AAPS PharmSciTech, Vol. 11, No. 3, September 2010 (© 2010)
DOI: 10.1208/s12249-010-9485-x

Research Article

Evaluation of Glycofurol-Based Gel as a New Vehicle for Topical Application of Naproxen

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Received 26 February 2010; accepted 30 June 2010; published online 23 July 2010

Abstract. In view of the good skin tolerability, glycofurol was used as a vehicle-based gel, and its effect in the topical penetration of Naproxen (NAP) was investigated. The aims of this study were to develop a suitable gel with bioadhesive property, spreadability, and viscosity for topical anti-inflammatory effect. Three gelling and adhesive agents were examined: Carbopol 974P, Gantrez AN 119, and polyvinylpyrollidone K30. Skin permeation rates and lag times of NAP were evaluated using the Franz-type diffusion cell in order to optimize the gel formulation. The permeation rate of NAP-based gel across the excised rat skin was investigated. A significant increase in permeability parameters such as steady-state flux (J_{ss}), permeability coefficient (K_p), and penetration index (PI) was observed in optimized formulation containing 2% Transcutol as an permeation enhancer. From skin irritation test, it was concluded that the optimized novel glycofurol-based gel formulation was safe to be used for topical drug delivery. The developed 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 inflammation focus, but unfortunately, most of them also inhibit gastric mucous cyclooxygenase-1, which produces 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 the 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 fascia, muscle, and synovium after topical application (5–7), which is a desirable feature for the relief of local symptoms with low dose, thereby reducing systemic side effects. Singh and Roberts (8) and Sioufi *et al.* (9) have shown that the concentration achieved in the subcutaneous tissues by NSAID gels is sufficient to provide a therapeutic benefit. 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 the best-tolerated classical NSAIDs, gastropathy appears following the use of a chronic oral administration kind of delivery system. However, alternative administration routes need to be considered in order to avoid the systemic side effects and gastric disorders that often occur after prolonged oral administration. 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. Water-insoluble drugs are often soluble in hydrophilic water miscible co-solvent, such as PEG 400 and glycofurol (glycofurol 75). By use of these excipients, it is possible to prepare a clinically relevant formulation for topical delivery. Besides, different studies had been done on glycofurol ability as an absorption enhancer (12).

Recent studies had investigated the use of Glycofurol or tetrahydrofurfuryl alcohol polyethylene glycol ether as a medium to obtain gels, with the help of thickening and adhesive agent, in order to make possible the dissolution of water-insoluble drugs (13). Carbopol (Cb) is one of the most used thickeners. Carbopol is a very high molecular weight polymer of acrylic acid and has also been used for their

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mucoadhesive properties. The rheological and mucoadhesive properties of Carbopol have been extensively studied in a relevant amount of work (14). The gelation properties of Carbopol dissolve a large number of water-insoluble drugs, for the formulation of semisolid systems which could be administered in different ways including ophthalmic, rectal, buccal, nasal, intestinal, vaginal, and dermal routes.

Gantrez AN (GZ) 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 (15,16). Percutaneous administration of bioadhesive gels allows ready application and ease of removal. The recent development of mucoadhesive dosage forms is due to the fact that a mucoadhesive drug formulation permits to localize a drug in a particular region, thereby increasing bioavailability and, at the same time, increasing the contact time between drug and mucosa. The primary approach to overcome skin resistance to drug penetration is the selection of vehicle and penetration enhancers, substances that facilitate penetration by reversibly altering the structure of the skin (17).

The aim of this work is the investigation of the gelation and the adhesion properties of Carbopol 974, Gantrez AN 119 and polyvinylpyrollidone (PVP)-based glycofurol 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.

EXPERIMENTAL

Materials

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). Diethylene glycol monoethyl ether, Transcutol® (TCL), was provided by Gattefossé (Saint-Priest, France). Semi-permeable cellulose membrane, molecular weight cut-off 12,000–14,000, was purchased from Sigma Chemical Co., USA, and polyvinylpyrollidone K30 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.

Methods

Gel Preparation

Different gels have been prepared according to the method of Bonacucina *et al.* (18). 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 glycofurol. The dispersions were homogenized using Ultraturax T 25 for 5 min at 9,000 rpm until a transparent dispersion was formed, degassed under vacuum, and then stored at room temperature for 1 day before being analyzed. For all the different gels prepared, the 5% (*w*/*w*) naproxen was completely dissolved in the medium at room temperature before the addition of the polymer, although more than 10% *w*/*w* of the drug might be solubilized in this system. No phase separation or precipitation was reported for all 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 any drug crystal particles. Smears of gels were prepared on glass slide and observed under the microscope for the presence of any particle or grittiness.

Determination of Drug Content and pH

For 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 high-performance liquid chromatography (HPLC) method described later.

The pH of the 5% *w*/*w* NAP gel was determined using Sartorius digital pH meter, (Sartorious PB11, USP), standardized using pH 4.0 and 7.0 standard buffers before use. Three batches of each polymer concentration were subjected to this determination.

Skin Adhesion Test

The skin 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). Glycofurol-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-sided 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 a very slight pressure (2 g) and was kept in this position for 1 min. Then the tensile test was performed at a constant extension rate of 20 mm/min until the 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 (19). All the measurements were performed in triplicate. Before performing the textural measurements, all samples were stored at 20°C for 24 h.

Gel Spreadability

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

A sample of 0.4 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 1 min. The diameters during each interval are given as the

area (square centimeter). The variations of the area as a function of weight were then analyzed as response factors (20,21). 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 different samples. The results obtained are 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 were to settle over 30 min 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 like τ_c (Casson's yield value) and $\eta_{ap.}$ 160 s^{-1} (apparent viscosity of the sample) are used as response factors; τ_c represents the initial resistance of the preparation to flow when it is subjected to an external shear force (22). It is a characteristic parameter of preparations comprising internal three-dimensional (3D) networks, typical of a gel (23). Quantitatively, it reflects the rigidity and cohesion between the molecules forming the internal 3D structure (24); $\eta_{ap.}$ 160 s^{-1} represents the ease with which the bonds comprising the structure break during flow (25). $\eta_{ap.}$ 160 s^{-1} determines the resistance of the samples to be extended over the skin (26).

In Vitro Permeation Studies

Release experiments employed the FDC-6 Transdermal Diffusion Cell Drive Console (Logan Instrument Corp., New Jersey, USA). The system is fitted with VTC-200 heater circulator with jacketed vertical glass Franz diffusion cells being the main unit. The artificial membrane (Cellulose tubing, Sigma Diagnostics, St. Louis, Missouri, 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 phosphate buffer solution (PBS; pH 7.4) was used as a receptor (27). The system was maintained at 37±0.5°C by 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 (28). Receptor samples, 5 mL were taken periodically, and the cells were replenished up to their marked volumes with 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 the drug content by HPLC, as described below. The cumulative amount of drug released was calculated as a function of time. Each experiment was performed at least three times, and the results were averaged (variation coefficient (CV) <5%).

HPLC Analysis of Samples from Receiver Solutions

Aliquots of 20 µl from each sample were injected into a HPLC system, equipped with a prepacked column (C18, 5 µm, 150×4.6 mm, Waters, Milford, USA). The HPLC system (Shimadzu VP Series) was equipped with 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 to 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%.

Skin Permeation Studies of Selected Formula

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 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*) TCL enhancer was prepared by the previous method (18). The enhancer used was Transcutol which shows great miscibility with glycofurol. Gel was prepared by adding, under stirring, NAP (5% *w*/*w*) to a glycofurol–TCL mixture. Gelling agent was then added and the preparation stirred till gelification took place. The amounts of drug permeated from the gel through rat skin were determined by HPLC. Each data point represent 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 h and replaced with the same volume of the receptor. The NAP concentration in the samples was assayed by HPLC, as described below. Sink conditions were met in all cases. Three parallel determinations were performed using skin from the same donor.

Data Analysis of Permeation Studies

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 (Q_t) was calculated from the following equation (29):

$$Q_t = \mathrm{V_r C}_t + \sum_{i=0}^{t-1} V_s \mathrm{C}_i \quad (1)$$

where C_t is the drug concentration of the receiver solution at each sampling time, C_i the drug concentration of the ith sample, and V_r and V_s are the volumes of the receiver solution and the sample, respectively. Data were expressed as the cumulative drug permeation per unit of skin surface area, Q_t/S (S=1.76 cm^2). For release data analysis, cumulative permeation (μg/cm^2) was correlated with a square root of time, and release rate was estimated as the slope of such plots (μg/cm^2/t$^{0.5}$).

The steady-state fluxes, J_{ss} (μg/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 (30):

$$J_{ss} = \frac{\Delta Q_t}{\Delta t \times S} \quad (2)$$

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

$$K_p = \frac{J_{ss}}{\mathrm{C_d}} \quad (3)$$

where K_p was the permeability coefficient, J_{ss} the flux calculated at steady state, and C_d represents the drug concentration which remains constant in the vehicle, and it assumed that under sink conditions the drug concentration in the receiver compartment is negligible compared to that in the donor compartment. Lag time (L) was determined from the x-intercept of the regression line. The effectiveness of penetration enhancer was determined by comparing the flux of NAP in the presence and absence of enhancer. It was defined as the penetration index (PI) that was calculated using the following equation:

$$\mathrm{PI} = (\text{drug flux of samples containing an enhancer}) / (\text{drug flux of control sample without an enhancer}). \quad (4)$$

Means, standard deviation (SD), 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 Glycofurol-Based Gel

Based on the results obtained from the previous studies, stability studies were performed on the selected formula; the

Table I. Spreadability, Apparent Viscosity, Bioadhesive Force, and pH of NAP Glycofurol Gel

Formulation code	Spreadability cm^{2}g$^{-1/2}$	τ_c (Pa)	$\eta_{ap.}$ 160 s^{-1}Pas	Bioadhesive force (gf)	pH
PVP 20%	2.41±0.23	562.71±33.4	0.475	47.7±17.4	4.56
PVP 25%	2.20±0.10	795.72±40.3	0.672	87.8±8.4	4.62
PVP 30%	1.77±0.50	1,123.87±63.8	0.950	68.7±6.7	4.58
Cb 1.5%	1.07±0.40	617.15±30.2	0.521	84.5±7.9	3.80
Cb 2.5%	0.43±0.02	863.44±39.2	0.729	119.7±7.6	3.76
Cb 4%	–[a]	1,471.51±81.2	12.39[b]	68.5±9.9	3.74
GZ 2.5%	0.31±0.01	507.97±37.3	0.429	88.3±10.5	4.80
GZ 5.0%	–[a]	1,109.11±94.9	0.937	103.9±8.9	4.76
GZ 7.5%	–[a]	1,332.98±77.9	1.127[b]	94.7±27.8	4.80
Cb 1%:15% PVP	1.44±0.5	905.22±56.2	0.765	64.0±18.9	4.10
GZ 1.0%:15% PVP	1.56±0.8	1,328.46±59.2	0.963	92.6±10.8	4.45
GZ 1.0%:15% PVP+1% TCL	1.50±1.0	1,376.23±83.9	1.023	79.3±17.8	4.32
GZ 1.0%:15% PVP+2% TCL	1.47±0.4	1,380.32±61.9	1.162	80.2±8.9	4.26
GZ 1.0%:15% PVP+4% TCL	1.32±0.5	1,467.90±58.9	1.353	82.8±8.9	4.16

τ_c Casson's yield value, η_{ap} 160 s^{-1} apparent viscosity at 160 s^{-1}, *PVP* polyvinylpyrollidone, *Cb* Carbopol, *GZ* Gantrez, *TCL* Transcutol
[a] Very sticky mass
[b] Viscosity determination using C-50 at 20 rpm

one showing suitable physical properties and appropriate release characteristics. This gel was stored in well-stoppered glass container for 6 months at room temperature, 20°C. The gels were visually inspected for any change in physical appearance of gels, *i.e.*, color, turbidity, odor, pH, drug content, and rheology. The effect of storage on the *in vitro* drug release was evaluated as well (31). The results obtained from the freshly prepared samples and after storage were compared using 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 Wistar albino rats weighing 200 to 225 g. The animals were kept under standard laboratory conditions, with 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 standard laboratory diet and water *ad libitum*. The hair on the dorsal side of the rats was removed with an electric hair clipper on the previous day of the experiment (32). The rats were divided into three groups (n=6). Group I served as control, without any treatment. Group II received topical 100-mg selected NAP gel formulation (1.0% GZ, 15% PVP, 2% TCL), and group III received 0.8% *v*/*v* aqueous solution of formalin as a standard irritant (33). The animals were applied with new NAP gel, or new formalin solution, each day up to 6 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 DISCUSSION

Evaluation of NAP Gels

Visual Appearance

All the four gels containing NAP were found to be transparent and uniform in consistency. All the formulations were evaluated microscopically for the presence of particulate matter. No appreciable particulate was seen under microscope. Hence, the gel formulations fulfilled the requirement of freedom from particulate matter and from 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 I).

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 similar manner. Estimations were made using 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 to deliver through the skin therapeutical effective amounts of a gel formulation of naproxen with glycofurol as a

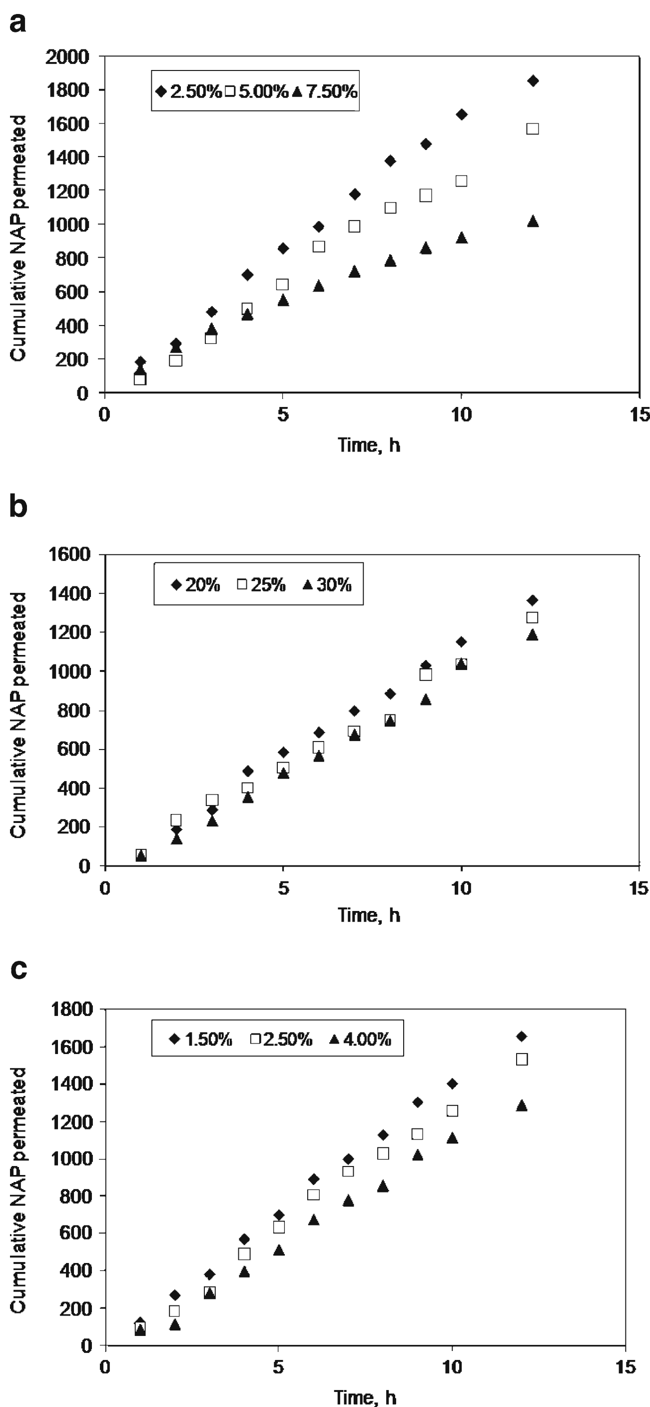

Fig. 1. Cumulative amount of NAP released per unit area (μg/cm^2) from different gels **a** Gantrez, **b** polyvinylpyrollidone, and **c** Carbopol and permeating through cellulose membrane as a function of time. Each data are the mean of three determinations

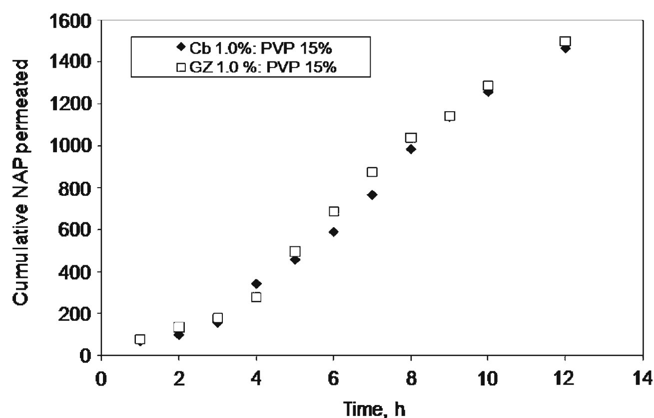

Fig. 2. Cumulative amount of NAP released per unit area (μg/cm^2) from binary gel composed of Cb and PVP or GZ and PVP and permeating through cellulose membrane as a function of time. Each data are the mean of three determinations

vehicle-based gel. Performed adhesion force studies highlighted that the employment of glycofurol-based gel permitted to obtain topical NAP gels with higher mucoadhesiveness. As bioadhesion measurements of the gels indicate, increasing the gelling agent concentration decreases the bioadhesion significantly ($P<0.05$). It may be concluded that the intermediate concentration of each polymer has the optimum adhesive force (25% PVP; 2.5% Cb, and 5% GZ). Increasing the gel concentration above this optimum concentration, bioadhesion has decreased. Carbopol and Gantrez-based gels showed the highest bioadhesive force at all concentrations tested compared with PVP. The bioadhesive force of Gantrez-based gel is less affected by increasing concentration of the polymer. In solid dosage forms, increasing the polymer concentration promotes the bioadhesion, but in the gels, there is a ceiling effect or optimum concentration for the polymer in which at greater concentrations, the bioadhesion decreases. This is because of the reduction in the solvent and increased coiling of the polymer chain (34).

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 were 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 the storage. The measurements were carried out at 32°C (administration site temperature).

Viscosity of the gel matrix is an important factor to consider in the evaluation of drug penetration from gels across skin or artificial membrane (35). 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 polymer content and that the diluted gels are more fluent than those containing higher polymer (30% PVP, 2.5% Cb, and 5% GZ). In fact, in the case of gel prepared with the highest polymer content, it was not possible to measure the extrudability because its high viscosity generated technical problems (Cb 4% and GZ 7.5%). The results revealed that these formulations are much less extrudable than the less concentrated prepared gels formulation.

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 will influence both the drug concentration gradient in solution and its partition coefficient between the gel and the membrane. Naproxen shows great solubility in the glycofurol ~250 mg/ml. Drug solubility increased with addition of Transcutol in the formulations. The influence of gel composition variations on the viscosity of glycofurol-based gel was evaluated because the viscosity of the gel matrix may 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 I).

Spreadability

In an attempt to determine the acceptability of the gels which is an important feature in cosmetic (36), 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=99.99), with the slope being used as the response factor (Table I), 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 spreading films are homogeneous with no visible fragmentation

Table II. NAP Flux from Glycofurol-Based Gel Using Cellulose Acetate Membrane and Correlation Coefficient of Regression Analysis of Release Data

Formulation	Permeation rate (μg/cm^2/h)	Correlation coefficient (r)	Release rate (μg/cm^2/$t^{0.5}$)	Accumulated amount at 24 h (μg/cm^2)
PVP 20%	116.998±32.3	0.9983	420.3372±31.4	3,092.5±116.3
PVP 25%	104.949±40.3	0.9958	376.3512±29.8	2,789.3±131.4
PVP 30%	103.018±29.9	0.9976	365.7475±30.3	2,359.8±127.9
Cb 1.5%	141.347±18.9	0.9743	547.9338±36.4	2,441.2±119.7
Cb 2.5%	130.534±19.3	0.9835	471.7512±48.8	2,398.4±141.3
Cb 4%	114.378±27.8	0.9768	407.8922±33.6	1,970.6±223.8
GZ 2.5%	161.168±29.2	0.9973	584.7866±32.8	3,462.4±212.8
GZ 5.0%	135.408±21.8	0.9957	487.1114±41.9	2,785.2±129.8
GZ 7.5%	84.601±37.6	0.9888	317.172±47.4	1,842.3±208.4
Cb 1%:PVP 15%	133.278±22.8	0.9888	462.9806±39.9	3,245.6±209.4
GZ 1.0%:PVP 15%	136.876±29.5	0.9889	478.1147±40.3	3,452.8±226.9

Data are given as mean±SD (n=3); permeation study times was 0–12 h
PVP polyvinylpyrollidone, *Cb* Carbopol, *GZ* Gantrez

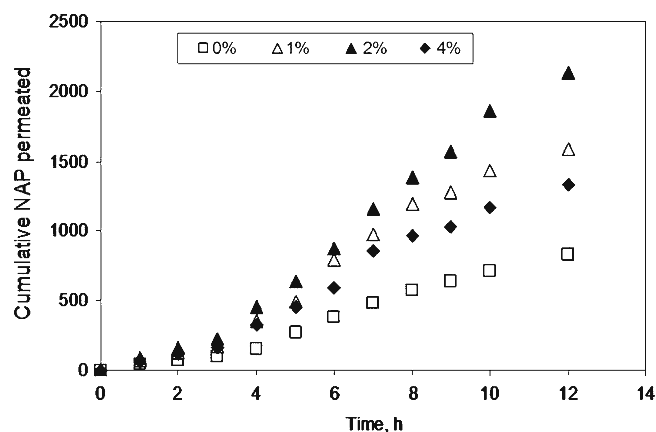

Fig. 3. *In vitro* skin permeation profile of NAP from 1% GZ:15% PVP gel, with and without different proportion of TCL as enhancer. Data are mean of three determinations

in any case, with gel samples containing PVP at all concentration showing the greatest spreadability. Gantrez gel shows 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 to get the acceptable and elegant gel formulation was done by using a mixture of PVP, which shows great spreadability, and Cb or Gz, which exhibits good viscosity and adhesion force. The properties of the NAP gel containing binary gelling agents are shown in Table I.

Gel formulation composed of 1% GZ and 15% PVP was selected as the control gel for further 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 is able to cross the biological membrane. The influence of the gel vehicle on the release of drug was investigated by comparing permeation release of the drug through each gel using 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-h time period. The formulations showed a linear relationship as long as sink conditions were maintained, indicating nearly zero-order release kinetics. Table II shows that the higher the concentration of the gelling agent was, the slower the drug permeation rate was, indicating that the higher concentration of the gelling agent provides higher resistance to drug permeation (17). The results of viscosity measurements confirm the permeation results. Other investigators, using photon correlation spectroscopy, have also reported a pronounced decrease in diffusion coefficient of a compound as the concentration of poloxamer 407 as gelling agent exceeded 10%. It was interpreted that these changes in diffusion coefficient were due to marked increase of mean micellar size and the polydispersity of the micelles (18,37).

Skin Permeation Study

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

Permeation of NAP from the Gel Containing Various Concentrations of TCL Across the Rat Skin

Figure 3 and Table III show the effect of penetration enhancer added to the selected NAP gel formulation. The enhancing effect of TCL was dependent on concentration. The effect of TCL at various concentrations (1%, 2%, and 4%) is shown in Fig. 3. In general, the enhancement effect of TCL at a 2% concentration was better than at 1%. An increase in concentration of TCL was found to increase the permeabilitiy coefficients (K_p). The permeation parameters of NAP–glycofurol gel (with and without enhancers) across rat skin are shown in Table III. Gel formulation containing 1% and 2% TCL significantly increased ($P<0.05$) NAP flux value at 1.76- and 3.02-fold, 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 (36,38). 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

Table III. Permeation Parameters of NAP from Glycofurol-Based Gel Composed of GZ 1.0%:PVP 15%, with and without Enhancer through Excised Rat Skin

Formulation	Drug flux (μg/cm^{2}h)	Lag time (h)[c]	K_p (cm/h)×10^3	Cumulative amount at 24 h (μg/cm^2)	Penetration index (PI)[b]
GZ 1.0%:PVP 15%	82.9±27.9	2.52±1.8	1.66±2.1	1,879±132.5	1.0
1% TCL	145.8±20.3	1.62±1.7	2.92±1.9	2,632±154.2	1.76
2% TCL	250.54±37.9	0.85±0.2	5.01±2.6	3,247±107.8	3.02
4% TCL	94.11±33.8	2.01±0.3	1.88±0.99	1,789±87.3	1.14

PVP polyvinylpyrollidone, *GZ* Gantrez, *TCL* Transcutol
[a] Each value represents the mean±SD (n=3)
[b] PI=flux with enhancement/flux control
[c] Lag time=the intercept on the time axis of the steady-state flux calculated by linear regression

systems may improve the penetration of drugs by modifying the thermodynamic activity of penetrants, *e.g.*, changes in partitioning tendencies (39) or by altering the skin barrier properties, thus reducing its diffusional resistance and promoting transdermal delivery of pharmacological substances (40). The latter effect can only be detected and investigated using human or animal skin, and this could explain the greater relative flux increase generally observed in *ex vivo* experiment (*e.g.*, changes in fluidity of extracellular lipids). The use of TCL although has little effect on gel viscosity, it does not have any unfavorable consequence on the drug diffusion rate. TCL, due to its high solubilizing power toward NAP, more of the drug would be available for partitioning with the gel/enhancer system into the skin leading to the higher NAP flux value. The enhancer concentration raises the drug concentration gradient in solution, thus favoring the passage of larger quantities of the drug into the stratum corneum, and allows greater solubilization in the aqueous phase of the skin tissues. Mura *et al.* found that TCL added to clonazepam transdermal gel may tend to create a drug depot in the skin, resulting in relatively low flux values (41).

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 physical stability of 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 (1.58±0.74), bioadhesive force (90.6±14.8), viscosity (1348.46±35.2), and *in vitro* permeation testing (142.18±27.5) were examined after 6 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/glycofurol-based gel components after application. It was observed that NAP gel showed a skin irritation score (erythema and edema) of less than 2, NAP gel was very well tolerated by the rat, and no signs of erythema and/or edema were seen even after 6 days. According to Draize *et al.*, compounds producing scores of 2 or less are considered negative, no skin irritation (42). Studies indicated that the novel NAP formulation was well tolerated by the mice, and it did not show any irritation.

CONCLUSIONS

Liquid Glycofurol can be successfully used as a medium to dissolve water-insoluble drugs, since it can easily transform into gel systems having great elasticity. On the basis of highest drug permeation, good adhesiveness, and spreadability, glycofurol-based gels containing NAP are a system with interest as topical bases formulations.

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