RESEARCH ARTICLE

Optimization of physical characterization, skin permeation of naproxen from glycofurol-based topical gel

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In view of good skin tolerability, glycofurol was used as a vehicle-based gel and its effect on the topical penetration of Naproxen (NAP) was investigated. The aim of this study was to develop a suitable gel with bioadhesive property, spreadability, and viscosity for a topical anti-inflammatory effect. Three gelling agents were examined: Carbopol 974P, Gantrez AN 119, and Polyvinylpyrroloidone PVP K30. Skin permeation rates and lag times of NAP were evaluated using the Franz-type diffusion cell, in order to optimize gel formulation. The permeation rate of the NAP-based gel across excised rat skin was investigated. A significant increase in permeability parameters such as steady-state flux (Jss), permeability coefficient (Kp), and penetration index (PI) were observed in the optimized formulation containing 2% Transcutol as a permeation enhancer. From the skin irritation test, it was concluded that the optimized novel tetraglycol-based gel formulation was safe to be used for transdermal drug delivery. The developed naproxen / glycofurol-based gel appeared promising for dermal and transdermal delivery of naproxen and could be applicable with water-insoluble drugs, which would circumvent most of the problems associated with drug therapy.

Key words: *Glycofurol, naproxen, permeation coefficient, physical stability, skin irritation test, topical delivery*

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the drugs most commonly used to reduce inflammation and pain. NSAIDs inhibit cyclooxygenase-2 at the inflammation focus, but unfortunately, most of them also inhibit gastric mucous cyclooxygenase-1, which causes gastric damage.[1] Several studies have shown the effectiveness of topical NSAIDs in treating acute and chronic soft tissue conditions.[2-4] The advantage of a topical NSAID gel over its oral equivalent is that therapeutic benefit can be achieved, while significantly reducing any potential systemic side effects. Recent studies have shown significant drug levels in deep tissues such as the fascia, muscle, and synovium after topical application,[5-7] which is a desirable feature for the relief of local symptoms with a low dose, thereby reducing systemic side effects. Singh and Roberts, and Sioufi *et al*., have shown that the concentration achieved in the subcutaneous tissues by NSAID gels is sufficient to provide a therapeutic benefit.[8,9] Furthermore, the plasma concentration achieved via topical delivery is 1 – 10% of that attained by oral medication and therefore has a significantly reduced risk of potentially serious side effects.

Naproxen is a non-selective cyclooxygenase-1/2 inhibitor when tested *in vitro*, but a slightly preferential cyclooxygenase-2 inhibitor when tested *ex vivo*.[10] Although it is one of best-tolerated classical NSAIDs, gastropathy appears following the chronic oral administration kind of delivery system used. Therefore, an improved naproxen formula with a high degree of skin permeation could be useful in the treatment of not only locally inflamed skin tissues,[11] but also painful states of supporting structures of the body — bones, ligaments, joints, tendons, and muscles. The solubility of naproxen in water is very low (0.3 mg/ml). It is therefore not possible to obtain homogenous hydrogel-based naproxen. It can only disperse, and a transparent aqueous gel cannot be obtained. On the contrary, water insoluble drugs are often soluble in hydrophilic water miscible co-solvents, such as PEG 400 and tetraglycol (glycofurol 75). In this article, Glycofurol, or Tetrahydrofurfuryl alcohol polyethyleneglycol ether is studied as a medium to obtain gels, with the help of a polymer as a thickening and adhesive agent, in order to make possible the dissolution of water-insoluble

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drugs. Carbopol is one of the most used thickeners, and is a very high molecular weight polymer of acrylic acid. Of late, Carbopols have also been used for their mucoadhesive properties and a relevant amount of study has been done on their rheological and mucoadhesive properties.[12,13]

Gantrez AN resins are linear alternating copolymers made by a charge transfer complex reaction of methyl vinylether and maleic anhydride. Five commercial grades of gantrez AN are available. Gantrez AN 119 is widely employed for pharmaceutical applications as a thickening and suspending agent, denture adhesive, and adjuvant for transdermal patches.[14,15] Percutaneous administration of bioadhesive gels allow ready application and ease of removal. The recent development of mucoadhesive dosage forms is due to the fact that a mucoadhesive drug formulation permits localization of a drug in a particular region, thereby increasing bioavailability and at the same time, increasing the contact time between the drug and mucosa. The primary approach to overcome skin resistance to drug-penetration is the skillful selection of vehicle and penetration enhancers, substances that facilitate penetration by reversibly altering the structure of the skin.[16]

The aim of this study is the investigation of the gelation and the adhesion properties of Carbopol 974, Gantrez AN 119, and PVP-based tetraglycol gel, in order to create systems that are able to load and dissolve a large number of drugs, and to obtain the targeted skin permeation profiles (high flux and short lag time) for naproxen.

MATERIALS AND METHODS

Glycofurol, was obtained from Hoffmann La-Roche (Basel, Switzerland). Poly (methyl vinyl ether-co-maleic anhydride) (Gantrez AN 119, MW200 000) was kindly gifted by ISP (Barcelona, Spain). Carbopol 974 was obtained from BF Goodrich (Cleveland, OH). Naproxen USP was obtained from Sigma Chemical Co., St. Louis. Diethylene glycol monoethyl ether, TCL (Transcutol®) was provided by Gattefosse´ (Saint-Priest, France). Semi-permeable cellulose membrane, molecular weight cut-off 12,000 – 14,000, was purchased from Sigma Chemical Co., USA. Polyvinyl pyrrolidone K30 was obtained from BASF, Germany. Methanol and acetonitrile were HPLC-grade and purchased from Merck (Darmstadt, Germany). All other chemicals were of analytical grade or equivalent quality.

Gel preparation

Different gels were prepared according to the method of Bonacucina *et al*.[17] Certain amount of PVP: 20, 25, and 30%, Carbopol: 1.5, 2.5, and 4.0%, and Gantrez AN 119: 2.5%, 5.0, and 7.5%, were dispersed well in tetraglycol. The dispersions were homogenized using Ultraturax T 25 for 5 minutes at 9,000 rpm until a transparent dispersion was formed. It was degassed under vacuum and then stored at room temperature for one day before being analyzed. In all the different gels prepared, 5% (w/w) of the naproxen completely dissolved in the medium at room temperature before the addition of the polymer, although more than 10 wt% of the drug might be solubilized in this system. No phase separation or precipitation was reported for any of the formulae.

Physical characterization of NAP formulations

The prepared gels were visually inspected for clarity, consistency, color, and transparency. The prepared gels were also evaluated for the presence of crystal particles of any drug. Smears of gels were prepared on a glass slide and observed under the microscope for the presence of any particles or grittiness.

Determination of drug content and pH

For the determination of drug content, about 1 g of the gel was weighed in a 100 ml volumetric flask and dissolved in methanol; it was diluted appropriately and analyzed by the HPLC method, described later in the text.

The pH of 5% w/w NAP gel was determined using a sartorius digital pH meter, (Sartorius 210, USP), and standardized using pH 4.0 and 7.0 standard buffer, before use. Three batches of each polymer concentration were subjected to this determination.

In vitro adhesion test

The *ex vivo* adhesion strength was measured in terms of the force needed to detach gels from rat skin. To evaluate the bioadhesive polymers, Bioadhesion was examined *in vitro* using excised skin of the neonate rat without any further treatment. The maximum force of detachment was measured on a tensile strength tester (Instron, A301, England). Tetraglycol-based gels (0.5 g) were homogeneously spread on a 2.5 × 2.5 cm glass disk and then the disks were fixed to the upper supports connected to the tensile strength tester using a double side adhesive. For each measurement a new mucosa sample was used. The gel was brought into contact with the excised skin of the neonate rat under very slight pressure (2 g) and was kept in this position for one minute. Then the tensile test was performed at a constant extension rate of 20 mm/min until complete detachment of the components was achieved. The force required to completely separate the two compartments was recorded as the adhesion force, which was designated as gram force, gf.[18] All the measurements were performed in triplicate. Before performing the textural measurements all samples were stored at 20°C for 24 hours.

Gel spreadability

The spreadability is represented by the thickness of the film, which the preparation leaves on the skin. Those producing thinner films, that is, higher spreadability, are naturally of greater interest.

A sample of 0.1 g of each formula was pressed between two slides (divided into squares of 5 mm sides) on which weights

of 50, 100, 200, and 500 g were placed at intervals of one minute. The diameters during each interval were given as the area (cm^2). The variations of the area as a function of weight were then analyzed as response factors.[19,20] The sample weight was fixed in order to perform the entire assay with all the samples, without surpassing the limits imposed by the glass, avoiding sliding, and easily differentiating the behavior of the different samples. The results obtained are an average of three determinations.

Rheological determination

The flow properties and the viscosity of the systems were determined at 25 ± 1 °C. A Cone and Plate Rheometer RS/Plus (Brookfield Engineering Laboratories Inc., USA) was used to measure the viscosities of the gels. The spindle C-50, using a gap value of 0.40 mm, was rotated at 200 rpm. The system was calibrated using Brookfield viscosity standard fluids. Samples of the gels had to settle over 30 minutes at the assay temperature (25 ± 1°C) before the measurements were taken. Samples were applied to the lower plate using a spatula to make sure that gel shearing did not occur. Parameters such as: τc (Casson's yield value) and $\eta_{ap.}$ 160 s^{-1} (apparent viscosity of the sample) were used as response factors; τc represented the initial resistance of the preparation to flow when it was subjected to an external shear force.[21] It was a characteristic parameter of preparations comprising of internal three-dimensional (3-D) networks, typical of a gel.[22] Quantitatively, it reflected the rigidity and cohesion between the molecules forming the internal 3-D structure;[23] η_{ap}. 160 s−1 represented the ease with which the bonds comprising the structure broke during flow.[24] η_{ap}. 160 s^{-1} determined the resistance of the samples to being extended over the skin.[25]

***In vitro* permeation studies**

Release experiments employed the FDC-6 Transdermal Diffusion Cell Drive Console (Logan Instrument Corp., NJ, USA). The system was fitted with VTC-200 heater circulator, with a jacketed vertical glass Franz diffusion cells being the main unit. The artificial membrane (Cellulose tubing, Sigma diagnostics, St. Louis, MO, USA) was mounted between the donor and receptor compartments of the diffusion cells. These cells provided a diffusional area of 1.7 cm^2 and the receptor compartment was 12 ml. The tested formulations (about 1 g) were loaded into the donor compartment before occluding the donor compartments using a parafilm. To maintain sink conditions, 30% (v/v) ethanol in a physiological buffer solution, PBS (pH7.4) was used as a receptor.[26] The system was maintained at 37 ± 0.5°C with the help of a water bath circulator and a jacket surrounding the cell, resulting in a membrane-surface temperature of 32°C, to mimic skin permeation experimental conditions.[27] Receptor samples of 5 mL were taken periodically and the cells were replenished up to their marked volumes with a fresh receptor. Addition of the receptor to the receiver compartment was performed with great care to avoid trapping air beneath the cellulose membrane. These samples were analyzed for drug content by high-performance liquid chromatography (HPLC), as described a little later in this text. The cumulative amount of drug released was calculated as a function of time. Each experiment was performed at least thrice and the results were averaged (C.V. < 5%).

***Ex vivo* skin permeation studies of selected formulae**

Skin preparation

The experiments were conducted according to the Guidelines for Animal Care and Treatment of the European Community. The protocol of this study was reviewed by the Research Ethics Committee (REC) of the Pharmacology Department affiliated to the Faculty of Medicine, King Saud University. A male rat (Sprague Dawley) was sacrificed by snapping the spinal cord at the neck. The hair of abdominal area was carefully removed with an electric clipper and a square section of the abdominal skin was excised. After incision, the adhering fats and other visceral debris in the skin were carefully removed from the undersurface with tweezers. The excised skin was used immediately. The skin was placed on the receiver chambers with the stratum corneum facing upward, and the donor chambers were then clamped in place. The excess skin was trimmed off, and the receiver chamber, defined as the side facing the dermis, was filled with 30% alcohol in PBS (pH 7.4) and kept at 37°C by a circulating-water jacket.

Effect of an enhancer on the permeation of NAP from the selected formulae

The 5% NAP gel containing 1 – 4% (w/w) transcutol, TCL, enhancer was prepared by the previous methods. The enhancer used was Transcutol, which showed great miscibility with glycofurol. The gel was prepared by adding, under stirring, NAP (5% w/w) to a glycofurol-TCL mixture. The gelling agent was then added and the preparation stirred till gelification took place. The amount of drug permeated from the gel through the rat skin was determined by HPLC. Each data point represents the average of three determinations. The formulation studied (1 g) was placed in the donor compartment and the cell was covered with aluminum foil. Samples of 5 ml were withdrawn from the receptor compartment at 1, 2, 3, 4, 6, 8, 10, 12, and 24 hours, and replaced with the same volume of the receptor. The NAP concentration in the samples was assayed by HPLC, as described a little later on. Sink conditions were met in all cases. Three parallel determinations were performed using skin from the same donor.

HPLC analysis of samples from receiver solutions

Aliquots of 20 μl from each sample were injected into an HPLC system, equipped with a pre-packed column (C18, 5 μm, 150 mm × 4.6 mm, Waters, Milford, USA). The HPLC system (Shimadzu VP series) was equipped with a system controller (SCL-10A VP, Shimadzu), and a variable UV detector (SPD-10A VP, Shimadzu). The quantification of naproxen was carried out at 274 nm. The samples were

chromatographed using an isocratic mobile phase consisting of acetonitrile : water, 420 : 580, (pH was adjusted to 3.1 using phosphoric acid, 150 μl/L) at a flow rate of 1 ml/min. A calibration curve with a concentration range from 0.2 – 10 μg/ml was used to measure the naproxen concentration of the samples and to validate the analytical technique. The analytical technique, validated intra- and inter-day (n = 6), was linear (P > 0.05) according to the statistics applied, precise with a percentage variation coefficient (CV%) between 2.1 and 4.8%, and accurate, with a relative error (% RE) between - 4.50 and 2.20%.

Data analysis of permeation studies calculation of the *in vitro* data

A calibration curve (peak area versus drug concentration) was constructed by running standard drug solutions in 30% alcohol in PBS for each series of chromatographed samples. In the *in vitro* testing, as a result of the sampling of large volumes from the receiver solution (and the replacement of these amounts with equal volumes of buffer), the receiver solution was constantly being diluted. Taking this process into account, the cumulative drug permeation (Qt) was calculated from the following equation:[28]

$$Q\,\mathrm{t} = \mathrm{Vr}\,\mathrm{Ct} + \sum_{i=0}^{t-1} Vs\,\mathrm{Ci}$$

where Ct was the drug concentration of the receiver solution at each sampling time, Ci the drug concentration of the *i* th sample, and Vr and Vs were the volumes of the receiver solution and the sample, respectively. Data were expressed as the cumulative drug permeation per unit of skin surface area, Qt/S (S = 1.76 cm^2). As the release data indicating nearly Zero order release kinetics.

The steady-state fluxes, (Jss, $\mu g/cm^2/h$) (the slope of the linear portion of the permeation curve), expressed as the mass of drug passing across 1 cm^2 of membrane over time, were calculated:[29]

$$Jss = \frac{\Delta Qt}{\Delta \mathrm{t} \times \mathrm{S}}$$

Apparent permeability coefficients (Kp, cm/h) were calculated according to the equation:

$$Kp = \frac{Jss}{Cd}$$

where Kp was the permeability coefficient, Jss the flux calculated at a steady state, and C_d represented the drug concentration that remained constant in the vehicle, and it was assumed that under sink conditions the drug concentration in the receiver compartment was negligible compared to that in the donor compartment. Lag time (L) was determined from the X-intercept of the regression line. The effectiveness of the penetration enhancer was determined by comparing the flux of NAP in the presence and absence of an enhancer. It was defined as the penetration index (PI) that was calculated using the following equation:

PI = (drug flux of samples containing an enhancer) / (drug flux of control samples without an enhancer).

The Higuch equation was used to analyze the release data of Naproxen from the different gel formulations:

$$Q = k\,t^{0.5}$$

Where Q was the amount of drug released per unit area (mg/cm^2), k was a constant, t was the time.

Means, standard deviation (S.D.), coefficient of variation (% CV), and linear regression analyses were calculated using Microsoft Excel 2007.

Statistical analysis

The differences in the results of *in vitro* release and *ex vivo* skin permeation studies were evaluated using one-way analysis of variance (to test the significant effect of different formulations on the obtained data) followed by post hoc analysis for significance at $P < 0.05$ (for pair-wise comparison of any two formulations) using the software SPSS (SPSS Inc., Chicago, USA).

Stability studies on the selected NAP tetraglycol-based gel

On the basis of the results obtained from the previous studies, stability studies were performed on the selected formula; that is, the one showing suitable physical properties and appropriate release characteristics. This gel was stored in a well stoppered glass container for six months at room temperature, 20°C. The gels were visually inspected for any change in their physical appearance, that is, color, turbidity, odor, pH, drug content, and rheology. The effect of storage on *in vitro* drug release was evaluated as well.[30] The results obtained from the freshly prepared samples after storage were compared using the student t test and the software utilized was Gragh Pad Instat V2.04 with 5% level of significance.

Skin irritation study

The skin irritation test was carried out on male Wister albino rats weighing 200 to 225 g. The animals were kept under standard laboratory conditions, with a temperature of 25±1°C and relative humidity of 55% ± 5%. The animals were housed in polypropylene cages, six per cage, with free access to a standard laboratory diet and water *ad libitum*. The hair on the dorsal side of the rats was removed by clipping, one day before this portion of the experiment.[31] The rats were divided into three groups (n = 6). Group I served as

the control, group II received topical 100 mg naproxen gel (optimized formulation;1.0 % GZ, 15% PVP, 2% TCL), and group III received 0.8% v/v aqueous solution of formalin as a standard irritant.[32] A new NAP gel, or new formalin solution, was applied daily for six days. Finally, the application sites were graded according to a visual scoring scale, always by the same investigator. The mean erythemal scores were recorded (ranging from 0 to 4) depending on the degree of erythema, as follows: no erythema = 0, slight erythema (barely perceptible-light pink) = 1, moderate erythema (dark pink) = 2, moderate-to-severe erythema (light red) = 3, and severe erythema (extreme redness) = 4.

RESULTS AND DISCUSSIONS

Evaluation of NAP gels

Visual appearance

All the four gels containing NAP were found to be transparent and uniform in consistency.

Presence of a particulate matter (grittiness)

All the formulations were evaluated microscopically for the presence of particulate matter. No appreciable particulate was seen under the microscope. Hence, the gel formulations fulfilled the requirement of freedom from particulate matter and grittiness. As desired for any topical preparation pH determination, the pH of each gel was noted and the results were taken as a mean of three determinations [Table 1].

Drug content estimation

NAP content of all the gels was estimated by withdrawing samples at random from three different sampling points in a single batch of the gel. Three batches were estimated in a similar manner. Estimations were made using the HPLC method of analysis, after dispersion of the gel in distilled water. The content of NAP in all the gels was found to be within limits (> 98.6%). Samples within a batch were uniform as evident from the low standard deviation value (< 3.5%).

Evaluation of physical properties of NAP gels

The present investigation was carried out to explore the possibility of delivering through the skin, therapeutically effective amounts of a gel formulation of naproxen with glycofurol, as a vehicle-based gel. Adhesion force studies performed highlighted that employment of the glycofurol-based gel permitted the attainment of topical NAP gels with higher mucoadhesiveness. As bioadhesion measurements of the gels indicated that increasing the concentration of the gelling agent decreased the bioadhesion significantly ($P < 0.05$). It could be concluded that the intermediate concentration of each polymer had the optimum adhesive force (25% PVP; 2.5% Cb, and 5% GZ). On increasing the gel concentration above this optimum concentration, bioadhesion had decreased. Carbopol and Gantrez-based gels showed the highest bioadhesive force at all concentrations tested, when compared with PVP. The bioadhesive force of the Gantrez-based gel was less affected by increasing the concentration of the polymer. In solid dosage forms, increasing the polymer concentration, promoted bioadhesion, but in the gels, there was a ceiling effect or optimum concentration for the polymer, wherein, at greater concentrations the bioadhesion decreased. This was because of the reduction in the solvent and increased coiling of the polymer chain.[33]

In the case of topical semisolid formulations it is very important to know the fluency and the product extrusion facility, because many passages (tubes) are required for their packaging and administration. All prepared gels are submitted to rheological tests in order to study their flow properties. It is important to analyze such data because these characteristics can influence the formulation stability during storage and in case of temperature changes. The measurements have been carried out at 37°C (administration site temperature).

Viscosity of the gel matrix is an important factor to consider in the evaluation of drug penetration of gels across the skin

Table 1: Spreadability, apparent viscosity, bioadhesive force and pH of NAP-glycofurol gel

Formulation code	Spreadability $cm^2/g^{1/2}$	τ_c (Pa)	η_{ap} 160/s^1 Pa s	Bioadhesive force (gf)	pH
PV 20%	2.41	562.71	0.475	47.7	4.56
PV 25%	2.20	795.72	0.672	87.8	4.62
PV 30%	1.77	1123.87	0.950	68.7	4.58
Cb 1.5%	1.07	617.15	0.521	84.5	3.80
Cb 2.5%	0.43	863.44	0.729	119.7	3.76
Cb 4%	--#	1471.51	12.39*	68.5	3.74
GZ 2.5%	0.31	507.97	0.429	88.3	4.80
GZ 5.0%	--#	1109.11	0.937	103.9	4.76
GZ 7.5%	--#	1332.98	1.127*	94.7	4.80
Cb 1%: 15% PVP	1.44	905.22	0.765	64.0	4.10
GZ 1.0%: 15% PVP	1.56	1328.46	0.963	92.6	4.45
GZ 1.0%: 15% PVP + 1% TC	1.50	1376.23	1.023	79.3	4.32
GZ 1.0%: 15% PVP + 2% TC	1.47	1380.32	1.162	80.2	4.26
GZ 1.0%: 15% PVP + 4% TC	1.32	1467.90	1.353	82.8	4.16

τ_c, Casson's yield value; η_{ap} 160 s^{-1}, apparent viscosity at 160 s^{-1}; *Viscosity determination using C-75 at 20 rpm; # Very sticky mass

or artificial membranes.[34] By analyzing the formulation behavior and their composition, it is possible to note that the extrudability (presented by τ_c, Casson's yield value) is inversely proportional to the polymer content and that the diluted gels are more fluent than those containing higher polymer content (30% PVP, 2.5% Cb, and 5% GZ). In fact, in the case of gels prepared with the highest polymer content, it is not possible to measure extrudability, because its high viscosity generates technical problems (Cb 4% and GZ 7.5%). The results reveals that these formulations are much less extrudable than gel formulations prepared with less concentration.

As viscosity decreased, it might improve diffusivity of NAP within the gel and facilitate flux. In addition, the solubility of the drug in the vehicle would influence both the drug concentration gradient in the solution and its partition coefficient between the gel and the membrane. Naproxen showed great solubility in glycofurol ~250 mg/ml. Drug solubility increased with the addition of transcutol in the formulations. The influence of gel composition variations on the viscosity of the glycofurol-based gel was evaluated because the viscosity of the gel matrix might play a role in controlling the release of the drug into the receptor medium. An appreciable viscosity increase was observed when the transcutol, TCL, content was increased Table 1.

Gel spreadability

In an attempt to determine the acceptability of the gels, which is an important feature in cosmetics,[35] we have determined the ratio between area and weight by the least-squares method. The best fit for each sample is obtained for the ratio of the area and the square root of the weight ($r^2 = 0.90$), with the slope being used as the response factor [Table 1], which is directly related to the spreadability. The tests are reproducible, and the CV% is less than 3%. At the end of the test, the films are homogeneous with no visible fragmentation in any case, with gel samples containing PVP, at all concentrations, showing the greatest spreadability. Gantrez gel has shown the least spreadability at all concentrations tested. Spreadability is inversely related to the polymer concentration. An increase in the polymer concentration increases the repulsion between chains, increases the cross-linking between chains, and reduces the spreadability. An attempt has been made to get an acceptable and elegant gel formulation using a mixture of PVP that showed great spredability, and Cb or Gz that exhibited good viscosity and adhesion force. The properties of the NAP gel containing binary gelling agents are shown in Table 1.

Gel formulation composed of 1% GZ and 15% PVP was selected as the control gel for the following set of studies in which the effect of penetration enhancers was evaluated.

In vitro diffusion through cellulose membrane

The formulations were successively submitted to *in vitro* drug release by the use of Franz diffusion cells in order to evaluate NAP release profiles. *In vitro* release studies of various gels were carried out to estimate the amount of drug that would be able to cross the biological membrane. The influence of the gel vehicle on the release of the drug was investigated by comparing the permeation release of the drug through each gel using the cellulose membrane to divide the donor and receptor compartments of the diffusion cell. Figures 1a-c and 2 show the amount of NAP permeated across a synthetic cellulose membrane from various gel formulations, as a function of time, over a 12-hour time period. The formulations showed a linear relationship as long as the sink conditions were maintained, indicating nearly zero-order release kinetics. Table 2 shows that the higher the concentration of the gelling agent was, the slower was the drug permeation rate, indicating that a higher concentration of the gelling agent provided higher resistance to drug permeation.[16] The results of viscosity measurements confirmed the permeation results. Other investigators, using photon correlation spectroscopy, had also reported a pronounced a decrease in the diffusion coefficient of a compound when the concentration of poloxamer 407, as a gelling agent, had exceeded 10%. It was interpreted that these changes in diffusion coefficient were due to a marked increase of mean micellar size and polydispersity of the micelles.[16,36]

Table 2: NAP flux from glycofurol-based gel using cellulose acetate membrane and correlation coefficient of regression analysis of release data. Data are given as mean ± SD (n = 3)

Formulation	Release rate µg/cm²/hr	Correlation coefficient (r)	Accumulated amount at 24 hours (µg/cm²)
PV 20%	116.998	0.9983	3092.5
PV 25%	104.9496	0.9958	2789.3
PV 30%	103.0185	0.9976	2359.8
Cb 1.5%	141.347	0.9743	2441.2
Cb 2.5%	130.534	0.9835	2398.4
Cb 4%	114.378	0.9768	1970.6
GZ 2.5%	161.1689	0.9973	3462.4
GZ 5.0%	135.4082	0.9957	2785.2
GZ 7.5%	84.60199	0.98886	1842.3
Cb 1%: PVP15%	1133.278	0.9888	3245.6
GZ 1.0%: PVP15%	136.8763	0.9889	3452.8

Skin permeation study

The sample with optimum gel characteristics and drug permeation through the cellulose membrane was further studied using an animal model diffusion barrier. The skin permeation of NAP from the selected gel (1% GZ and 15% PVP, 5% NAP) is shown in Figure 3. As expected, the drug penetration rate through excised rat skin was slower than that through the cellulose membrane and longer times were necessary to establish a uniform concentration gradient within the membrane, to reach the quasi-stationary state.

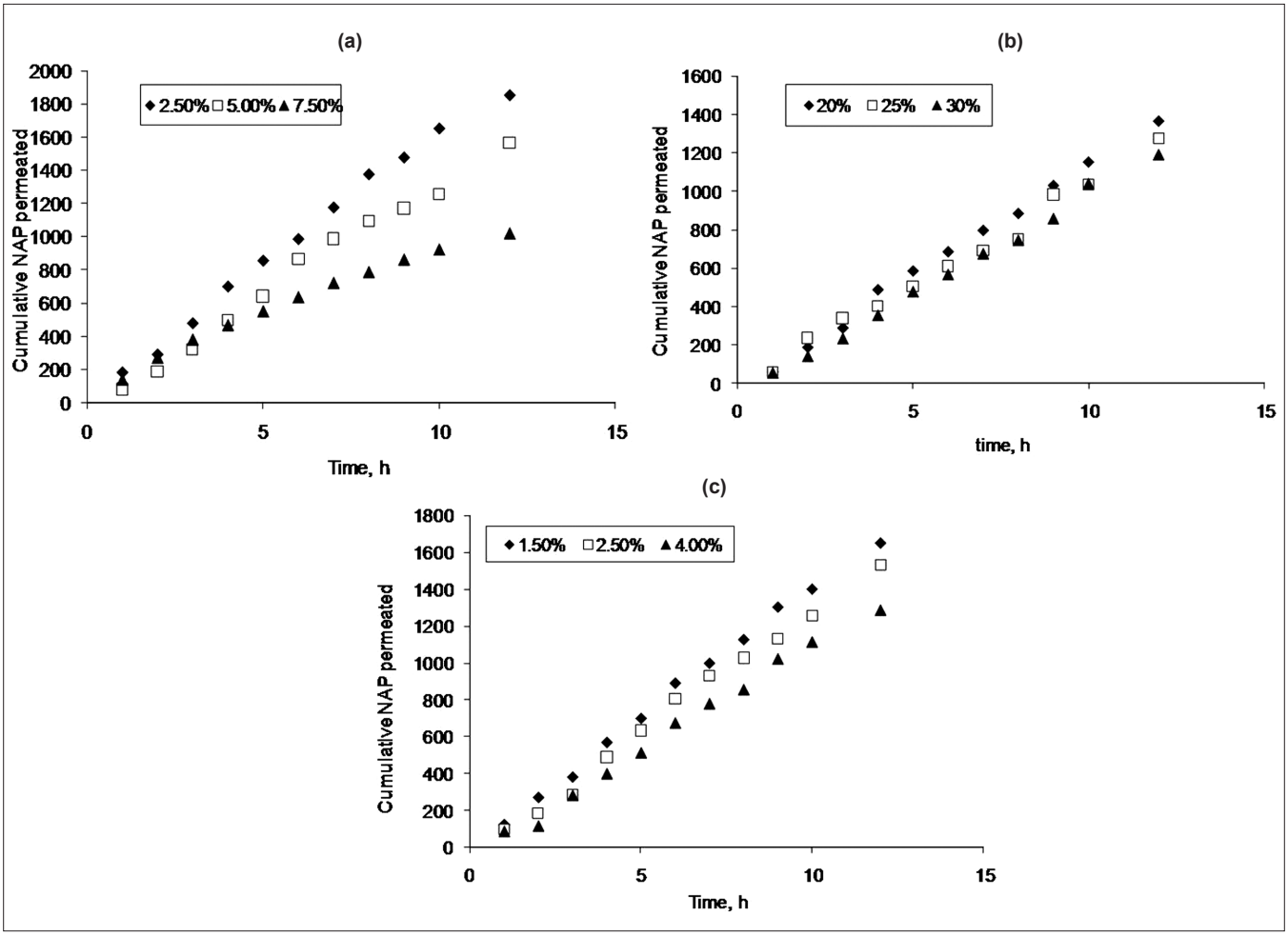

Figure 1: Cumulative amount of NAP released per unit area, µg/cm2 from different gels (a) Gantrez, GZ; (b), polyvinylpyrollidone, PVP; (c) Carbopol, Cb and permeating through cellulose membrane as a function of time. Each data is the mean of three determinations.

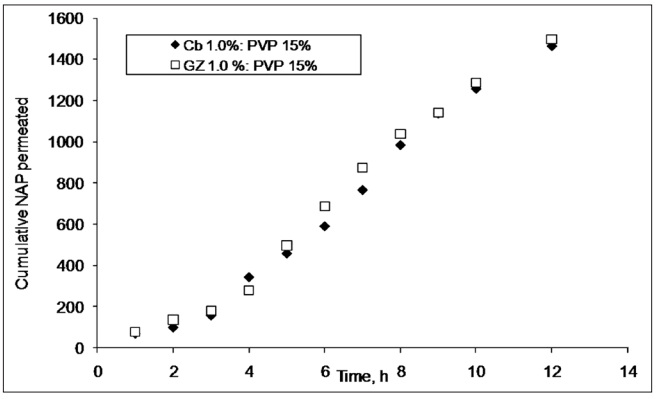

Figure 2: Cumulative amount of NAP released per unit area, µg/cm^2, from a binary gel composed of Cb and PVP or GZ and PVP, and permeating through cellulose membrane as a function of time. Each data is the mean of three determinations

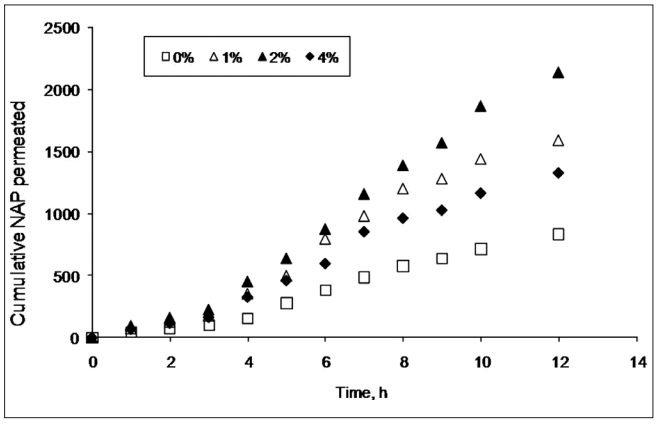

Figure 3: *In vitro* skin permeation profile of NAP from 1% GZ : 15% PVP gel, with and without different proportions of TCL as enhancer. Data are mean of three determinations

Table 3: Permeation parameters a of NAP from glycofurol-based gel composed of GZ1.0 %: PVP 15%, with and without enhancer through excised rat skin

Formulation	Drug flux (µg/cm² h)	Lag time (h)[c]	*K*p (cm/h) x 10³	Cumulative amount at 24 hours µg/cm²	Penetration index (PI)[b]
GZ1.0%: PVP15%	82.9	2.52	1.66	1879	1.0
1% TC	145.8	1.62	2.92	2632	1.76
2% TC	250.54	0.85	5.01	3247	3.02
4% TC	94.11	2.01	1.88	1789	1.14

[a]Each value represents the mean ±S.D. (n = 3); [b]PI: penetration index = Flux with enhancement / Flux control; [c]Lag time: the intercept on the time axis of the steady state flux calculated by linear regression

Permeation of NAP from the gel containing various concentrations of TCL, across the rat skin, Figure 3 and Table 3, shows the effect of the penetration enhancer added to the selected NAP gel formulation.

The enhancing effect of TCL was dependent on the concentration. The effect of TCL at various concentrations (1, 2, and 4%) is shown in Figure 3. In general, the enhancement effect of TCL at a 2% concentration was better than at a concentration of 1%. An increase in concentration of TCL was found to increase the permeabilitiy coefficients (Kp). The permeation parameters of NAP–tetraglycol gel (with and without enhancers), across the rat skin, are shown in Table 3. Gel formulation containing 1 and 2% TCL, significantly increased $P < 0.05$ NAP flux value 1.76- and 3.02-folds, respectively, when compared with the control formulation. Although, 4% TCL increased the NAP flux value, the change was not significant when compared with the control formulation.

It has been reported that the concentration of enhancer in a formulation markedly influences the promotion of transdermal drug delivery.[35,37] Thus, the amount of enhancer present in the skin is an important factor in the enhancing effect. The addition of penetration-enhancing compounds to transdermal delivery systems may improve the penetration of drugs by modifying the thermodynamic activity of the penetrants, for example, changes in partitioning tendencies[38] or by altering the skin barrier properties, thus reducing its diffusional resistance and promoting transdermal delivery of pharmacological substances.[39] The latter effect can only be detected and investigated using human or animal skin, and this can explain the greater relative flux increase generally observed in *ex vivo* experiments (e.g., changes in fluidity of extracellular lipids). Although, the use of TCL has little effect on gel viscosity, it does not have any unfavorable consequence on the drug diffusion rate. As TCL has a high solubilizing power toward NAP, more of the drug would be available for partitioning with the gel / enhancer system into the skin leading to a higher NAP flux value. The enhancer concentration raises the drug concentration gradient in the solution, thus favoring the passage of larger quantities of the drug into the stratum corneum, and allowing greater solubilization in the aqueous phase of the skin tissues. Mura *et al*., has found that TCL added to clonazepam transdermal gel may tend to create a drug depot in the skin, resulted in relatively low flux values[40]. in the stability studies on the selected NAP gel. on the selected NAP tetraglycol-based gel

Stability studies on the selected NAP glycofurol-based gel

The selected gel formulation did not show any appreciable change in gel clarity and color, ratifying the physical stability of the prepared gel formulations. Further, no obnoxious odor was perceptible from the gel formulation. Upon visual inspection, no macroscopical physical changes were observed during storage. Spreadability, bioadhesive force, viscosity, and *in vitro* permeation testing were examined after six months of storage and showed no significant difference when compared to the fresh ones (using Student's t test the $p > 0.05$).

Primary NAP gel irritation study

The skin irritation studies were carried out to evaluate the tolerability of the NAP / tetraglycol-based gel components after application. It was observed that the NAP gel showed a skin irritation score (erythema and edema) of less than 2. The NAP gel was very well-tolerated by the mice and no signs of erythema and / or edema were seen even after three days. According to Draize *et al*., compounds producing scores of two or less are considered negative (no skin irritation).[41] Studies indicated that the novel NAP formulation was well-tolerated by the mice and it did not show any irritation.

CONCLUSION

Liquid Glycofurol can be successfully used as a medium to dissolve water insoluble drugs, as it can be easily transformed into gel systems, having great elasticity. On the basis of the highest drug permeation, good adhesiveness, and spreadability, the glycofurol-based gel containing NAP is a system of interest, as a topical base formulation.

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