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Effect of essential oils as penetration enhancers on percutaneous penetration of furosemide through human cadaver skin

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Abstract

In this study, matrix-type transdermal patches containing furosemide were prepaid using ethylcellulose and polyvinylpyrrolidone by solvent evaporation technique. Effect of polyvinylpyrrolidone and penetration enhancers on the in vitro permeation of drug through human cadaver skin was investigated. Incorporation of essential oils and propylene glycol enhanced the moisture content, moisture uptake capacity and permeation of furosemide across skin barrier. Among the penetration enhancers used, clove oil 10 % w/w and combination of clove oil 10 % w/w + propylene glycol 10 %w/w found to be most effective and give high permeation rate of drug. Stability studies did not show any degradation of the drug. In conclusion, stable and effective furosemide transdermal patches can be prepared using essential oils as penetration enhancers.

Keywords: Transdermal patches, furosemide, essential oils, permeation study.

Introduction

Transdermal delivery of drugs provides many advantages over conventional administration including enhanced efficacy, increased safety, greater convenience, and improved patient compliance. This can avoid the 'peak and valley' effect of oral or injectable therapy and can enable more effective treatment by delivering drugs at a steady rate into bloodstream over an extended period of time. It also reduces the dosage-related side effects because the amount of drug delivered into the biological system in a very controlled manner and avoids first-pass metabolism (Cleary 1984, Kydonieus 1987). This route of administration may be particularly significante in infants and children because of their greater surface area to weight ratio (Sintov et al. 2003). The system designs for transdermal patches include matrix, microresrevoir, reservoir, adhesive and membrane-matrix hybrid. Matrix type transdermal patches remain the most popular as they are easy to manufacture (Mukherjee et al. 2005).

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Furosemide (5-(aminosulfonyl)-4 –chloro-2-[(2 furanylmethyl) amino] benzoic acid) is potent diuretic agent that induces a powerful diuresis, followed by the loss of sodium, potassium and chloride into urine, by acting on thick ascending limb of the loop of Henle (Giebisch 1985). Its usual daily dose for adults is 20-80 mg, while for paediatric use ranges form 1 mg/kg up to a maximum of 40 mg daily. It is commonly used in the treatment of cardiac and pulmonary disorders in premature infants and neonates. The half-life of furosemide is about 2 hr and its bioavailability has been reported to be about 60-70 %. Furosemide is administrated peroral or parentally although its physicochemical and pharmacokinetic characteristics like low molecular weight, lipid solubility, elimination half-life and low melting point are in agreement with the ideal properties of molecule for effective penetration of the stratum corneum (Barry 2001). The furosemide containing Hydroxypropyl methylcellulose gel (Agyralides et al. 2004) and ethylene- vinyl acetate matrix (Cho et al. 2005) for transdermal delivery purposes have been reported. The main objective of this study was to prepare and evaluate the transdermal drug system for furosemide based on ethylcellulose matrix and to study the effect of polyvinyl pyrrolidone and essential oils on the physicochemical and in vitro permeation parameter of furosemide.

Materials and Methods

Materials

Furosemide (Hemdeep Organic Pvt. Ltd., Ankleshwar), Ethyl cellulose-20 cps (Colorcon Pvt Ltd., Goa, India) were received as gift samples. Polyvinyl Pyrrolidone K-30 (S. D. Fine Chem. Ltd., Mumbai), Propylene glycol, (S. D. Fine Chem. Ltd., Mumbai), Di-n-butyl phthalate (Central Drug House Pvt. Ltd., Mumbai), Menthol oil, lemon grass oil, clove oil and Eucalyptus oil (Rajesh Chemical. Co. Mumbai) were purchased. All other reagents were of analytical grade.

Preparation of transdermal films

Matrix type transdermal patches containing furosemide (3.70 mg/ cm^2) were prepared using ethyl cellulose (800 mg) and PVP (50mg) by solvent evaporation technique in petri dish. The polymer was weighed and dissolved in acetone–chloroform (3:2) solvent system and di-n-butyl phthalate (10 %w/w of polymer) was used as a plasticizer. Drug and enhancer were added to the polymer solution. The resultant homogeneous solution was poured into a petri dish. Controlled solvent evaporation was achieved by inverting a funnel over the petri dish for 24 h. The dry films were wrapped in aluminium foil and kept in desiccator until used.

Partition coefficient of drug

The partition coefficient study was performed using n-octanol as oil phase and phosphate buffer pH 8.0 as aqueous phases. The two phases were mixed (1:1) and were saturated with each other on a mechanical water bath shaker at 32 ^{0}C for 24 h. The saturated phase was separated by centrifugation at 2000 rpm. Equal volume of the two phases were taken in conical flask and to each, 100 mg of drug was added. Flask was shaken at 32 ^{0}C for 6 h to achieve a complete partitioning. The two phases were separated and analyzed for drug content spectrophotometerically (UV Pharmaspec-1700, Shimadzu, Japan) at 277.5 nm. The partition coefficient of drug (Ko/w) was calculated using the following expression,

$$Ko/w = \frac{\text{Concentration in octanol}}{\text{Concentration in pH 7.4}}$$

Solubility study

The solubility studies were performed in phosphate buffer pH 8.0 by adding excess amount of drug in each case and keeping on a water bath shaker for 24 h at 32 ^{0}C. After appropriate dilution, solution was analyzed spectrophotometerically at 277.5 nm.

Physicochemical properties of film

Thickness

The thickness of films was determined using micrometer gauge (Mitoyoto, Japan). Each was measured at two different places and the experiment was duplicated.

Drug content analysis

The uniformity of drug distribution was determined by taking known area of two different places of the each film. The films were dissolved in 2 ml of casting solvent and subsequently diluted with phosphate buffer pH 8.0. After appropriate dilution, solutions were analyzed spectrophotometerically for furosemide at 277.5 nm.

Moisture content of films

The prepared films were weighed individually and kept in desiccators containing activated silica at room temperature for 24 h till a constant weight was attained. The percentage of moisture content was calculated as the difference between initial and final weight with respect to final weight (Arora 2002).

Moisture uptake by films

A weighed film kept in desiccator at room temperature for 24 h was taken out and exposed to 84 % RH in a stability chamber (Lab Care, Mumbai) until a constant weight of film was obtained. The percentage moisture uptake was calculated as the difference between final and initial weight with respect to initial weight (Arora 2002).

Folding endurance

This was determined by repeatedly folding the film at the same place until it broke. The number of times the film could be folded at the same place without breaking/craking gave the value of folding endurance (Devi et al. 2003).

In vitro permeation study

Preparation of cadaver skin

Sample of whole adult human skin (40 age) was obtained from breast reduction operation (provided by Navodaya Medical College, Raichur). Subcutaneous fat was carefully trimmed off and then rinsed with normal saline. Skin was wrapped in aluminium foil and kept in plastic bag and stored at -20 °C until used.

Procedure

Permeation studies were performed for different formulations across cadaver skin, using phosphate buffer pH 8.0 as in vitro fluid in receptor compartment of modified diffusion cell at 32 ^{0}C. The whole assembly was kept on magnetic stirrer and the solution was stirred continuously using a magnetic bead. The sample was withdrawn at different time interval and replaced with equal volume of diffusion media. Samples were analyzed in UV spectrophotometer at 277.5 nm.

Fourier transfer infrared spectroscopy

Furosemide and formulations were mixed separately with IR grade KBr in the ratio of 1:3 and corresponding pellets were prepared by applying 5.5 metric ton pressure in hydraulic press. The pellets were scanned over a wave number range of 4000-400 cm^{-1} in FTIR (Shimadzu –8400, Japan) instrument.

Stability studies

The stability studies were conducted according to ICH guidelines by storing the TD films at 40±2^{0}C/75% RH in stability chamber (Lab Care, Mumbai) for 6 months. The samples were withdrawn after 6 months and analyzed for drug content in a UV spectrophotometer at 277.5 nm.

Statistical analysis of data

The results were analyzed by one-way ANOVA with Turkey post *t*-test using Graph Pad Prism software-5 version (Graph Pad software Inc., San Diego, CA, USA).

Table 1. Composition of transdermal films

Formulation	Enhancer
F1	-
FP2	-
FP3	Clove oil 10% w/w
FP4	Lemon grass oil 10% w/w
FP5	Menthol oil 10% w/w
FP6	Eucalyptus oil 10% w/w
FP7	Clove oil 10% w/w+Propylene glycol 10% w/w
FP8	Lemon grass oil 10% w/w+Propylene glycol 10% w/w
FP9	Menthol oil 10% w/w+Propylene glycol 10% w/w
FP10	Eucalyptus oil 10% w/w+Propylene glycol 10% w/w

Result and Discussion

In preliminary studies, the EC films were found to be brittle. The study revealed that, addition of di-n-butyl phthalate at 10 % w/w of polymer produce smooth, uniform and flexible films. Hence, further studies were carried using 10 % of di-n-butyl phthalate in formulations. In order to maintain the sink condition during permeation study, solubility of furosemide was determined in phosphate buffer pH 8.0. Solubility of drug was found to be 6.12 ± 0.6 mg/ml. To learn the partioning of drug between skin and in vitro study fluid (pH 8.0), the partition study were performed in triplicate and logarithmic value was found to be 1.446 ± 0.07. It indicates that drug possesses sufficient lipophilicity for percutaneous transfer.

Thickness

Thickness of film ranged between 0.110 and 0.120 mm. However, incorporation of any of the essential oil and propylene glycol (PG) did not show any significant ($p>0.05$) variation in the thickness of films.

Moisture content studies

The results of moisture content studies of different formulations are shown in Table 2. The moisture content of the menthol oil formulation and menthol oil / PG formulation (FP5 and FP9 respectively) was higher than other formulations. Moisture content in the formulations with

respect to essential oil was found to be in the following order: menthol oil > lemon grass oil> clove oil > eucalyptus oil. Addition of PG further enhanced the moisture content of the formulations relative to those found with films containing only essentials oils.

Moisture uptake studies

Table 2 shows moisture uptake of various formulations. Incorporation of essential oils (10 %) tended to increase the moisture uptake of patches depending on the type of essential oil in the patch. Moisture uptake (%) of menthol oil patches (FP5 and FP9) were more than other formulations containing other essential oils. Moisture uptake in the formulations was found to be in following order: menthol oil> lemon grass oil> clove oil > eucalyptus oil. Addition of PG further enhanced the moisture uptake of the formulations relative to those found with films containing only essentials oils.

Drug content analysis

Drug content for all formulations are shown in Table 2. The drug content of all the formulations was ≥ 98.47 % with low standard deviations (≤1.4%) indicating that the method employed to prepare films in this study was capable of giving films with uniform drug distribution and insignificant batch variability ($p>0.001$).

Folding endurance

Folding endurance of all the film is shown in Table 2. Folding endurance was found to be higher in formulations FP8 and FP9 than other films. Folding endurance of the films with respect to essential oils was found to increase in following order: menthol oil> lemon grass oil> clove oil > eucalyptus oil.

Table 2. Physicochemical properties of transdermal films.

Formulation	Drug content (%)±SD,n=4	Thickness (mm)±SD,n=4	Moisture uptake capacity (%)±SD,n=4	Moisture content (%)±SD,n=4	Folding endurance (No. Of folds), n=4
F1	98.83±0.6	0.101±0.005	1.3±0.3	1.1±0.3	2
FP2	98.47±1.2	0.113±0.0050	2.4±0.2	1.9±1.21	4
FP3	99.20±1.3	0.116±0.0058	3.7±1.5	2.8±1.1	5
FP4	98.54±0.83	0.110±0.0056	5.5±0.1	3.3±0.2	6
FP5	98.83±1.2	0.116±0.016	7.5±0.2	5.5±0.43	8
FP6	99.12±0.71	0.112±0.002	3.5±1.2	2.6±0.5	4
FP7	98.60±0.8	0.117±0.001	6.8±1.1	4.2±1.2	22
FP8	99.61±1.2	0.118±0.001	8.5±0.51	5.2±0.6	24
FP9	98.94±1.4	0.115±0.004	12.11±0.15	8.5±0.2	27
FP10	98.99±0.75	0.120±0.002	5.6±1.11	3.2±1.13	21

In vitro permeation studies

Permeation study was carried out using human cadaver skin as permeation barrier. The permeation profiles of the drug are shown in the Figures 1 and 2. The steady state permeation flux shown in table 2,were determined from the slope of the linear portion of cumulative amount of permeation (Q) verses time (t) plot. Comparison of flux values of F1 and FP2 indicated a significant ($p<0.05$) increase in the permeation of the drug; so addition of PVP shows higher flux compared to formulation without PVP. PVP has antinucleating effect that

converts crystalline drug into higher energy amorphous state with improved solubility, and the enhancement in solubility of drug increases thermodynamic activity that facilitates permeation of drug across the skin. Pyrrolidones are also reported to fluidize the lipids in the stratum corneum and decreasing barrier resistance (Yoneto et al. 1995, Yoneto et al. 1998). Further to improve the permeation of the drug, different essentials oils were added to the formulations (FP3-FP6). Addition of essential oils further increased ($p< 0.05$) the permeation of drug and the flux was higher with clove oil (FP3). For lipophillic drugs terpenes increases in partioning are likely due to bulk solvent effect (Cornwell et al. 1996, Williams 1991). PG in combination with penetration enhancers, found to be highly effective. In our study formulations (FP7- FP10) showed an increase in flux with addition of PG.

Table 3. In vitro flux of furosemide through human cadaver skin.

Formulation	Human Cadaver Skin (mcg cm^2/h) ± SD, n=3	Values of Higuchi model (R^2)
F1	18.75 ± 2.94	0.9991
FP2	40.76 ± 6.93*	0.9660
FP3	184.24 ± 4.83**	0.9914
FP4	154.64 ± 8.11**	0.9639
FP5	116.95 ± 2.63**	0.9643
FP6	66.45 ± 3.53**	0.9935
FP7	218.54 ± 10.28**	0.9829
FP8	180.67 ± 12.20**	0.9913
FP9	155.77 ± 7.89**	0.9627
FP10	100.28 ± 9.02**	0.9807

* ($p<0.05$) when compared with F1
** ($p<0.05$) when compared with FP2

Comparison of flux values of F1 and FP2 indicated significant ($p<0.05$) increase in permeation of the drug with PVP. Similarly, flux value increases significantly ($p<0.05$) with addition of essential oils. Propylene glycol (PG) /terpenes synergy may produce enhanced lipid bilayer disruption (Cornwell et al. 1996). Terpenes have been given the designation of generally recognized as safe (GRAS) by the FDA (Williams 1991). The mechanism by which terpene increase stratum corneum permeability by disrupting intercellular lipid bilayers (Barry 1989, Cornwell 1991). Terpenes, neat or in combination with propylene glycol (PG) or ethanol have been extensively investigated as skin permeation enhancers for hormone and other drugs (Barry, 1993, Kobayashi et al. 1994, Cornwell 1995). However in the present study clove oil (eugenol), lemongrass oil (citral) menthol oil (menthol) and eucalyptus oil (cineole) were effective in enhancing the skin permeation alone and in combination with propylene glycol. Among these formulations FP3 and FP7 were found to show higher flux values than other.

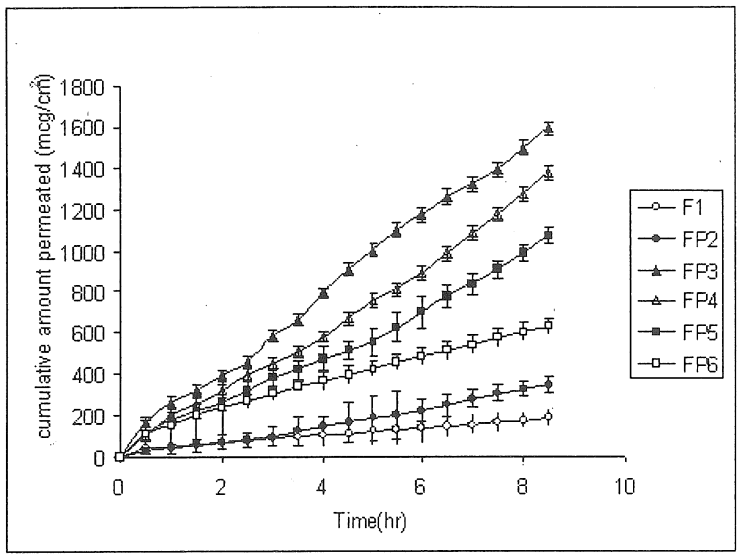

Figure 1. Effect of essential oils on the permeation of furosemide through human cadaver skin.

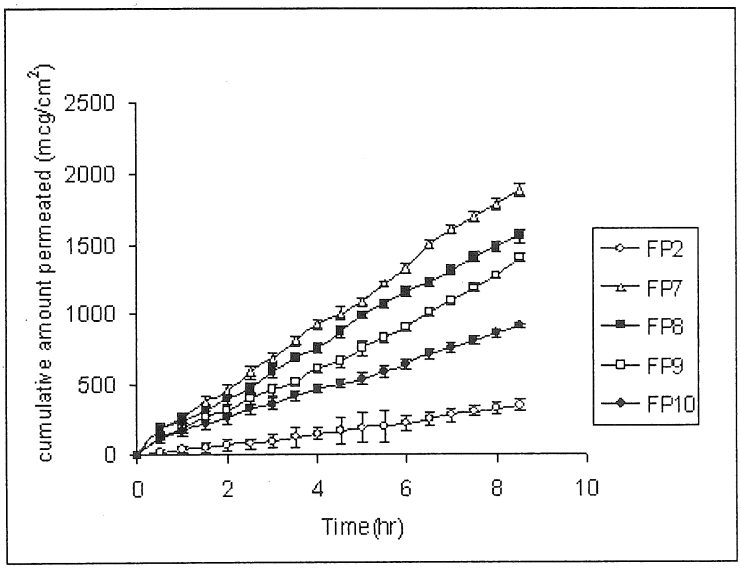

Figure 2. Effect of combination of essential oils with propylene glycol on the permeation of furosemide through human cadaver skin.

Drug-excipient interaction studies

Drug-excipient interactions were studied by FTIR technique. Figure 4 shows the IR spectra of furosemide, drug and polymer, drug, polymer and PVP. IR spectra of drug (A) shows characteristic band at 3351-3397 1/cm (NH_2), 3283.58 1/cm (-NH), 3050 1/cm (aromatic C-H stretching), 2980 1/cm (O-H of COOH group), 2800 1/cm (-CH streching of-CH_2), 1671.20 1/cm (-CO of -COOH), 1582-1451 1/cm (C=C ring streching) and 581 1/cm (CL). From the spectra of different formulations with that of drug implied that all the excipients are compatible

with furosemide. Changes in area of peaks occur simply due to mixing of components without any physical - chemical interactions (Mukherjee et al. 2005).

Analysis of release data

For matrix controlled release systems, the Higuchi equation is described as per (Mehidizadeh et al. 2005).

$$Q = (2ADCs\ t)^{1/2} \ldots\ldots\ldots\ldots (1)$$

Where Q is the cumulative amount of drug released per unit area of the matrix, A is the total drug concentration in the matrix dissolved and undissolved, D is the diffusion coefficient of the drug in the matrix, Cs is the solubility or saturation concentration of the drug in the matrix, and t is time. Table 3 summarizes correlation coefficient of the data analysis. The high correlation (R^2>0.9627) value indicates that release of furosemide from the patches was in compliance with Higuchi diffusion model. This relationship observed in system in which the drug is either fully dissolved or suspended and thus membrane used has no effect on the release kinetics of the drug.

Stability study

The formulations were stored at 40±2°C / 75%RH in stability chamber (Lab-Care, Mumbai) for six months. After six months, formulations were tested for drug content. Drug content of the patches after stability studies was found to be 99.9 % to 99.1 % and did not show any significant variations. These result indicate that drug remain stable after stability studies.

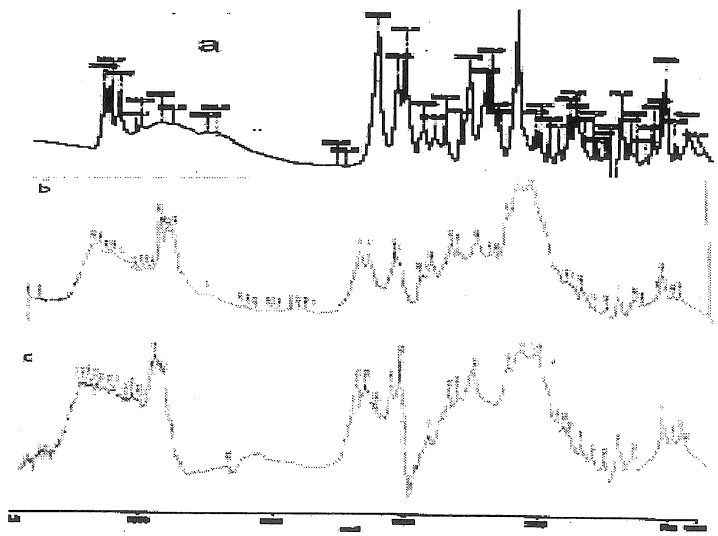

Figure 4. FTIR spectra of (A) Furosemide (B) drug loaded polymeric patch (C) drug loaded polymeric patch with PVP

Conclusion

In conclusion, essential oils can be incorporated in transdermal patches of ethylcellulose containing polyvinylpyrrolidone for improving the permeation of furosemide. Among the

essential oils used, clove oil 10 % w/w and combination of clove oil 10 % w/w + propylene glycol 10 %w/w was more effective in enhancing the in vitro permeation of furosemide. Stable and effective furosemide transdermal patches can be prepared using essential oils as penetration enhancers.

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