus time relationships obtained after application of the tested formulations in rabbits are shown in Fig. (5). **Provos et al.** (10) compared the penetration rate of a hydrophilic and lipophilic drug from TOW gels, as used here, through human skin in vitro. They concluded that the penetration rate for both drugs was comparable with formulations using other commonly used vehicles.

As shown in Fig. (5), the maximal serum concentration, Cmax for TOW gel was significantly higher in comparison with the other formulations MC gel and hydrophilic base. For all formulations tmax value were around 4 h. The areas under the serum concentration versus time profiles (AUC_{0-8h}) were calculated by utilizing the trapezoidal method and are shown in table (4). ACU_{0-8h} values were significantly higher for TOW gel in comparison with the other formulations. A good correlation has been observed between the in-vitro and in vivo release data of TOW gel and the hydrophilic base. The in-vitro release data indicates that the amount of (NA) released from TOW gel was 1.65 time as that released from the hydrophilic base. Also the in –vivo data shows that the AUC for TOW gel was 1.65 time as that from the hydrophilic base.

Table (3) : Mathematical treatment of the in -vitro release data of Naproxen from different tested bases

Base	Zero-order kinetic K (mg $min.^{-1}$)	r	First-order kinetic K ($min.^{-1}$)	r	Higuchi Model D. 10^{-8} (Cm^2/sec)	r
TOW Gel	0.038	0.878	0.79 9.10-3	0.896	4.59	0.959
Emulsoid Gel	0.013	0.963	0.269.10-3	.0.103	0.57	0.994
Hydrophilic Base	0.022	0.960	0.461.10-3	0.567	1.59	0.995
Hydrogel	0.029	0.925	0.60 5.10-3	0.931	2.70	0.981

K= Specific rate constant r = Correlation coefficient D = Diffusion coefficient

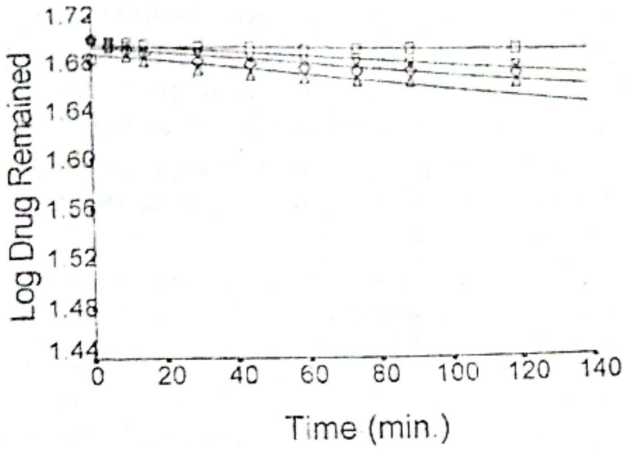

Fig. (3) First-order plot between amount of (NP) remaining in different bases and time, Tow gel (Δ), MC gel (◊), Hydrophilic base (∇) and. o/w emulsion base (□)

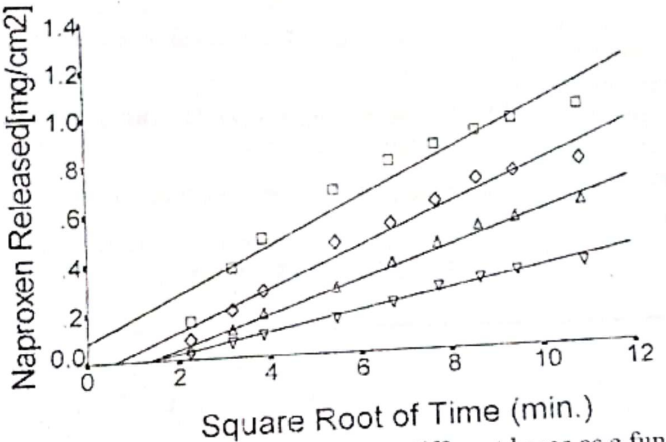

Fig. (4) *In vitro release* of Naproxen from different bases as a function of the square root of time. (□) Tow gel, (◊) MC gel, (Δ) hydrophilic base and (∇) w/o emulsion base.

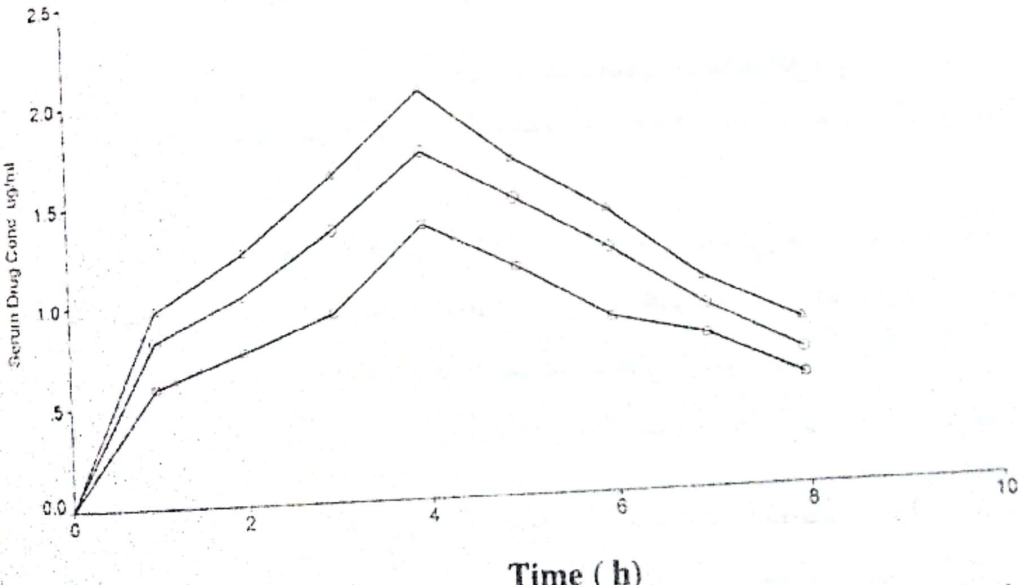

Fig. (5) : Mean serum concentration - time profiles of Naproxen after single application of the tested formulations in rabbits. (□) hydrophilic base, (O) hydrogel and (Δ) Tow gel.

Table (4) : Bioavailability parameters (± s.d) after a single topical application of 50 mg Naproxen with TOW gels, a hydrogel and a hydrophilic base, on rabbits (n=4)

Parameters	TOW gel	Hydrogel	Hydrophilic base
AUC ($\mu g\ ml^{-1}h$)	10.06±1.23	8.58±1.12	6.43±1.18
C_{max} ($\mu g ml^{-1}$)	1.97±0.15	1.42±0.12	1.32±0.24
T_{max} (h)	4	4	4

Finally, it is concluded that TOW gels may be considered as an interesting alternative for transdermal delivery of (NA), since dermatological products containing local anti inflammatory or analgesic agents require good skin penetration of the therapeutic agents to attain their desirable clinical effects.

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Received : April 29, 1998
Accepted : May 20, 1998

الإتاحة العملية والبيولوجية لعقار النابروكسين من هلاميات شفافة ومقارنتها مع بعض القواعد السطحية التقليدية

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في هذا البحث تم صياغة هلاميات شفافة تتكون من زيت فى ماء وكذلك قواعد تقليدية لمستحضرات سطحية ودراسة إتاحة العقار منها عبر غشاء السلوفان . ولقد تحقق معدل إتاحة عال من الهلاميات الشفافة مقارنه بباقى القواعد الأخرى ولقد وجد أن معدل الإتاحة يعتمد أساساً على تركيز العقار وأيضاً يزداد معدل إتاحة العقار بزيادة معامل التوزيع التجزئى (Partition coefficient) بين القاعدة ووسط الإتاحة وذلك من جميع القواعد .

وبتحليل حركية إتاحة العقار وجد أنه يتبع معادلة الأنتشار المنضط (Higuchi equation)وقد تم دراسة الإتاحة البيولوجية للعقار على الأرانب للنيوزيلاندية وتم تعين المساحة تحت المنحنى وكذلك أعل تركيز للعقار فى المصل والوقت اللازم لحصول على أعلى تركيز . وقد ثبت تعاظم النتائج التى تم الحصول عليها من الهلاميات الشفافة مقارنه بباقى القواعد السطحية ولقد ثبت أن هناك علاقة بين الإتاحة البيولوجية والإتاحة المعملية.