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In Vitro and *in Vivo* Evaluation of Transdermal Absorption of Naproxen Sodium

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Abstract: Naproxen sodium is a non steroidal antiinflammatory drug, used in the treatment of inflammatory and degenerative disorders of the musculoskeletal system. It is widely prescribed for the treatment of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, gout, extra-articular disorders, bursitis, tendonitis, and non articular rheumatic condition. Naproxen sodium has some side effects when taken orally, epigastric pain, heartburn, nausea, diarrhoea, vomiting, peptic ulcer, and hepatic impairment. The aim of this study was to formulate topical gel containing 1% of naproxen sodium using various ratios of different type of gel forming agents as pectin, hydroxypropylmethyl cellulose low and high viscosity (HPlv&hv), carboxymethyl cellulose (CMC), and carbopol 934 with and without penetration enhancers; isopropyl myristate (IPM) and sodium lauryl sulphate (NaLS). Also the duration of anti-inflammatory activity of new gel formulation of 1% naproxen sodium was measured using carrageenan-induced paw oedema in rats. Furthermore, the pharmacokinetics percutaneous absorption of 1% naproxen sodium from three different gel formulations prepared with pectin, HPlv and carbopol as gel forming agent without penetration enhancers, was studied in six healthy human volunteers. While orally administered naproxen sodium tablet (marketed as Naprofen®) was used as a control. Bioavailability was estimated from plasma concentration which determined up to 72 hr after drug administration. The drug plasma concentration was higher from pectin gel (15.944µg.hr/ml) followed by HPlv (14.4199µg.hr/ml) then carbopol (8.5781µg.hr/ml) with the corresponding rate of drug elimination (Kel) of 0.085 hr^{-1}, 0.13hr^{-1} and 0.11hr^{-1}respectively. The PK parameters, such as the maximum blood concentration (C_{max}), time to reach the peak blood concentration (T_{max}), mean residence time (MRT), area under the curve ($AUC_{0-\infty}$) and terminal elimination half-life ($t_{1/2}$) were significantly ($p < 0.001$) different following transdermal administration of 1% naproxen sodium gel prepared with pectin without penetration enhancers compared with oral administration of reference naproxen sodium tablet Naprofen®.

Key words: *In vitro*, *in vivo,* transdermal absorption, naproxen sodium.

INTRODUCTION

The transdermal route has many advantages for the administration of drugs for local and systemic therapy. Recent development in transdermal drug delivery systems have been extensively studied as drug delivery methods showing promising systemic and topical efficacy (Okamoto *et al.*, 1986; Hadgraft and Ridout 1988; Potts and Francoeur 1990; Yamada and Uda 1987) the outermost layer of skin, the stratum corneum (SC), forms a strong barrier to most exogenous substances including drugs. The barrier function of the SC is attributed to its multilayered wall-like structure, in which terminally differentiated keratin-rich epidermal cells (corneocytes) are embedded in an intercellular lipid-rich matrix (Hui, *et al.*, 2005) .One approach to deliver an effective dose of drug through skin is to reversibly reduce the barrier function of skin with the aid of penetration enhancers or accelerants (Hadgraft, *et al*. 1999). The therapeutic efficacy of a drug, following its application onto the skin, mainly depends on its ability to penetrate the skin at such extent to elicit the desired pharmacological activity. Since most drugs show unsuitable physicochemical properties to penetrate effectively the skin different strategies have been developed to increase drug skin permeation. Penetration enhancers have been extensively used to increase drug percutaneous absorption the prodrug should exhibit an adequate aqueous stability (Valenta *et al.*, 2000; Finnin and Morgan, 1999; Godwin and Michniak, 1999) although they show some disadvantages (Hadgraft, 1989) The treatment of skin diseases as well as of musculoskeletal disorders might benefit from topical administration, obtaining a substantial reduction of the systemic side effects and an improvement of the patient compliance. Drug topical administration is still a challenge in pharmaceutics and

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drug delivery due to the difficulties in controlling and, not less important, determining the exact amount of drug that reach the different skin layers (Schoafer-Korting *et al.*, 2007).The active ingredient as well as the vehicle physicochemical characteristics are retained to be the main features responsible for the drug differential distribution in the skin (Beetge *et al.*, 2000; Jacobi *et al.*, 2006; Teichmann *et al.*, 2007).

The NSAIDs has prominent anti-inflammatory, analgesic and antipyretic properties. Oral therapy of NSAIDs is very effective, but the clinical use is often limited because of their potential to cause adverse effects such as irritation and ulceration of the gastro-intestinal (GI) mucosa. It has been reported that gastric irritation induced by NSAIDs can be influenced by the route of administration (Cioli *et al.*, 1979). Administration of these agents via the dermal route can bypass these disadvantages of the oral route and may maintain relatively consistent plasma levels for long term therapy from a single dose (Berba *et al.*, 1991).

Naproxen sodium [(S)-6-methoxy-alpha-methyl-2naphthaleneacetic acid sodium salt] is an NSAID with analgesic and antipyretic properties (Sevelius *et al.*, 1980; Capone *et al.*, 2007; Moyer 1986; Segre 1980) used for the treatment of musculoskeletal disorders (e.g., rheumatoid arthritis, osteoarthritis and ankylosing spondylitis) with non-optimal characteristics to be delivered through the skin (Swart *et al.*, 2005). The mechanism of action of the naproxen anion, like that of other NSAIDs, is primarily due to the inhibition of prostaglandin biosynthesis through the inhibition of the cyclooxygenase (COX) enzymes COX-l and COX-2 (Capone *et al.*, 2007).

Different strategies have been used to increase local soft tissue bioavailability of naproxen topically administered (Rautio *et al.*, 1998; Bonina *et al.*, 2001). In order to increase therapeutic efficacy after topical application of drugs, it is necessary to employ percutanous absorption enhancer and /or appropriate vehicles. An attempt has been made, to enhance the transdermal permeation of naproxen sodium by using different types of gel forming agents as delivery vehicles.

The present study aims to evaluate the efficacy of naproxen sodium gel formulations prepared with five different gel forming agents as pectin, CMC, HPhv, HPlv and carbopol with and without penetration enhancers (isopropyl myristate (IPM) and sodium lauryl sulphate NaLS) for transdermal delivery of naproxen sodium across the rat skin *in vitro*, and to determine whether therapeutically relevant delivery rates could be achieved under these conditions when applied transdermally *in vivo* on healthy volunteers.

MATERIALS AND METHODS

Materials:

Naproxen sodium powder and pectin were obtained as gift sample from El-Nile Pharmaceutical Chemical Company, Cairo, Egypt. Hydroxpropyl methyl cellulose powder high and low viscosities 15000, 4000 respectively, were purchased from Sigma Chemical Co. (USA). Carboxymethyl cellulose and Carbopol 934 were kindly supplied by Egyptian International Pharmaceutical Industries Company (E.I.P.I.Co), Egypt. All other chemicals were commercially available products of analytical grade.

Preparation of Naproxen Gel Formulations:

Various gel formulations are listed in Table (1). To each of the following gel bases, the naproxen sodium is added at 1%w/w concentration and also the different ratios of penetration enhancers were added by mean of gentle levigation.

Preparation of Carboxymethyl Cellulose (CMC) Gels:

The weighed amount of CMC powder were sprinkled gently in 100 ml beakers, containing boiling dist.water and stirred magnetically at a high speed (Dubuque Lowa USA), stirring was continued until a thin hazy dispersion, without lumps, were formed. Leaving the gel overnight in the refrigerator (Pharmaceutical exipients, 2003).

Preparation of Hydroxypropylmethyl cellulose (HPMC) Gels:

The weighed amount of HPMC powder, both viscosity, were sprinkled gently in 100 ml beakers, containing a portion of hot water at 80°C and stirred magnetically at high speed (Dubuque Lowa USA). Stirring was continued until a thin hazy dispersion, without lumps, was formed the remaining amount of water was added on cold and mixing was continued till smooth homogenous gel is formed. Leaving the gel overnight in the refrigerator (Pharmaceutical exipients, 2003).

Preparation of Pectin Gels:

The weighed amount of pectin powder were sprinkled gently in 100 ml beakers containing a portion of warm water and stirred magnetically at high speed (Dubuque Lowa USA). Stirring was continued until a thin hazy dispersion, without lumps, was formed the remaining amount of water was added on cold and mixing was continued till smooth homogenous gel is formed. Leaving the gel overnight in the refrigerator may be necessary for complete gel dispersion.

Preparation of Carbopol 934 Gels:

Gels were prepared by dispersing 1% w/w Carbopol 934 in distilled water , being kept under mechanical stirring at high speed. The dispersion was then neutralized (pH 7.4) by addition of 1% w/w triethanolamine. Any entrapped air in the gel was allowed to escape by allowing the gels to stand overnight.

In Vitro Release Studies:

The dissolution test was performed using standard USP apparatus II with some modifications (Marzouk, 1999) by using modified paddle over watch glass using buffer solution of pH 7.4. The dissolution medium was 300 ml phosphate buffer pH 7.4. The temperature was maintained at 37 ± 0.5°C. The rotation speed was 50 rpm. Samples of 5ml was withdrawn at predetermined time intervals 5, 10, 30, 40, 45, 50, 60 minutes for gel containing polymer concentration 2% w/w and 5, 10, 30, 40, 45, 60 ,90,120,150,180 minutes for gel containing polymer concentration 4,6% w/w and replaced with fresh and preheated at 37±0.5°C buffer solution each time. Samples were measured spectrophotometrically at 272 nm. All experiments were performed in triplicate (n = 3). The amount released was calculated from the regression line of the standard curve developed in the same medium. All naproxen gel formulae were subjected to kinetic analysis by fitting the release data to different kinetic models to explain the release kinetics of naproxen from various gel preparations.

Curve fitting:

Curve fitting was performed using Microsoft Excel 2003 version. The dissolution data were fitted to equation. Release exponent "n" was calculated The dissolution data were fitted to the following equation (Eq. (1) (Kormeyer *et al.*, 1983; Peppas, 1985; Ritger and Peppas, 1987a,b):

$Mt / M\infty = kt^n$ ----------Eq.1

Where $Mt/M\infty$ is the fraction of drug released at time t, k is the kinetic constant of the system, and n is the exponent characteristic of the mode transport (Table 2). The release exponent takes various values depending upon different geometries (Ritger and Peppas, 1987b).

Table 1: composition of naproxen gels (% w/w).

Gel forming agent	Gel forming agent wt	PE wt		Nap.wt	Dist.Water
		IPM	NaLS		
Carb.	2	5/10	5/10	1	Ad 100 g
Pect.	2	5/10	5/10	1	Ad 100 g
Pect.	4	--	--	1	Ad 100 g
Pect.	6	--	---	1	Ad 100 g
HPhv	2	5/10	5/10	1	Ad 100 g
HPhv	4	--	--	1	Ad 100 g
HPhv	6	--	--	1	Ad 100 g
HPlv	2	5/10	5/10	1	Ad 100 g
HPlv	4	--	--	1	Ad 100 g
HPlv	6	--	--	1	Ad 100 g
CMC	2	5/10	5/10	1	Ad 100 g
CMC	4	--	--	1	Ad 100 g
CMC	6	--	--	1	Ad 100 g

PE; penetration enhancer; IPM;isopropyl myristate; NaLS;sodium lauryl sulphate
Nap; naproxen; Pect; pectin; Carb; carbopol 934; CMC; carboxy methyl cellulose
HPhv;hydroxyl propyl methyl cellulose viscosity 15000; HPlv; hydroxyl propyl methyl cellulose viscosity 4000

Table 2: Transport Mechanisms from a polymer tablets Under Sink Conditions

Diffusional release exponent (n)	Overall solute diffusion mechanism
0.5	Fickian diffusion (Higuchi release)
0.5 < n < 1.0	Non-Fickian (anomalous)
1.0	Time-independent linear transport (Zero-order release)
n >1.0	Super Case II Transport

In Vitro Permeation Studies Through Rat Skin.

Skin Membrane Preparation:

The abdominal hair of wistar male rats, weighing 160 ±25 g, was shaved using electric and hand razors. After anesthetizing the rat with ether, the abdominal skin was surgically removed from the animal, and adhering subcutaneous fat was carefully cleaned. To remove extraneous debris and leachable enzymes, the dermal side of the skin was in contact with a saline solution for 1 hr before starting the diffusion experiment.

Permeation Studies:

The rate and extent of skin permeation of naproxen sodium from gel formulations that showed the best dissolution profile (gel prepared with 2% w/w gel forming agent with and without penetration enhancers) were determined using a modified glass diffusion cell fitted with excised rat skin (Hsu *et al.*, 1994). The skin was mounted on the receptor compartment with the stratum corneum side facing upwards into the donor compartment and the dermal side facing downwards into the receptor compartment. The donor cell was filled with 1 g of 1% naproxen sodium gel with and without various types and concentration of penetration enhancers and occluded by paraffin. The receptor compartment was filled with 50 ml of pH 7.4 phosphate buffer and its temperature was maintained at 37±0.5 °C. The effective diffusion area was 4.9 cm^2. Approximately 3 ml of the receptor medium was withdrawn at determined intervals and replaced immediately with an equal volume of receptor solution to maintain a constant volume. The sample withdrawn from the receptor compartment was then analyzed spectrophotometrically at λmax 272 nm. Each data point represents the average of three determinations.

Data Treatment:

In vitro, the cumulative amount of the drug penetration through rat skin was plotted as a function of time and a linear regression analysis was used to determine the flux and lag time of the drug. The effectiveness of penetration enhancers can be determined by penetration index (PI) which is expressed as PI=flux of drug with enhancers/ flux of drug without enhancers (Wu *et al.*, 1996).

The skin flux was determined from Fick's law of diffusion:

$Js = dQr/Adt$ ----------Eq.2

Where Js is the steady-state skin flux in $\mu g/cm^2/h$, dQr is the change in quantity of the drug passing through the skin into the receptor compartment in µg, A is the active diffusion area in cm^2, and dt is the change in time. The flux was calculated from the slope of the linear portion of the profiles.

The lag-time was determined by extrapolating the linear portion of the curve to the abscissa (Niazy, 1996). The permeability coefficient (P) was calculated as (Scheuplein, 1978)

$P = Js/Cs$ ----------Eq.3

Where Cs is the saturated solubility of drugs in donor solutions.

Statistical analysis was carried out using analysis of variance (ANOVA), the level of significance was taken as $P < 0.05$. A correlation analysis was performed with the help of the Instate program, and correlation coefficients were examined for significance ($P < 0.05$) using Tukey (post test) for multiple comparison.

Duration of Antiinflammatory Activity of Naproxen Gel:

Schier *et al.* (1986) studied the duration of antiinflammatory action of meclofenamic acid and indomethacin topical formulations in mice. This method was not suitable for evaluating the antiinflammatory activity of a drug for >10 hr due to the limited duration of the inflammatory response after carrageenan injection in mice. Another method has been suggested by Chi *et al.*, 1990 for the determination of duration of antiinflammatory activity in rats, wherein the extended duration of antiinflammatory activity of the new gel formulation of naproxen sodium was measured by applying the gel to the paw much earlier than the carrageenan injection.

Six groups, each containing six rats, were selected and 0.5 g of 1% naproxen sodium in pectin gel without enhancer was applied on the plantar region of their left hind paw at 0, 1, 2, 3, 4, 6, 8, 12, 24 and 30 hr prior to the injection of 0.1 ml of the 1% carrageenan solution. Three hours after the carrageenan injection, the percent swelling of the paw was measured in each rat, and the average percent inhibition of oedema formation at each dosing interval was calculated using Eq. 4

Percent inhibition = [1-percent swelling of drug-treated group/percent swelling of control group] ×100. ----------Eq.4

Six rats were used as control animals, and they received the gel base alone 3 hr prior to the injection of carrageenan.

Pharmacokinetics Study of Naproxen Sodium Gel:

Protocol:

The gel formulation that gave best *in vitro* permeation flux through rat skin (1% naproxen sodium in 2% pectin gel) was subjected to *in vivo* study. Furthermore the effect of gel forming agent type (1% naproxen sodium in HPlv gel and carbopol gel) on transdermal absorption of drug through human skin was evaluated. Also one marketed product, naprofen® 250 mg tablet (El-Nile Pharmaceutical Co., Egypt) was orally administered to six healthy human male volunteers in order to identify the pharmacokinetic properties and relative bioavailability of naproxen sodium for all the formulations.

In vivo experiments were carried out on six human volunteers of healthy Egyptian male in the age range 25–35 years. The volunteers were fully informed of the nature of the study and the procedures involved. The participants did not suffer from any disease and were not on any medication at the time of the study. On the first day of the study, immediately, after taken the initial blood sample, 1 g gel was applied to the skin on the ventral surface of the forearm, and was rubbed in for 1 minute, over an area approximately 5x 20 cm^2.

Heparinized venous blood samples were drawn just before administration and at 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72 hr postdose. Plasma was separated and frozen prior to assay for naproxen sodium by HPLC. All volunteers fasted until 3 h after drug administration. A 15-day washout period was left between the dosing days.

Drug Analysis:

Naproxen sodium (determined as naproxen ;i.e., the free-acidic form of the naproxen sodium salt) plasma level was simultaneously determined by using an HPLC method developed and validated by personnel of Fundacion A.C., Liomont, Mexico City, Mexico (Jose Antonio *et al.*, 2009) with slight modification in which propylparaben sodium(El-Nasr Pharmaceutical Co., Egypt) was used as the internal standard. The method included 0.5 ml of plasma, 0.01 ml of internal standard (propylparaben sodium 20µg/ml), and 0.5 ml $ZnSO_4$ 0.014 M. These components were mixed together in a 2.0 ml conical tube and vortexes (Vortex mixer, Medtronic, P-selecta, 246539, Switzerland) for 1 minute. The tube was cooled in a freezer (-75°C ± 5°C)(General Electric, 500 P123, USA) for 1.5 minutes and then centrifuged at 1440g (Centrifuge Janetzki T30, Germany) for 12 minutes at room temperature (25°C). The supernatant was separated and injected into the chromatographic system (model HP1100, Hewlett-Packard, Les, Ulis, UK). Naproxen concentrations were determined using analytical column LICHrospher100RP18(Sum Putielesize, 125 x 4 cm) and eluted with a mobile phase consisting of a mixture (79.5:20.5 v/v) of an aqueous buffer solution (ammonium phosphate 5 mM; pH 6.0 ± 0.2) and acetonitrile. The column temperature was 25°C. Flow rate was maintained at 1.0 ml/min and naproxen was detected by a UV detector (Agilent Technologies) set at a wavelength of 230 nm. Typical retention times for naproxen, and the internal standard were 9, and 2 minutes, respectively. Naproxen peak areas were used for its quantification. Under these conditions, the method was linear in the range of 25 to 250 ng /ml (25, 50, 70, 90, 170, 245, and 250 ng /ml; lower limit of quantification =25 ng /ml) for naproxen. Accuracy for naproxen was between 92.1% and 106.4%; the relative SD was always <7%.

Quality control (QC) samples were included in every analytical run (during both method validation and analysis of the study samples) to verify performance.

Calculations of the Pharmacokinetic Parameters and Statistical Analyses:

$AUC_{0-\infty}$ is the area under the plasma concentration vs. time curve, calculated using the trapezoidal rule for the time interval 0 to the last measurable point, 72 h. The peak plasma concentration (C_{max}) and time to reach the maximum drug plasma concentration (T_{max}) was determined from visual inspection of the concentration–time plots. The relative bioavailability of topical naproxen gel formulations, compared to Naprofen® tablet, was calculated according to the formula:

$$\text{Relative bioavailability (\%)} = \frac{[AUC]_T}{[AUC]_R} \times 100$$

Where $[AUC]_R$ is the area under the curve of Naprofen® tablet, and $[AUC]_T$ is the area under the curve of topical naproxen gel formulations.

Each experiment was repeated six times and the mean value with standard deviation was presented. Student's t-test was performed to see any significant difference in pharmacokinetic parameters between the control and test preparations.

RESULTS AND DISCUSSION

In Vitro Release Studies:

Dissolution profiles of 1% naproxen sodium gel formulations prepared with 2, 4, and 6% polymer (pectin, carbopol, CMC, HPhv and HPlv) without penetration enhancer are shown in Figs. (1, 2 and 3) respectively. As shown in the figures the fastest release of naproxen sodium are shown from gel prepared with low % of gel forming agent as 100% released of naproxen from HPlv is reached only after 10 minutes. While by using more % of the same gel forming agent the % released of drug will be much delayed as it reached 95% after 3 hours with HPlv. The effect of addition various penetration enhancers with different ratio on the release of naproxen sodium from gel formulations prepared with 2% of the five selected gel forming agent are shown in Figs. (4-8). It is clear that the penetration enhancer IPM and NaLS delay the release of naproxen sodium from most of gel formulations when used in both ratios (5 and 10%).As 100% release of naproxen sodium was showed after 45 minutes from gel formulation prepared with pectin and 5%IPM, while it reaches 100% after only 20 minutes when used pectin base only without any penetration enhancers. On the contrary, the gel formulation that prepared with 2% carbopol show slightly enhancing when used with 10% NaLS as 100% naproxen sodium was released after one hour in contrast to 84% released from gel prepared with carbopol only.

The release exponent (*n*) values varied from 0.0988 to 1.168 which indicates that drug release from the prepared gel mostly follows a Fickian drug release pattern unless with few formulation shows super Case II Transport and others show non-fickian drug release pattern. The correlation coefficient (R^2) values ranged from 1 to 0.9297. All the release kinetic parameters of naproxen sodium from the studied formulae are given in Table (4).

In Vitro Permeation Studies Through Rat Skin:

Aliphatic esters, such as IPM, have been reported to act as permeation enhancers (Sato *et al.*, 1988; Ozawa *et al.*, 1988). Also, Na-laurylsulfate is surfactant has role as penetration enhancer (Junginger and Verhoef, 1998) .In order to evaluate the effect of IPM and NaLS on the percutaneous absorption of naproxen sodium, experiments from gels containing the 5, 10% IPM were carried out. The diffusion experiments showed that naproxen sodium is able to permeate abdominal rat skin from formulations prepared with 2% gel forming agent of pectin, HP hv, HP lv and CMC and 2% carpopol without and with 5, 10% penetration enhancers IPM and NaLS. The steady-state flux (J), permeability coefficients of the formulations and the penetration index (PI) of each enhancer were calculated and listed in Table (4). These results demonstrate that 5, 10 % NaLS and IPM were ineffective accelerant for the drug as naproxen sodium flux was markedly decreased from all formulations. The most outstanding permeation showed with using pectin as gel forming agent without any penetration enhancer as the naproxen sodium flux reaches 82 ($\mu gcm^{-2}hr^{-1}$). Whereas the NaLS and IPM showed mild accelerant activity with formulation that prepared using 2% carbopol as the naproxen sodium flux was slightly increased to 14, 12 $\mu gcm^{-2}hr^{-1}$ with 10% IPM and NaLS, respectively.

Duration of Antiinflammatory Activity of Naproxen Sodium Gel:

Table (5) shows the percent inhibition of the oedema formation after the topical application of 1% naproxen sodium gel prepared using 2% pectin and without penetration enhancer at various times between 0-30 hr prior to the carrageenan injection. When the gel was applied on the rat paw between 6 hr prior to the carrageenan injection, the maximum inhibition was observed at 3 hr after the carrageenan injection (60.72%). It is clear that the time required for maximum inhibition (T_{max})was observed at 6 hr prior carragenan injection .When the gel was applied 12-30 hr prior to the carrageenan injection, gradually decreased in inhibition was observed at 3 hr after the carrageenan injection (7.225% at 30 hr prior injection). These results clearly indicate the prolonged antiinflammatory activity of 1% naproxen sodium gel prepared.

Pharmacokinetic Parameters:

The selection of an appropriate base is very important in increasing the efficacy of a topically applied drug. Topical application of non-steroidal anti-inflammatory drugs such as naproxen is very useful for local activity in various arthritic conditions which also avoids GI disturbances. Hence in the present study,

percutaneous absorption of naproxen from pectin gel, carbopol gel, and HPlv gel was evaluated to assess the effect of base on the absorption of naproxen. As the plasma concentration is the most important determinant of the pharmacological effect, simultaneous estimation of plasma concentration of naproxen was undertaken at the same time intervals at which the plasma concentration of naproxen sodium reference tablet Naorofen ® was estimated, in the same human volunteers.

Table 3: Dissolution kinetic parameters of naproxen sodium from gel formulations prepared with 2% different gel forming agents

		parameters		
Gel forming agent	Enhacer ratio	Release exponent n	Correlationcoefficient R^2	Kinetic constant K
2% HPhv	Without enhancer	0.3612	0.9994	0.3339
	5% IPM	0.26461	0.95613	0.42629
	10%IPM	0.1455	0.9016	0.50501
	5%NaLS	0.1952	0.9986	0.51386
	10%NaLS	0.1596	0.9607	0.59597
2% HPlv	Without enhancer	0.21996	1.0000	0.5965
	5% IPM	0.3064	0.94078	0.3382
	10%IPM	0.2929	0.9018	0.36263
	5%NaLS	0.2668	0.9848	0.3531
	10%NaLS	0.1458	0.9857	0.59959
2% Pectin	Without enhancer	0.6537	0.9431	0.1531
	5% IPM	0.2788	0.99206	0.3432
	10%IPM	0.5291	0.96939	0.1807
	5%NaLS	0.2677	0.99	0.39837
	10%NaLS	0.2824	0.9865	0.3611
2% CMC	Without enhancer	0.0988	0.9914	0.70183
	5% IPM	0.2132	0.98054	0.4641
	10%IPM	0.3352	0.9297	0.24198
	5%NaLS	0.1669	0.9875	0.54329
	10%NaLS	0.1175	0.9684	0.64957
2% Carbopol	Without enhancer	0.7436	0.99101	0.04463
	5% IPM	0.5743	0.9936	0.08123
	10%IPM	1.168	0.9649	0.00708
	5%NaLS	0.56301	0.9968	0.08653
	10%NaLS	0.3549	0.9708	0.21407

Table 4: Effect of gel forming agent type and penetration enhancer on naproxen sodium skin permeation parameters[a].

Gel forming agent	Enhancer ratio	Flux ($\mu gcm^{-2}hr^{-1}$)	P($cm\ hr^{-1}$)	PI
2% HPlv	Without enhancer	36 ± 1.1	0.0036	--
	5% IPM	16±1.9	0.0016	0.44
	10%IPM	10±2	0.001	0.277
	5%NaLS	8±0.8	0.008	0.22
	10%NaLS	10±1.3	0.001	0.277
2% HPhv	Without enhancer	30±1.1	0.003	--
	5% IPM	31±0.56	0.0034	1.1
	10%IPM	16±1.5	0.0016	0.5
	5%NaLS	4±2.9	0.0004	0.13
	10%NaLS	8±1.87	0.0008	0.26
2% Pectin	Without enhancer	82±3.4	0.0082	--
	5% IPM	42±5	0.0042	0.5
	10%IPM	18±2.4	0.0018	0.2
	5%NaLS	12±2.2	0.0012	0.14
	10%NaLS	14±2.9	0.0014	0.17
2% CMC	Without enhancer	30±4.3	0.003	--
	5% IPM	10±3.1	0.001	0.33
	10%IPM	10±2	0.001	0.33
	5%NaLS	6±1.1	0.0006	0.2
	10%NaLS	7±1	0.0007	0.23
2%Carbopol	Without enhancer	8±0.9	0.0008	--
	5% IPM	10±2.2	0.001	1.25
	10%IPM	14±3.2	0.004	1.75
	5%NaLS	6±4	0.0006	0.75
	10%NaLS	12±5.3	0.0012	1.5

[a]Values are the mean ± S.D. of three determinations at 37°C; PI; penetration index; P, permeability coefficient.

Table 5: Duration of anti-inflammatory effect of 1% naproxen sodium in pectin gel.

Time (hr)	Percent swelling	Percent inhibition
control	40 ± 3.1	-
0	35.62 ±1.75	10.95
1	30.512 ±.11	23.72
2	24.44 ±1.23	38.9
3	21.13 ±1.09	47.17
4	18.92 ±1.24	52.7
6	15.71 ±2.54	60.72
8	23 ±2.16	42.5
12	27.2 ±2.02	32
24	30.9 ±2.9	22.75
30	37.1 ±13.1	7.225

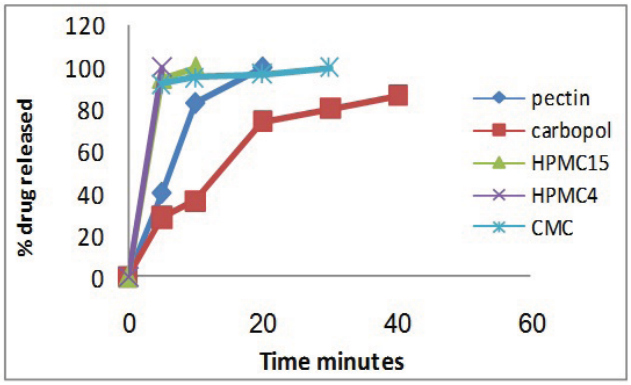

Fig. 1: Dissolution profile of 1% naproxen sodium from 2% different gel bases without enhancers.

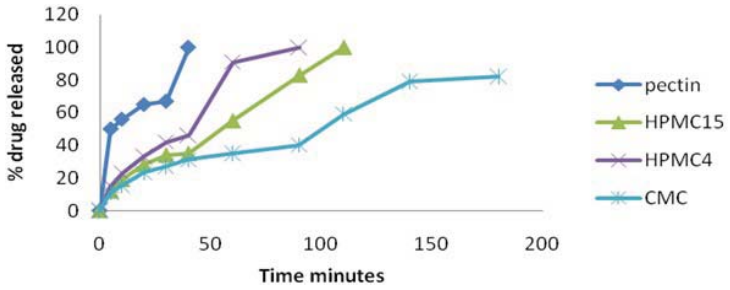

Fig. 2: Dissolution profile of 1% naproxen sodium from 4% different gel bases without enhancers.

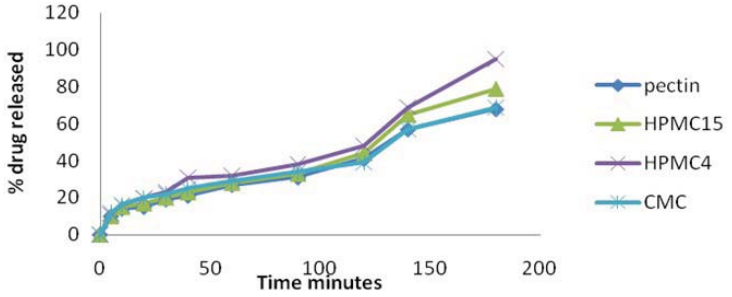

Fig. 3: Dissolution profile of 1% naproxen sodium from 6% different gel bases without enhancers

Mean plasma concentration-time curves of the 4 naproxen sodium formulations are depicted in Fig. (9) and PK parameters (C_{max}, T_{max}, $t_{1/2}$, and AUC $_{0-\infty}$) are summarized in Table (6). The results clearly indicated that the absorption profile of naproxen is better from pectin gel base when compared to that from the other two bases. The drug plasma concentration obtained from pectin gel base was significantly ($P < 0.001$) higher as compared to that from the other two bases at all time points (Fig. 9). The order of absorption based on the

plasma concentration of naproxen from three different gel bases are as follows: pectin gel > HPlv gel > carbopol gel. These findings were in accordance with the results of Sastry *et al.*, 1995. After application of 1% naproxen sodium in pectin gel, the mean plasma naproxen concentration was found to exceed 0.1µg/ml after 3 hr, the highest mean concentration was1.27 µg/ml at 6 hr with correlation with previous results obtained from anti inflammatory duration study on rats and the mean plasma concentration 72 hr after application was 0.45µg/ml. While after application of 1% naproxen sodium in HPlv gel, mean plasma naproxen concentration was found to exceed 0.1µg/ml after 1 hr, the highest mean concentration was 0.887 µg/ml at 8 hr and the mean plasma concentration 72 hr after application was 0.06µg/ml. It is clear that after application of 1% naproxen sodium in carbopol gel, a similar picture was found, mean plasma naproxen concentration was found to exceed 0.1µg/ml after 1 hr, the highest mean concentration was 0.786 µg/ml at 6 hr and the mean plasma concentration 72 hr after application was 0.039µg/ml. In addition, Table (6) shows the bioavailability % (test /reference) for tested formulations were ranged from 19.5 to 36.

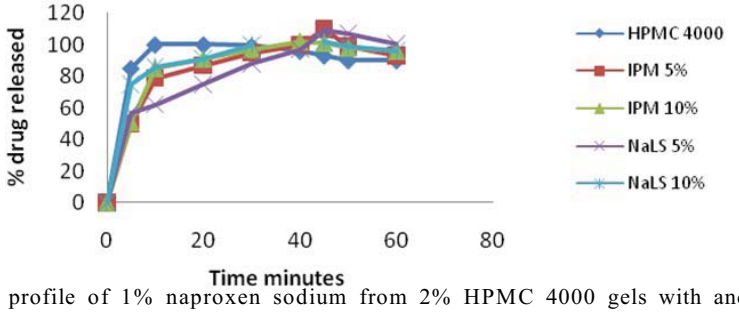

Fig. 4: Dissolution profile of 1% naproxen sodium from 2% HPMC 4000 gels with and without different penetration enhancers.

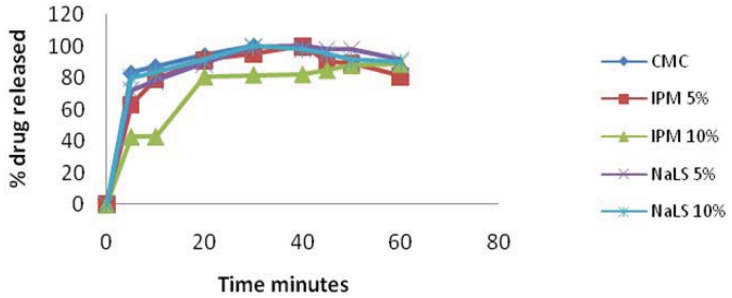

Fig. 5: Dissolution profile of 1% naproxen sodium from 2% CMC gels with and without different penetration enhancers.

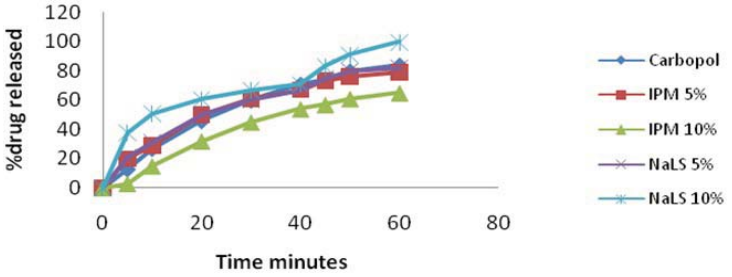

Fig. 6: Dissolution profile of 1% naproxen sodium from 2% Carbopol 934 gel with and without different penetration enhancers.

Significant differences (P<0.001) were indicated for the area under the plasma–time curve (AUC) parameter between all of the formulations. 1% naproxen sodium in pectin gel showed the highest bioavailability followed by 1% naproxen sodium in HPlv gel, and 1% naproxen sodium in carbopol gel. Interestingly, the estimated $t_{1/2}$ of naproxen for all formulations was in the range of mean values from 5.035 to 8.08 hours (Table 6). These values appeared to indicate a slightly shorter $t_{1/2}$ versus the reported naproxen (or naproxen sodium) $t_{1/2}$ which ranges from 12 to 17 hours (Jose Antonio *et al.*, 2009).

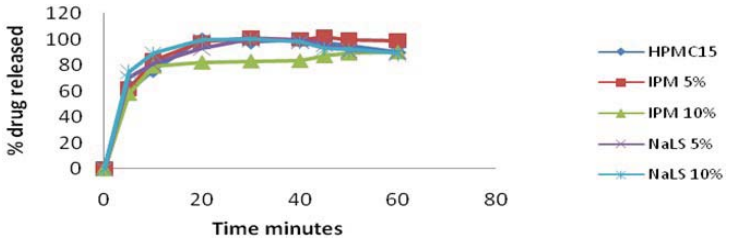

Fig. 7: Dissolution profile of 1% naproxen sodium from 2% HPhv gels with and without different penetration enhancers.

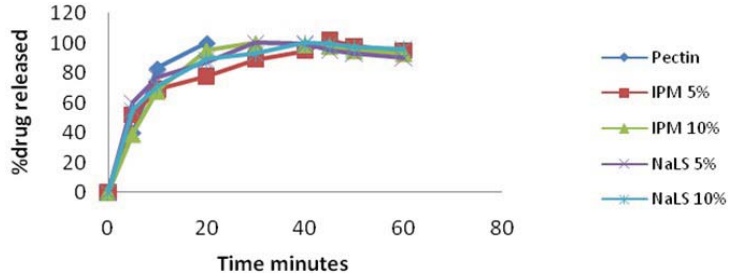

Fig. 8: Dissolution profile of 1% naproxen sodium from 2% pectin gels with and without different penetration enhancers.

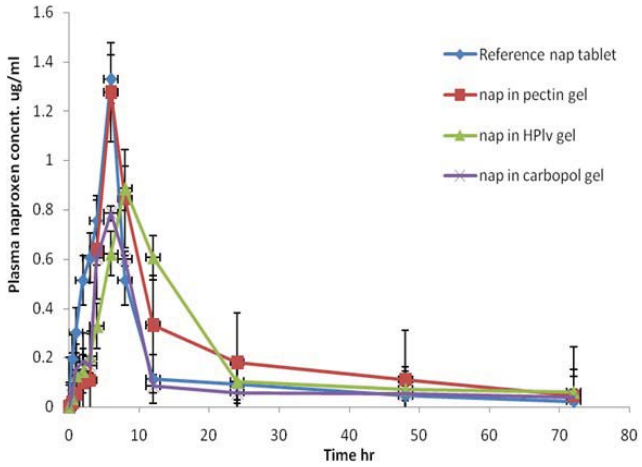

Fig. 9: Mean plasma naproxen concentration-time curve for the test formulations (1% naproxen sodium in pectin, HPlv and carbopol gel) and reference oral-tablet (Trademark Naprofen 250 mg El-Nile Pharmaceutical Co., Egypt) Values are mean (± SD n = 6).

Table 6: Pharmacokinetic parameters of naproxen after administration of oral reference tablet and three topical gel tests to human volunteers.

nap formulation	Pharmacokinetic parameters				
	C_{max}	T_{max}	$t_{½}$	$AUC_{0-\infty}$	Bioavailbility
	µg/mL	h	h	µg/mL. h	%
Reference nap tablet	1.329	6	9.7416	10.9483	--
1%nap pectin gel	1.276	6	8.0838	15.9441	36.4
1% nap HPlv gel	0.887	8	5.0375	14.4199	32.92
1%nap carbopol gel	0.786	6	5.7916	8.5782	19.588

Conclusion:

The experiments have demonstrated that the type of gel forming agent have great effect on the permeation of naproxen sodium through human and rat skin. It is also obvious that the penetration enhancers NaLS and

IPM when added clearly decreased the permeation of naproxen sodium across the skin *in vitro* and *in vivo*. Hence topical application of naproxen in the pectin gel without penetration enhancers may be of potential use for local and systemic activity and will also avoid GI disturbances.

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