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Percutaneous absorption of bendroflumethiazide from gel and membrane-controlled gel systems: an in vitro and in vivo study

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Abstract

A number of gel formulations were developed for bendroflumethiazide (BFTZ). The gels were composed of BFTZ dissolved in an alcohol–polyol–water medium and mixed with polyacrylic acid. Based on preformulation studies it was determined that a transdermal therapeutic system (TTS) could be constructed with 5% BFTZ, 40% ethanol (EtOH), 20% of a 1:1 mixture of 2-pyrrolidone and *N*-methyl-2-pyrrolidone (pyrol), 2% polyacrylic acid (PA) and 33% purified water, sandwiched between an aluminum foil backing and a porous polypropylene rate-control membrane. The device was tested, with and without the rate-control membrane, by in vitro diffusion across whole thickness SKH-1 hairless mouse skin and by in vivo percutaneous absorption in New Zealand White rabbits. In vitro results indicate that this device will deliver BFTZ at penetration rates of 0.66 and 0.98 $\mu g/cm^2$ per h across hairless mouse skin, with and without the rate-control membrane, respectively. In vivo results show that blood levels of 50–300 ng/ml could be reached over a 12-h dosing period. Stability studies on the gel formulation and the TTS indicate that elevated storage temperatures will change the viscosity of the gel matrix and the release of BFTZ from the device. © 1997 Elsevier Science Ireland Ltd.

Keywords: Bendroflumethiazide; Gel; Hairless mouse skin; Membrane controlled; Percutaneous absorption; Rabbit

1. Introduction

A number of potent chemicals are currently being administered percutaneously through a number of effective delivery systems. The transdermal therapeutic patch is one such system that controls the rate of drug delivery by using a suitable solvent–polymer reservoir with or without a diffusion rate-control artificial membrane (Cleary, 1984). The solvent portion is made up of

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Table 1
Synthetic and rate-control membranes

Membrane name	Membrane description	Specifications
SpectraPor2	Natural cellulose	2–14 nm Pore size, 80 μm thick, semipermeable, hydrophilic
Metricel alpha 450	Regenerated cellulose	450 nm Pore size, 100 μm thick, nonaqueous
Celgard 2400	Polypropylene	20–200 nm Pore size with 38% porosity, 0.001″ thick, hydrophobic
Normed film	Polyester urethane	Nonporous, 0.0007″ thick
EVA dense	Ethylene vinyl acetate copolymer	Nonporous, 0.002″ thick
Silastic sheeting	Silicone	Nonporous, 0.005″ thick

either water-soluble alkanols (Durrheim et al., 1980), polyols (Flynn and Smith, 1972; Di Colo et al., 1980; Sheth et al., 1986), nonaqueous hydrocarbons (Wolff et al., 1988) and fatty acid esters (Bronaugh et al., 1981). The polymer portion can be hydrophilic and sponge-like or hydrophobic and minolithic. The former includes polyvinyl alcohol (Chien, 1987; Guy and Hadgraft, 1987), polyacrylic acid (Nagai et al., 1983; Dalvi and Zatz, 1984; Nagai et al., 1985), collagen (Thacharodi and Rao, 1996) and gelatin (Gabriel and Simonelli, 1988). The latter includes silicone elastomers (Keith, 1983; Chien, 1987; Guy and Hadgraft, 1987), polyvinyl acetate (Laughlin et al., 1987) and the methacrylate resins (Pywell et al., 1986; Kim et al., 1987). The feasibility of using a skin penetration enhancer in the drug reservoir has been widely explored with additives such as ethanol (Good et al., 1985; Higuchi et al., 1987; Yum et al., 1987; McDaid and Deasy, 1996; Wilding et al., 1996), dimethyl sulfoxide (Astley and Levine, 1976; Chandrasekaran et al., 1977), pyrrolidone (Southwell and Barry, 1984; Akhter and Barry, 1985; Barry and Bennett, 1987; Viegas et al., 1988), laurocapram (Stoughton, 1982; Stoughton and McClure, 1985; Wotton et al., 1985; Morimoto et al., 1986; Diez-Sales et al., 1996; Niazy, 1996), diethylene glycol monoethyl ether (Ritschel et al., 1991; Panchagnula and Ritschel, 1991; Watkinson et al., 1991; Pavliv et al., 1994), propylene glycol (Turi et al., 1979; Barry, 1987; Miller et al., 1993) and fatty acids (Cooper et al., 1985; Francoeur et al., 1990; Ogiso and Shintani, 1990).

Bendroflumethiazide (BFTZ) is a diuretic administered orally in doses of 2.5, 5 and 10 mg. In larger doses it acts as an antihypertensive. It is well absorbed orally, producing plasma concentrations of 76–100 ng/ml and has a biological half-life of 3–4 h (Reynolds, 1982). The selection of BFTZ in this academic study was based on its lipophilicity ($\log P = 1.56$), short half-life and low molecular mass (421.21 Da). Polyacrylic acid is an acrylic acid polymer cross-linked with polyalkenyl ether. It has vast cosmetic and therapeutic applications and its use as a topical gel is of some interest (Nagai et al., 1983, 1985). In this study we incorporated dissolved BFTZ into a polyarylic acid gel and studied its release in vitro across abdominal and dorsal hairless mouse skin and in vivo across the abdominal skin of rabbits.

Table 2
Solutions used in the hairless mouse skin diffusion study

Solution components	% Solvent in each test solution		
	A	B	C
Ethanol	46	27.5	40
PEG 400	24	—	—
2-pyrol	—	13.75	10
M-pyrol	—	13.75	10
Deionized water	30	45	40

Note: 150 mg BFTZ in 3 ml is 5% w/v in concentration.

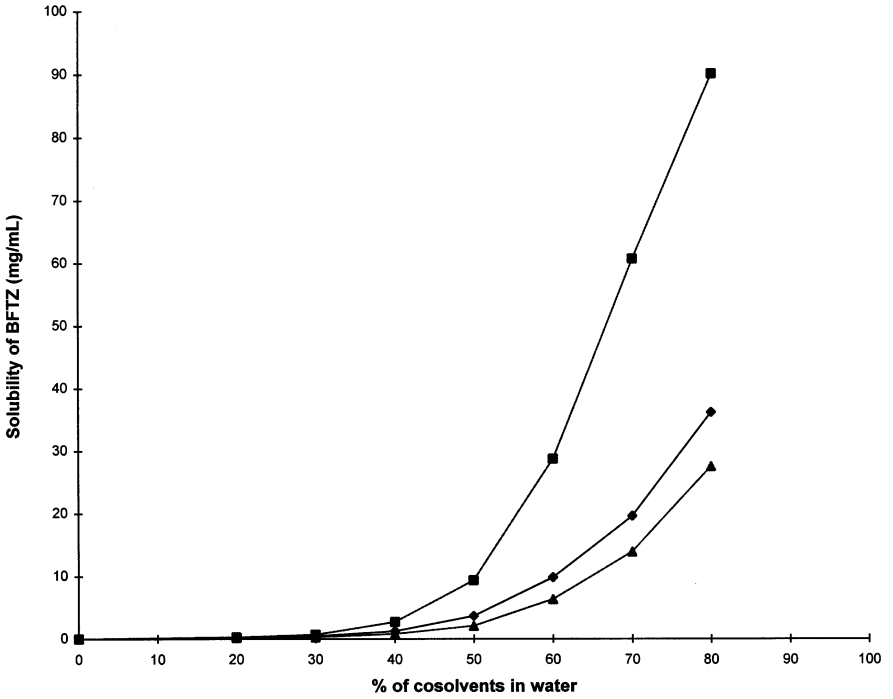

Fig. 1. Solubility curves of BFTZ in 2:1 EtOH:polyol in water. Polyol = GLY (▲), PG (◆) and PEG (■).

2. Materials and methods

2.1. Materials

Bendroflumethiazide (BFTZ) was donated by E.R. Squibb and Sons, Princeton, NJ; Carbopol 934 is from B.F. Goodrich Company, Cleveland, OH; 2-pyrol and *M*-pyrol are from GAF Corporation, Wayne, NJ; Celgard 2400 is from Questar, Charlotte, NC; Normed film is from Norwood Medical Products Division, Malvern, PA; ethylene vinyl acetate copolymer is from ALZA corporation, Palo Alto, CA; SpectraPor2 membrane is from Spectrum Medical Industries, Los Angeles, CA; Metricel alpha 450 is from Gelman Sciences, Ann Arbor, MI; and Silastic Sheeting 500-1 is from Dow Corning Corporation, Midland, MI. All other chemicals used were reagent grade.

2.2. Analysis of bendroflumethiazide

Ultraviolet spectrophotometry (Florey and Russo-Alesi, 1976; Mills and Roberson, 1987) was employed for all preformulation, in vitro diffusion and stability assays. A Perkin–Elmer lambda 3B instrument, with a 1-cm quartz cell was used to measure the absorbance of BFTZ in methanol, at 273 nm. High-performance liquid chromatography (Hikal, 1986) was used to analyze samples obtained from the in vitro hairless mouse skin diffusion studies and the plasma from the in vivo rabbit studies. The chromatograhic system consisted of a Waters 6000A pump, a reverse phase 5 μm C-18 column eluted at 1 ml/min with 40% acetonitrile in 0.015 M phosphoric acid, a Waters 420AC fluorescence detector and a Hewlett Packard 3390A integrator. Retention times of 12 and 6 min were recorded for BFTZ and furosemide (internal standard), respectively.

2.3. Solubility of bendroflumethiazide

The saturation solubility of BFTZ in water, ethanol (EtOH), propylene glycol (PG), polyethylene glycol 400 glycerine (GLY) and a 50:50 mixture of 2-pyrrolidone and *N*-methyl-2-

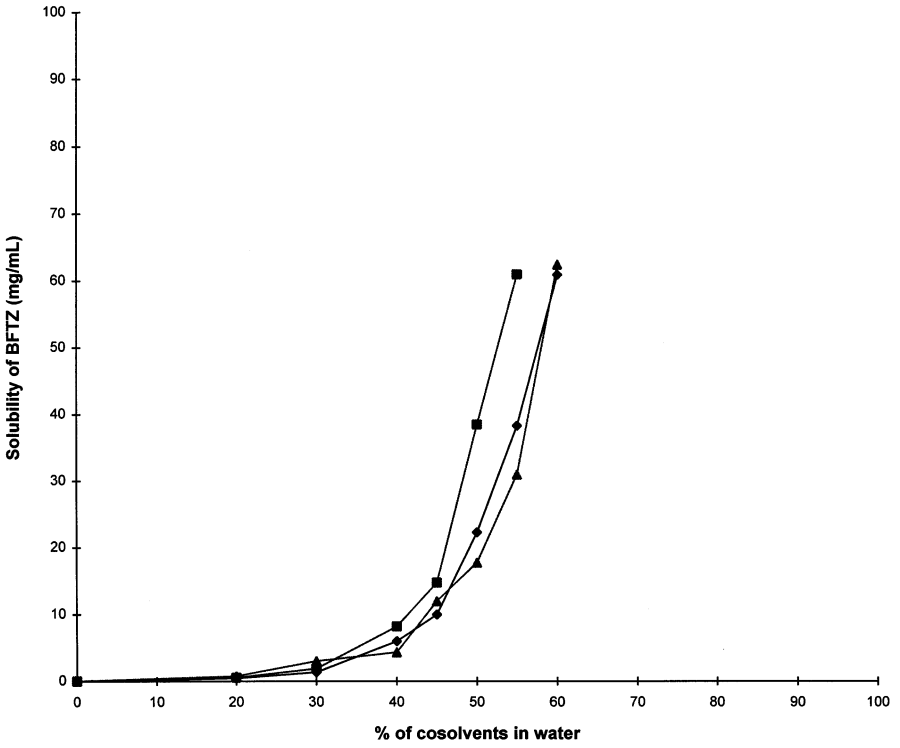

Fig. 2. Solubility curves of BFTZ in EtOH:pyrol in water. Ratio = 1:2 (▲), 1:1 (■) and 2:1 (◆).

pyrrolidone (pyrol) was first determined by mixing an excess amount of drug in 2–5 ml of pure solvent and shaking the mixtures for 24–48 h at 25°C. The saturated solutions were then filtered through a 0.45-μm nylon filter and the filtrates diluted in methanol and assayed by UV spectrophotometry. The solubility of BFTZ was similarly determined in each of the following cosolvent combinations in water:

1. a 2:1 ratio of EtOH and either PG, PEG or GLY;
2. a 2:1 ratio of EtOH and pyrol;
3. a 1:1 ratio of EtOH and pyrol;
4. a 1:2 ratio of EtOH and pyrol.

Plots of solubility (mg/ml) vs percentage of cosolvents in water were constructed.

2.4. In vitro diffusion apparatus

The Franz vertical diffusion apparatus was used in all the in vitro experiments. Each flow-through cell had a 3-ml donor capacity, a 7.5-ml receptor capacity and a 15-mm diameter opening. The cell has two sample ports. The upper sample port was connected to a 3-cm^3 plastic syringe containing receptor medium. Aliquots of 0.1–0.2 ml were withdrawn from the lower port with the aid of an insulin syringe, as fresh receptor medium was simultaneously being introduced through the upper port. The receptor medium used was 5% w/v Tween 80 in distilled water, maintained at 37°C and stirred at 600 rpm with a 10-mm teflon stir bar. The validity of using Tween 80 as a surfactant in the solubilization of thiazide compounds was explained by Aboutaleb et al. (1977). The test gel formulation with the membrane was placed and clasped, between the donor and receptor compartments. When the hairless mouse skin was used, the gel formulation or the TTS was placed on the epidermal surface the skin, which was fixed between the two compartments. The receptor phase was sampled at appro-

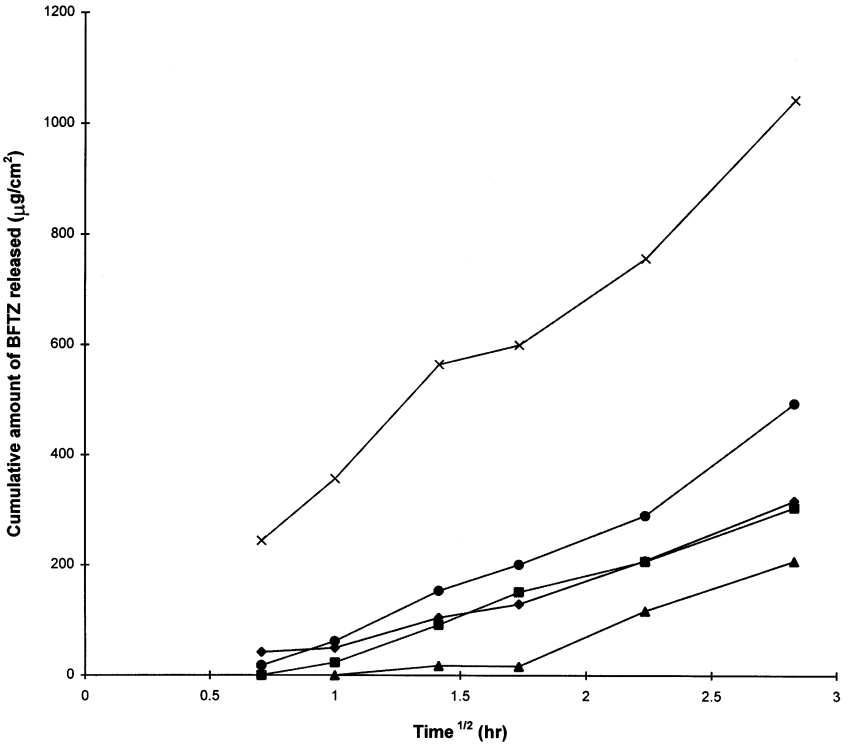

Fig. 3. Cumulative amounts of BFTZ released from gel formulations using the cellulose membrane. Key: 2:1 EtOH:PG (◆); 2:1 EtOH:GLY (▲); 2:1 EtOH:PEG (■); 1:1 PG:pyrol (×); and 1:1 PEG:pyrol (●).

priate time intervals, and assayed to determine the diffusion rates of BFTZ.

2.5. Preparation of gel formulations and their transdermal systems

Polyacrylic acid (PA) was weighed and placed in a glass mortar and uniformly mixed with the required volume of water. The resultant milky white gel was deaerated under vacuum for approximately 15 min. A few drops of 5% ammonium hydroxide solution were added to the mixture to obtain a viscous and transparent gel. BFTZ was dissolved in the selected cosolvent mixture and gently incorporated into the gel with continuous but slow mixing. Gels of 1 and 5% w/w BFTZ concentration were prepared. The finished product was divided in two portions. One portion was packaged in half-ounce wide-mouth glass jars and the second portion was used to prepare the transdermal therapeutic units. Each TTS unit was composed of an aluminum backing, 1 g gel spread within a 15-mm diameter circle, and a 2 × 2 cm membrane-controlled sheet. The units were placed in sealed aluminum pouches and stored in PVC plastic bags before using. The choice of the membrane-controlled barrier and the PA concentration was based on the following set of experiments.

2.6. Selection of the membrane-controlled barrier

Membranes of different porosity and hydrophilicity were selected possible rate-control membranes for the prepared BFTZ gel formulations. Table 1 summarizes the properties of each

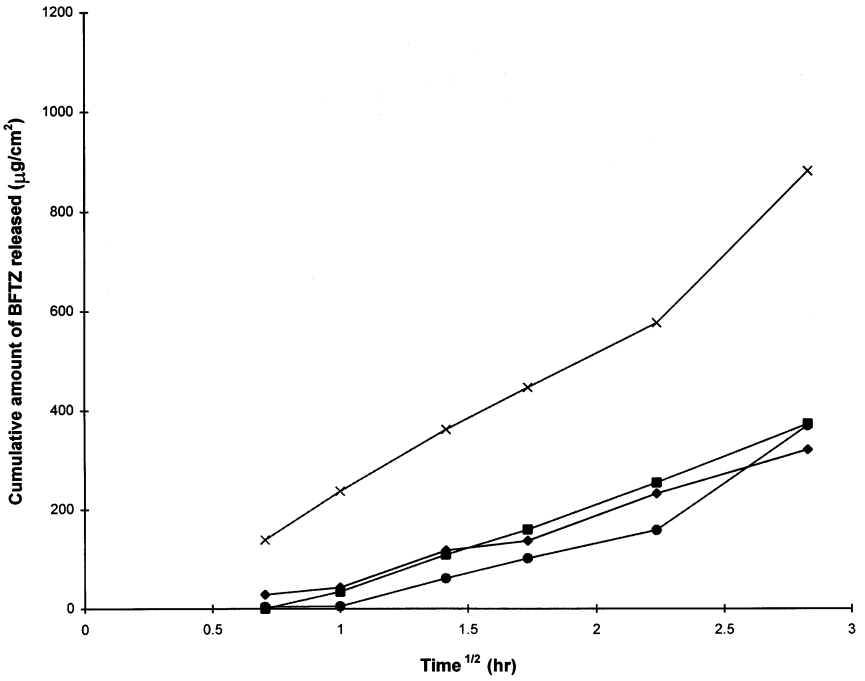

Fig. 4. Cumulative amounts of BFTZ released from gel formulations using the polypropylene membrane. Key: 2:1 EtOH:PG (◆); 2:1 EtOH:PEG (■); 1:1 PG:pyrol (×); and 1:1 PEG:pyrol (●).

membrane. The membranes were cut into squares of 2×2 cm and immersed in distilled water overnight, before use. Each square was blotted dry and placed between the donor and receptor compartments of the Franz vertical cell assembly. Based on the results in our solubility experiments, we prepared two gel formulations:

- 1% BFTZ, 5% PA, 47% of a cosolvent system of EtOH mixed with PG/PEG/GLY (2:1 ratio) and 47% deionized water;
- 1% BFTZ, 5% PA, 16% PG/PEG, 16% pyrol and 62% deionized water.

The diffusion studies were performed as previously explained.

2.7. Selection of optimum polyacrylic acid concentration

Gels containing 3, 5 and 7% by weight of PA were made as described above. Each gel contained 1% BFTZ, 47% EtOH and PEG (2:1 ratio) in appropriate amounts of deionized water. The diffusion of BFTZ from these gels was determined as explained above.

2.8. In vitro diffusion across SKH-1 hairless mouse skin

A number of 2–6-week-old male SKH-1 hairless mice (Charles Rivers Laboratory) were sacrificed by carbon dioxide asphyxiation. Full thickness skin was excised from the back and abdomen of the animal with the aid of sharp scissors. The skin was immersed in sterile normal saline and the subcutaneous fat was teased off with the aid of blunt forceps. The skin specimen, abdominal or dorsal, was inserted epidermal side up between the donor and receptor compartments of the Franz cell assembly. Three test solutions containing 5% BFTZ were used in the first part of the study (Table 2). We selected these solutions based on the results obtained from the solubility and membrane diffusion studies. The diffusion of BFTZ from 3 ml of test solution and across the abdominal and dorsal skin was measured. Aliquots were taken from the receptor phase at time intervals of 0.5, 1, 3, 5, 8, 12 and 24 h and analyzed for drug content. In the second part of

Table 3
Diffusion constants of BFTZ gel formulations

Gel formulation	Diffusion constants (cm^2/h)	
	Cellulose membrane	Polypropylene membrane
1% BFTZ, 5% polyacrylic acid, 47% EtOH:PG (2:1), 47% water	1.35×10^{-4}	1.58×10^{-4}
1% BFTZ, 5% polyacrylic acid, 47% EtOH:PEG (2:1), 47% water	1.67×10^{-4}	2.46×10^{-4}
1% BFTZ, 5% polyacrylic acid, 47% EtOH:GLY (2:1), 47% water	1.64×10^{-4}	—
1% BFTZ, 5% polyacrylic acid, 16% PG, 16% pyrol, 62% water	1.01×10^{-3}	8.71×10^{-4}
1% BFTZ, 5% polyacrylic acid, 16% PEG 400, 16% pyrol, 62% water	3.67×10^{-4}	2.14×10^{-4}
1% BFTZ, 3% polyacrylic acid, 47% EtOH:PEG (2:1), 49% water	2.39×10^{-4}	2.34×10^{-4}
1% BFTZ with 5% polyacrylic acid, 47% EtOH:PEG (2:1), 47% water	1.67×10^{-4}	2.46×10^{-4}
1% BFTZ with 7% polyacrylic acid, 47% EtOH:PEG (2:1), 45% water	7.38×10^{-5}	1.67×10^{-5}

the study, a gel was prepared containing 5% BFTZ, 2% PA, and the cosolvent system, from part one of the study, that had the highest penetration rate. The diffusion study was similarly carried out using 1 g gel, with and without the placement of the polypropylene rate-control membrane between the gel and the skin.

2.9. In vivo availability in rabbits

New Zealand White rabbits of 2.5 kg average weight were used for this study. The animals were fasted for at least 12 h before use. Each rabbit was dosed in the marginal ear vein, with a fast-acting barbiturate anesthetic (thiamylal sodium 10–15 mg/kg). Upon reaching unconsciousness, the ventral part of the neck area was operated to expose the external jugular vein. The vein was catherized with a polypropylene tube (0.024″ i.d. and 0.05″ o.d.) and flushed with normal saline containing 200 units/ml of heparin sodium. The abdomen of the rabbit was then shaved and the animal was allowed sufficient time to regain consciousness. The gel formulation used in the in vitro mouse study was applied to a 5×5 cm area of the abdomen, covered with a non-porous silicone membrane and wrapped with a gauze bandage. The amount of gel used was equivalent to a dose of 100 mg BFTZ per kg rabbit weight. The study was repeated with the gel and the polypropylene membrane (TTS). Blood samples of 1 ml were withdrawn through the catheter at time intervals of 0, 0.5, 1, 1.5, 2, 3, 4, 5, 8, 12 and 24 h and analyzed for drug content.

2.10. Pharmacokinetics of BFTZ in rabbits

Three rabbits were selected and operated as above. They were intravenously dosed through the marginal ear vein with a 10 mg/ml BFTZ solution in 55% EtOH:PEG 400 (2:1 ratio) and 45% sterile water for injection. The amount of solution injected was equivalent to a dose of 10 mg BFTZ per kg rabbit weight. Blood samples of 1 ml were withdrawn and analyzed as before. The data collected from this study were used to compute the appropriate kinetic parameters with the aid of a PCNONLIN Estimation Program VO2-F (Statistical Consultants, Lexington, KY).

2.11. Stability study

Gel formulations prepared in jars and in transdermal patch units were stored at temperatures of 5, 25, 37 and 50°C. The following stability parameters were evaluated at time intervals of 0, 4, 8, 16 and 24 weeks. Appearance: the gel in the jar and patches were visually inspected for color changes and syneresis. Drug content: approximately 100 mg gel was removed from the jar and patch, accurately weighed, dissolved in methanol at 40°C, mixed, centrifuged, and the supernatant assayed for drug content. Viscosity: a Brookfield synchroelectric viscometer with a number 7 spindle was used. The stress (spindle torque) was measured at different rates (rpm). Rheograms were plotted for each sample. BFTZ release: the patches were removed from the alu-

Table 4
In vitro diffusion of BFTZ across abdominal and dorsal skin of hairless mouse from three solvent systems

Time (h)	Cumulative amount of BFTZ penetrated in $\mu g/cm^2$ (S.E.M.)											
	A				B				C			
	Abdominal		Dorsal		Abdominal		Dorsal		Abdominal		Dorsal	
0.5	0.00		0.00		0.05	(0.02)	0.00		0.06	(0.07)	0.11	(0.07)
1	0.06	(0.03)	0.00		0.21	(0.09)	0.00		0.64	(0.39)	0.16	(0.10)
2	0.24	(0.23)	0.00		0.37	(0.04)	0.11	(0.11)	2.37	(1.11)	0.40	(0.23)
3	0.92	(0.91)	0.32	(0.24)	0.79	(0.43)	0.12	(0.10)	2.64	(0.24)	1.26	(0.26)
5	2.43	(1.85)	0.49	(0.26)	1.09	(0.48)	0.26	(0.13)	9.18	(3.78)	2.43	(0.30)
8	4.28	(2.93)	1.38	(0.19)	2.41	(0.76)	1.02	(0.34)	16.48	(5.20)	6.24	(2.12)
12	8.51	(4.31)	4.53	(1.22)	5.05	(1.15)	4.70	(0.65)	27.82	(6.96)	13.59	(3.41)
24	32.58	(13.64)	17.29	(5.12)	22.83	(6.49)	21.39	(1.40)	89.52	(15.39)	56.32	(13.53)

minum pouch and then placed on the Franz diffusion cell. The release of BFTZ from the gel matrix was determined as before.

3. Results and discussion

3.1. Solubility of bendroflumethiazide

The saturated solubilities (in mg/ml) of BFTZ in the pure solvents are 0.04 (water), 52.91 (EtOH), 38.84 (PG), 3.05 (GLY), 361.28 (PEG) and 447.25 (pyrol). Figs. 1 and 2 illustrate the solubility curves of BFTZ (in mg/ml) in different percentages of EtOH, polyol and pyrol solutions. It is evident from Fig. 1 that the EtOH and PEG 400 combination will dissolve at least twice the amount of BFTZ than EtOH with PG or GLY. Similarly, Fig. 2 shows that a solution with a 1:1 ratio of EtOH:pyrol dissolved more BFTZ than those with the 1:2 and 2:1 ratios. Examination of these curves shows that a 5% drug load (50 mg/ml) would correspond to a 65% EtOH:PEG (2:1) solution or a 55% EtOH:pyrol (1:1) solution or a 60% EtOH:pyrol (2:1) solution in water. On the other hand, a 1% drug load (10 mg/ml) can be made with a 45–65% concentration of any cosolvent mixture in water.

Table 5
In vitro diffusion of BFTZ across abdominal and dorsal skin of hairless mouse from gel, with and without the rate-control membrane

Time (h)	Cumulative amount of BFTZ penetrated in $\mu g/cm^2$ (S.E.M.)		
	Without membrane		With membrane
	Abdominal	Dorsal	Abdominal
0.5	0.55 (0.28)	0.04 (0.04)	0.67 (0.19)
1	0.95 (0.31)	0.20 (0.18)	0.89 (0.23)
2	1.01 (0.32)	0.52 (0.27)	1.20 (0.31)
3	1.30 (0.35)	0.67 (0.20)	1.26 (0.40)
5	2.78 (0.81)	2.29 (0.85)	2.07 (0.28)
8	5.78 (1.23)	5.04 (1.73)	4.31 (0.51)
12	12.22 (1.71)	(5.02)	8.61 (1.60)
24	42.38 (4.16)	51.25 (13.09)	48.20 (14.33)

3.2. Data analysis

The square root equation (Higuchi, 1962) was used to analyze the release of BFTZ from the gel.

$$Q = 2C_0\sqrt{\frac{Dt}{\pi}}$$

The equation explains that the amount of drug released (Q) per square root of time (t), is dependent on the initial concentration of drug in the gel (C_0) and the diffusion coefficient of drug in the gel (D). The data collected agreed with the limitations associated with this equation, which are: (1) the drug must be released from one side of the gel; (2) the drug must be uniformly dissolved within

Table 6
In vitro percutaneous parameters

Parameter	Without membrane		With membrane
	Abdominal	Dorsal	Abdominal
Penetration rate, J_s (μg/cm^2 per h)	0.9788	0.9990	0.6636
Lag time (h)	0.91	1.54	0.41
Permeability coefficient, k_p (cm/h)	1.96×10^{-5}	1.99×10^{-5}	1.33×10^{-5}
Diffusion constant within skin, D (cm^2/h)	2.93×10^{-6}	1.74×10^{-6}	6.54×10^{-6}
Skin/vehicle partition coefficient, k_m	0.027	0.046	0.008

the gel; and (3) less than 30% of the drug should be released during the testing period. The equation is rewritten to calculate the diffusion coefficient of drug in the test gels (Sprang-Brunner and Speiser, 1975; Fares and Zatz, 1995):

$$D=\frac{\pi}{t}\left(\frac{Q}{2C_0}\right)^2$$

3.3. Evaluation of rate-control membranes

Figs. 3 and 4 illustrate the cumulative amount of BFTZ released across the cellulose and polypropylene membranes, respectively. The release of drug across the regenerated cellulose membrane was negligible and no diffusion of drug was observed across the three nonporous membranes. Table 3 summarizes the diffusion constants of the different BFTZ gels across the cellulose and polypropylene membranes. It is evident from the data that there is no difference between the two rate-control membranes. Comparison of the different gels shows that those containing the pyrol solution with PG had higher diffusivity constants than those that contained EtOH and GLY or PG or PEG (significant at $p=0.01$ using two-way analysis of variance (ANOVA) analysis). The polypropylene rate-control membrane was selected, because it is microporous and hydrophobic. The natural cellulose membrane is microporous, hydrophilic and semipermeable and allows the gel matrix to partially swell and expand.

Table 7
Computed pharmacokinetic parameters

Correlation coefficient of biexponential curve (pooled data for three rabbits)	0.987
A (μg/ml)	19.179
B (μg/ml)	5.339
α (h^{-1})	2.970
$\alpha t_{1/2}$ (h)	0.233
β (h^{-1})	0.669
$\beta t_{1/2}$ (h)	1.036
k_{10} (h^{-1})	1.699
k_{12} (h^{-1})	0.771
k_{21} (h^{-1})	1.170
AUC_∞ (μg·h/ml)	14.435
V_d (ml/kg)	407.85

3.4. Evaluation of optimum polyacrylic acid concentration

The diffusion constants of the gel formulations containing 3, 5 and 7% of PA were calculated (Table 3). Of the three concentrations compared, the one containing 7% PA had the lowest diffusion constant. We selected a 2% concentration because there was little difference in the viscosity and drug release between the gels containing 2, 3 and 5% PA. Based on the data obtained from the solubility and diffusion studies, a gel and TTS formulation was prepared which comprised of 5% BFTZ, 2% PA, 40% EtOH, 20% pyrol and 33% purified water. The diffusion constant of this formulation was calculated to be 9.32×10^{-4} cm^2/h. The selection and choice of this cosolvent system and gel formulation was further investigated in the skin diffusion experiments.

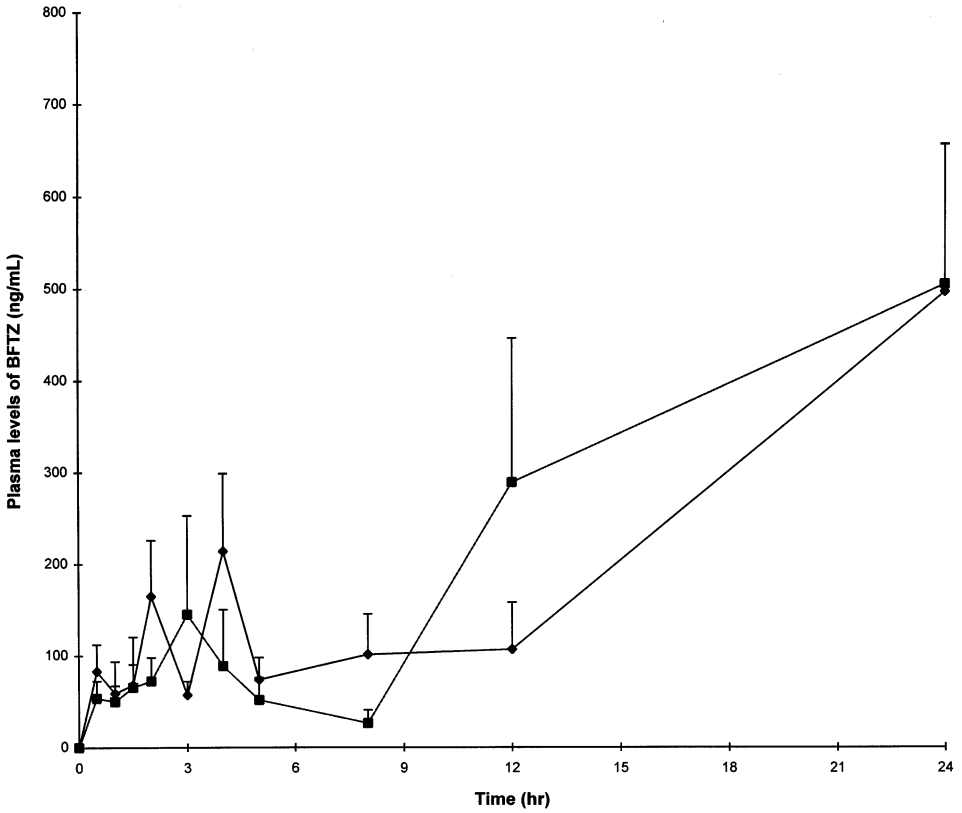

Fig. 5. Plasma concentration of BFTZ after transdermal administration (± S.E.M.), with (◆) and without (■) the use of the rate-control membrane.

3.5. In vitro diffusion across hairless mouse skin

Table 4 summarizes the cumulative amounts of BFTZ transferred across the abdominal and dorsal sections of the hairless mouse skin, when the three test solutions (Table 2) were used. Table 5 summarizes the cumulative amounts of BFTZ transferred across the abdominal and dorsal sections of the hairless mouse skin, when the gel formulation was used. The in vitro data for the first 12 h of the experiment were fitted to the following diffusion model equations (Flynn and Smith, 1972; Ogiso et al., 1993; Shah, 1996). The calculated percutaneous parameters are listed in Table 6.

$$D = \frac{\delta^2}{6\tau}$$

$$J_s = \frac{Dk_m C_0}{\delta} = k_p C_0$$

where J_s is the penetration rate (μg/cm^2 per h); k_m is the skin/vehicle partition coefficient of the drug; D is the diffusion constant within the skin (cm^2/h); τ is the lag time (h); δ is the thickness of the hairless mouse stratum corneum, reported as 40 μm (Higuchi and Yu, 1987); k_p is the permeability coefficient through the stratum corneum (cm/h); and C_0 is the initial concentration of the drug in the solution or gel (μg/cm^3). The total amount of drug diffused for the 24 h testing period was the highest for solution C > A > B, agreeing with our observations in the preformulation diffusion experiments. Solutions A and C both contain the volume of EtOH, but solution C contains the pyrol penetration enhancers. It was also observed

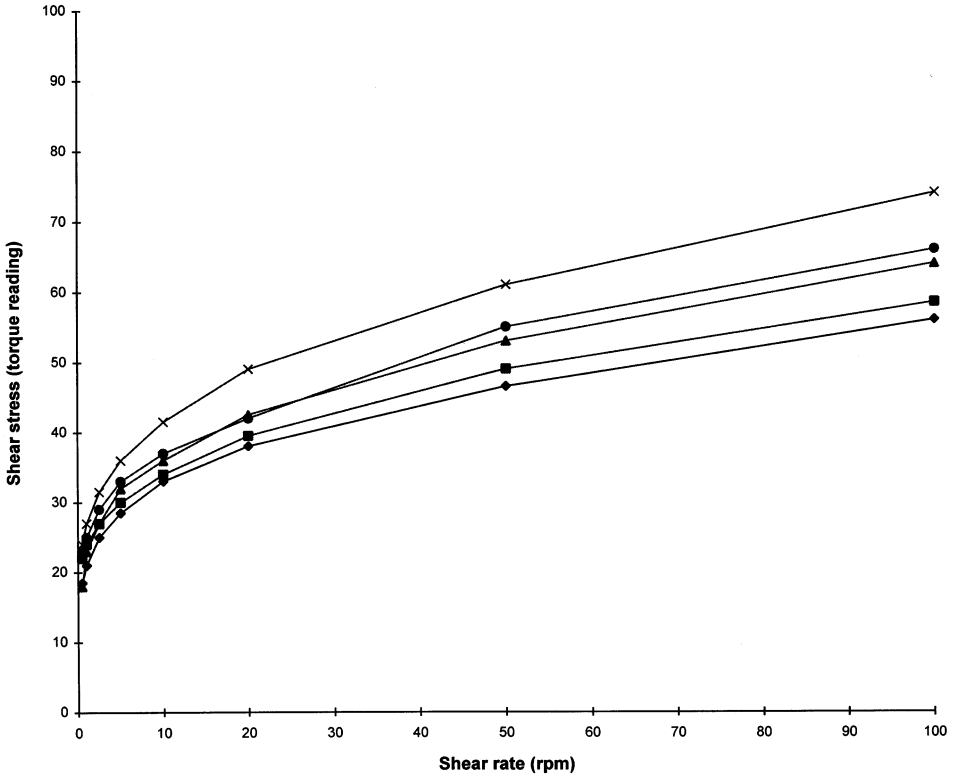

Fig. 6. Effect of storage at 50°C on the flow properties of the gel: week 0 (◆); week 4 (■); week 8 (▲); week 16 (●); and week 24 (×).

that the abdominal skin was slightly more permeable to drug than the dorsal skin. When the gel containing solution C was prepared and tested, the penetration rates for BFTZ across the abdominal and dorsal skin were 0.999 and 0.978 $\mu g/cm^2$ per h, respectively. The presence of the polypropylene membrane reduced the rate to 0.664 $\mu g/cm^2$ per h.

3.6. Pharmacokinetics of bendroflumethiazide in rabbits

The concentration of BFTZ in rabbit plasma, following a single intravenous injection follows a biexponential curve for a two-compartment model. The computed pharmacokinetic parameters for the pooled data of the three rabbits are in Table 7. The short biological half-life of 1.04 h supports the rationale for developing a controlled delivery system.

3.7. In vivo availability of bendroflumethiazide in rabbits

Fig. 5 illustrates the rabbit plasma levels of BFTZ, following transdermal delivery from a gel, with and without the use of the polypropylene membrane. Levels of 50–300 ng/ml were reached over a 12-h period. At the 24-h time interval, concentrations of 500 ± 150 ng/ml were observed. The data does not indicate whether steady-state was reached at 24 h. There was no difference in BFTZ blood levels when the gel and the TTS were statistically compared.

3.8. Stability study

3.8.1. Gel appearance

The gel remained colorless and transparent at the 5 and 25°C storage conditions. The appearance of a pale yellow to straw color in samples

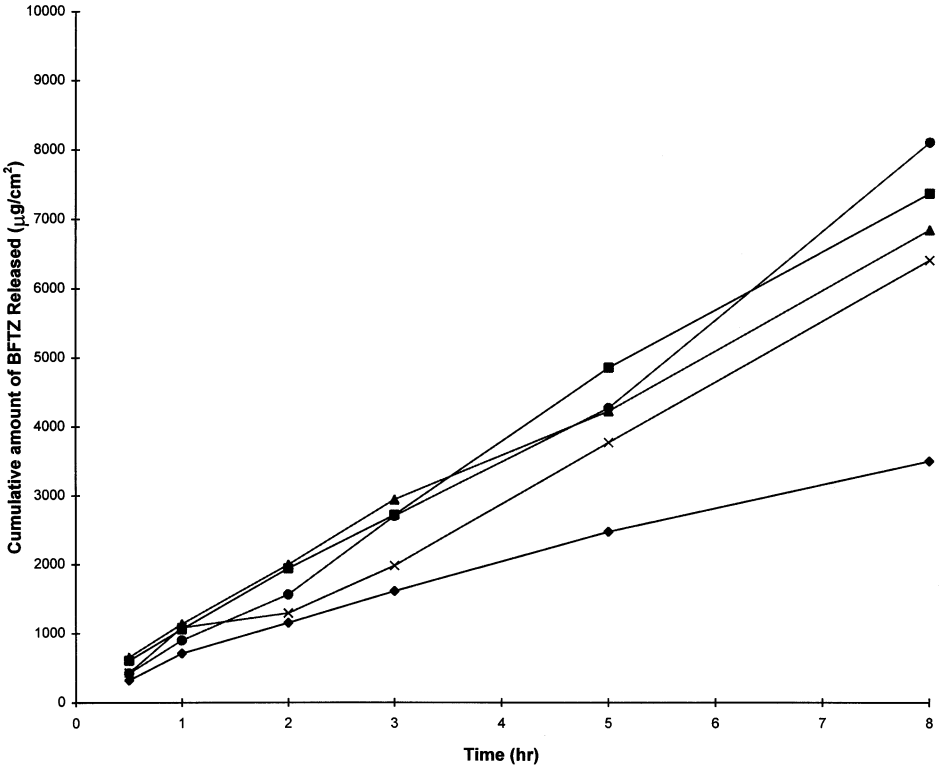

Fig. 7. Cumulative BFTZ release from TTS stored at 50°C: week 0 (◆); week 4 (■); week 8 (▲); week 16 (●); and week 24 (×).

stored at 37 and 50°C is most likely due to 2-pyrol, which is known to slightly darken at temperatures above 43°C. Some samples stored at 5°C were turbid due to the precipitation of drug in the gel.

3.8.2. Drug content

BFTZ was found to be stable and uniformly distributed in the gel when stored at 25, 37 and 50°C. Refrigerated samples (5°C) produced large variation in the drug recovery and sample-to-sample uniformity. This variation may be due to the precipitation of BFTZ in the gel at refrigerated temperatures and its unequal redistribution and dissolution when allowed to stand at room temperature.

3.8.3. Viscosity

The polyacrylic acid gel formulation produces a pseudoplastic behavior under shear. High temperatures (50°C) will shift the curve upwards, indicating an increase in viscosity of the gel matrix (Fig. 6).

3.8.4. BFTZ release

In vitro release of BFTZ from TTS samples stored at 37 and 50°C, was higher at week 4–24 when compared to week 0 (Fig. 7). The difference can be correlated to the increase in viscosity of the gel matrix and its shrinkage within the TTS. This would result in an increase in C_0, which is the initial concentration of BFTZ per unit volume of the gel matrix.

4. Conclusion

In the present study, we evaluated the development of a transdermal gel for BFTZ. We demonstrated that BFTZ can be delivered across excised hairless skin and into the blood stream of the rabbit. The delivery of drug via the lipid and

pore pathways of the skin was enhanced by the vehicle-power effect of the ethanol–pyrol–water system. The rate of percutaneous absorption of drug was attributed to the release properties of the gel and the diffusion across the stratum corneum and not due to the rate-control properties of the polypropylene membrane.

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