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RESEARCH PAPER

Development and In Vitro Evaluation of Diltiazem Hydrochloride Transdermal Patches Based on Povidone–EthylCellulose Matrices

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ABSTRACT

To select a suitable formulation for the development of transdermal drug-delivery system of diltiazem hydrochloride. Transdermal patches of the drug, employing different ratios of polymers, ethylcellulose (EC), and povidone (PVP) were developed and evaluated for the potential drug delivery using depilated freshly excised abdominal mouse skin. The influence of different film compositions on in vitro drug permeation into receptor fluid were studied using a modified Franz diffusion cell. The cumulative amount of drug was found to be proportional to the square root of time, i.e., Higuchi kinetics. From this study, it was concluded that the films composed of povidone:ethylcellulose (1:2) should be selected for the development of transdermal drug-delivery system of diltiazem hydrochloride, using a suitable adhesive layer and backing membrane, for potential therapeutic use.

Key Words: Transdermal patches; Diltiazem hydrochloride.

INTRODUCTION

The strategies used by the pharmaceutical industry for discovery of a potential new drug-delivery system have changed dramatically in recent years. These changes in strategy present new challenges and opportunities for the application of new methodologies in the drug-delivery processes. The study of the controlled release of drug for their extended and safe use has become an important field of this research and drug-delivery to the systemic circulation through the intact skin is of utmost importance in its kind, which is yet to be successfully used in case of a large number of drugs. Formulations on skin can be classified into two categories according to the target site of action of the containing drugs. One has systemic action after drug uptake from the cutaneous microvascular network and the other exhibits local

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effects in the skin.[1] The pharmacological effect of the former formulations are greatly influenced by the penetration of drug molecules through stratum corneum. Analysis of drug-release kinetics through skin, therefore, is important in the assessment of transdermal drug-delivery system, and in vitro skin permeation experiments are valuable and necessary for studying the rate and mechanisms of percutaneous absorption of drugs.[2]

Diltiazem is classified as a calcium channel blocker. It has a mean plasma half-life of 3.5 hr and only 40% of the orally administered drug reaches the circulation due to hepatic metabolism.[3] The present search was directed to examine the permeability of diltiazem hydrochloride from polymeric films through mouse skin into in vitro fluid, and success of this study may provide more insight into the way of bypassing the hepatic first-pass effect of the drug.

The aim of the present study was to develop different transdermal polymeric films with PVP and EC containing the drug diltiazem hydrochloride and to evaluate in vitro release of the drug at a controlled rate to provide a therapeutically effective drug level for a longer period of time from the transdermal patches. This study was further amalgamated with investigation of different physical properties of these patches.

Moreover, here an attempt was made to establish the best possible combination of polymeric ratio to formulate transdermal patches with maximum sustained drug-releasing capability as well as stability in terms of its physical characteristics from the experimental polymeric films.

MATERIALS AND METHODS

Preparation of Films

Films composed of different ratios of EC and PVP were prepared. Dibutyl phthalate (30% w/w of dry weight of polymers) was used as a plasticizer. Backing membrane was prepared by pouring 3 mL of 4% w/v PVA solution into a mold, which was prepared by wrapping aluminium foil over the bottom of a both side open four-walled glass box, and then molds were kept at 60°C for 6 hr. Drug matrix was prepared by dissolving requisite amounts of EC and PVP, as depicted below, in chloroform, which was taken 5% of the weight of the polymer and 30% w/w of the polymer composition; dibutyl phthalate was added. Formulations were RG 1 (PVP:EC, 3:2), RG 2 (PVP:EC, 2:3), RG 3 (PVP:EC, 1:4), RG 4 (PVP:EC, 2:1), RG 5 (PVP:EC, 1:2), and RG 6 (PVP:EC, 1:5) and each contained diltiazem hydrochloride, 5% w/w of the polymers. An accurately weighed amount of diltiazem hydrochloride was homogeneously dispersed in it and it was casted on the backing membrane and dried at 40°C for 2 hr. The dry films were removed and kept in a dessicator until use.

Physical Characteristics of the Prepared Films

The following physical studies were conducted: (i) moisture content, (ii) moisture uptake, and (iii) flatness.

(i) **Moisture content:** The film was weighed and kept in a dessicator containing calcium chloride at 40°C in a drier for 24 hr. Then the film was weighed again and again until it showed a constant weight. The moisture content was the difference between the constant weight taken and the initial weight (Table 1).

(ii) **Moisture uptake:** A weighed film kept in a dessicator at 40°C for 24 hr was taken out and exposed to two different relative humidities of 75% (saturated solution of sodium chloride) and 93% (saturated solution of ammonium hydrogen phosphate) in two different dessicators, respectively, at room temperature. Then the weights were measured periodically to constant weights (Table 2).

(iii) **Flatness:** Longitudinal strips were cut out from the prepared medicated film, the lengths of each strip were measured, and then variation in the lengths due to the nonuniformity in flatness was measured

Table 1. Determination of moisture content of different formulations ($n = 10$).

S. No.	Formulation	PVP:EC	% Increase
1	RG 1	3:2	3.870 ± 0.08[a]
2	RG 2	2:3	3.269 ± 0.19
3	RG 3	1:4	2.019 ± 0.26
4	RG 4	2:1	3.975 ± 0.17
5	RG 5	1:2	2.860 ± 0.98
6	RG 6	1:5	1.928 ± 0.23

[a]Data represents mean ± S.D.

(Table 3). Flatness was calculated by measuring constriction of strips and a zero-percent constriction was considered to be equal to a hundred-percent flatness.

$$\text{Constriction (\%)} = \frac{l_1 - l_2}{l_2} \times 100$$

where l_1 = final length of each strip and l_2 = initial length.

In Vitro Skin Permeation Studies

Modified Franz diffusion cell was used for these studies. Six-to-eight-week-old male Laca mice were sacrificed by cervical dislocation after depilation. The study was conducted in accordance with the Declaration of Helsinki and "Animal Care and Facilities" in *Principles and Methods of Toxicology*.[4] A square section of abdominal skin was excised, the skin was lifted, and then the adhering fat and visceral debris were removed carefully from the surface underneath. Then the skin, facing dermal side upward was tied with a thread on holder. A portion of film was cut out, measured, and placed on the dermal side of the skin in the donor compartment facing the drug matrix side to the skin and backing membrane upward. This holder containing the skin and the formulation was then placed on the reservoir compartment of the diffusion cell containing 20% PEG 400, maintaining 37°C thermostatically, and stirred by magnetic stirrer. The cell was covered with aluminium foil to avoid the problem of drug photosensitivity.

Now samples (5 mL every time) were withdrawn at regular periods and fresh receptor fluid solution was added. Absorbances of samples were read spectrophotometrically at 236.8 nm against blank (20% v/v PEG 400 solution).[5,6] Cumulative amounts of drug per unit area were calculated from the standard curve.

The in vitro drug-release study was conducted using a USP dissolution apparatus[7] in the in vitro receptor media keeping 1 cm^2 of the film. Samples were taken until spectrophotometric reading became constant. Amounts were calculated from the standard curve.

Table 2. Determination of moisture uptake (in wt%) of different formulations ($n = 10$).

S. no.	Formulation	PVP:EC	Relative humidity 75%	Relative humidity 93%
1	RG 1	3:2	8.117 ± 0.18	12.829 ± 0.26[a]
2	RG 2	2:3	7.008 ± 0.16	10.975 ± 0.33
3	RG 3	1:4	6.211 ± 0.43	10.087 ± 0.42
4	RG 4	2:1	8.998 ± 0.90	13.889 ± 0.61
5	RG 5	1:2	6.785 ± 0.66	9.872 ± 0.25
6	RG 6	1:5	6.024 ± 0.49	9.369 ± 0.23

[a]Data represents mean ± S.D.

RESULTS AND DISCUSSION

Release of the drug from transdermal patches is controlled by the chemical properties of drug and delivery form, as well as the physiological and physicochemical properties of the biological membrane.[8] In this study, polymeric films of different combinations of EC and PVP released variable amounts of diltiazem hydrochloride through mouse skin into the in vitro study fluid. The means ($n = 5$) of cumulative amounts of drug released per cm^2 of the film after 24 hr from the preparations RG 1

Table 3. Determination of flatness of different formulations.

S. no.	Formulation	PVP:EC	Film length (of strips)	Amount of constriction in strips	Thickness ($n = 10$)	Flatness
1	RG 1	3:2	1.2 cm	0	52 ± 0.32 μm[a]	100%
2	RG 2	2:3	1.2 cm	0	56 ± 0.18 μm	100%
3	RG 3	1:4	1.2 cm	0	53 ± 0.37 μm	100%
4	RG 4	2:1	1.2 cm	0	67 ± 0.55 μm	100%
5	RG 5	1:2	1.2 cm	0	63 ± 0.29 μm	100%
6	RG 6	1:5	1.2 cm	0	60 ± 0.21 μm	100%

[a]Data represents mean ± S.D.

(PVP:EC, 3:2), RG 2 (PVP:EC, 2:3), RG 3 (PVP:EC, 1:4), RG 4 (PVP:EC, 2:1), RG 5 (PVP:EC, 1:2), and RG 6 (PVP:EC, 1:5) were found to be 3.5358, 4.8723, 4.2790, 5.2951, 3.9117, and 4.9669 μg, respectively. But in general, there were gradual falls in cumulative amounts of drug released after 5–10 hr (as depicted in Fig. 1) and a change in kinetic pattern was noticed. This may be because the amounts of drug released were less than those in about the first 5–10 hr, where we observed that the release pattern had the tendency to go for the first-order kinetics from zero-order kinetics, as it is evident when data were plotted as log concentration against time (Fig. 2). Release of a drug from a transdermal drug delivery system mainly involves factors of diffusion.[9] Diffusion is related to transport of drug from dosage matrices into the in vitro study fluid depending on concentration.[5] As gradient varies, the drug is released, and the distance for diffusion becomes increasingly greater. This could be an explanation why the drug diffuses comparatively at a slower rate as the distance for diffusion increases.

Higuchi developed an equation for the release of a drug from a homogeneous-polymer matrix-type delivery system that indicates the amount of drug released is proportional to the square root of time.[10] If the release of drug from the transdermal film, when plotted against square root of time, shows a straight line, it indicates that the release pattern is obeying Higuchi's kinetics. In our experiments, in vitro release profiles of all the different formulations of transdermal patches did not fit zero-order behavior truly and they could be best expressed by Higuchi's equation (Fig. 3) for the release of drug from a homogeneous-polymer matrix-type delivery system that depends mostly on diffusion characteristics.

Rao and Diwan[11] demonstrated the in vitro releasing patterns of diltiazem hydrochloride and indomethacin from EC–PVP films through rat abdominal skin and they recommended a EC:PVP (4:1) combination for the development of transdermal drug-delivery system for both diltiazem hydrochloride and indomethacin. In our study, however, we observed the EC:PVP (2:1) combination could be one of the choices for the development of transdermal patches of diltiazem hydrochloride. In their study, they varied the amounts of drug in the formulations as well as used rat skin to study the in vitro permeability. In the permeation experiment, repeated experiments with the skin of some species of animals were found to vary release patterns about 20% of each other, and the variation becomes greater when skin from different animals are compared.[12–14] In their study, they established the best in vitro

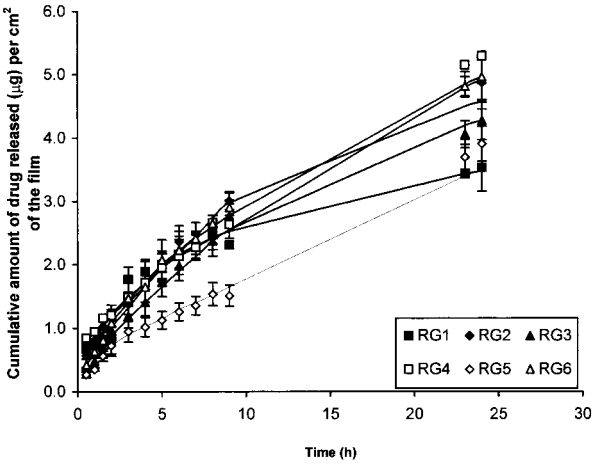

Figure 1. In vitro skin permeation profile of diltiazem hydrochloride incorporated film, RG 1 (PVP 60%, EC 40%), RG 2 (PVP 40%, EC 60%), RG 3 (PVP 20%, EC 80%), RG 4 (PVP 66.67%, EC 33.33%), RG 5 (PVP 33.33%, EC 66.67%), and RG 6 (PVP 16.67%, EC 83.33%), through mouse abdominal skin in 20% PEG 400 in distilled water. Data shows mean ± SEM (where $n = 5$).

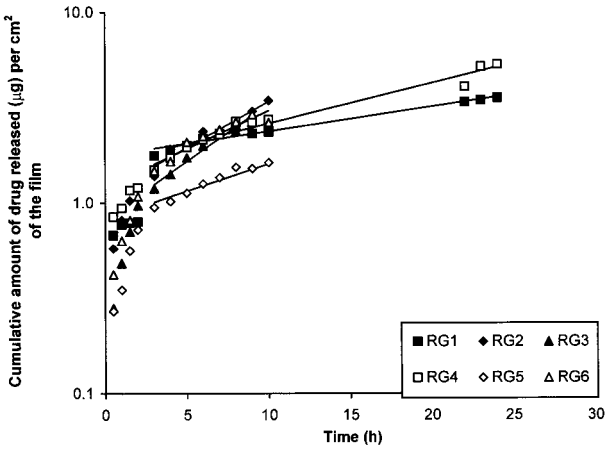

Figure 2. In vitro skin permeation profile of diltiazem hydrochloride incorporated film, RG 1 (PVP 60%, EC 40%), RG 2 (PVP 40%, EC 60%), RG 3 (PVP 20%, EC 80%), RG 4 (PVP 66.67%, EC 33.33%), RG 5 (PVP 33.33%, EC 66.67%), and RG 6 (PVP 16.67%, EC 83.33%), through mouse abdominal skin in 20% PEG 400 in distilled water.

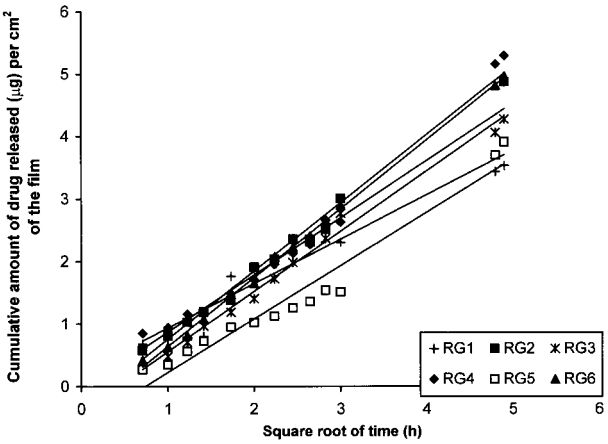

Figure 3. In vitro skin permeation profile of diltiazem hydrochloride incorporated film, RG 1 (PVP 60%, EC 40%), RG 2 (PVP 40%, EC 60%), RG 3 (PVP 20%, EC 80%), RG 4 (PVP 66.67%, EC 33.33%), RG 5 (PVP 33.33%, EC 66.67%), and RG 6 (PVP 16.67%, EC 83.33%), through mouse abdominal skin in 20% PEG 400 in distilled water.

release profile of EC and PVP at a ratio of 4:1, in the case of both of the drugs, and they claimed that an increase in release rate might be due to leaching of hydrophilic fraction. Here it is noticeable that both indomethacin (practically insoluble in water) and diltiazem hydrochloride (freely soluble in water) have different solubilities. So, increase in release rate may not be due to the leaching of hydrophilic fraction, and rather, it may be due to the nonhomogenous dispersion of drugs in the polymer matrix. Thus, the dissolution of hydrophilic fraction (where accumulation of drug in both the cases might be due more to heterogenous dispersion) released the drug at an increased rate. In our study, the average drug content per square centimeter of film indicated that the drug was homogenously dispersed (Table 4).

Again, selection of study fluid is also important for in vitro studies related to transdermal drug-delivery system. Biphasic characteristics of the study fluid is desirable as the diffusion of the drug molecules through skin is enrouted through both aqueous and nonaqueous heterogenous media. PEG 400 and water or normal saline are commonly chosen to provide biphasic characteristics of the liquid.[4] PEG 400 is considered a noninteracting solvent for receptor media.[6]

Instead, only water or buffer does not provide the biphasic action of the solvent on the skin barrier and may definitely change some hydrodynamicity of the in vitro system. Thus, mass flow of water and heterogenous dispersions of drugs more partitioning to the PVP layer might cause such an increase in the release rate of drugs from PVP–EC membranes.

Table 4. Determination of drug concentration in in vitro drug-release study.

S. no.	Formulation	Average drug concentration ($\mu g/cm^2$) ($n = 10$)
1	RG 1	6.3 ± 0.28[a]
2	RG 2	6.1 ± 0.63
3	RG 3	6.5 ± 0.15
4	RG 4	6.2 ± 0.42
5	RG 5	6.3 ± 0.09
6	RG 6	6.5 ± 10.16

[a]Data represents mean ± S.D.

Physical studies conducted on different polymeric films containing diltiazem hydrochloride favored the combination of these polymers for preparation of transdermal patches, and 100% flatness of all the formulations indicate (Table 3) no amount of constriction in formulated transdermal membrane strips. Thus, this could better maintain a smooth surface when it is applied onto skin. Moisture content and moisture uptake (Tables 1 and 2) were found to increase with the increase of hydrophilic polymer, PVP. Significant changes in properties like reduced crushing strength, increased total porosity, and increased pore diameter on hydrophilic polymer containing polymer matrix due to water uptake were reported.[15] Moisture content in our preparations were found to be low and it varies very little in formulations. This little moisture content helps the formulations stable from preventing a completely dried, brittle product. Low moisture uptake also protects the material from microbial contamination and bulkiness of the patches.

When the average rate constants (Fig. 4) ($n = 5$) were studied, it was observed that the formulations RG 1 (PVP:EC, 3:2), RG 4 (PVP:EC, 2:1), and RG 5 (PVP:EC, 1:2) showed comparatively lower rates of release of the drug amongst the formulations studied. Considering the factors of sustained release properties, these three formulations could be the choice for manufacturing the transdermal patches of diltiazem hydrochloride with EC and PVP polymers. But when these three formulations were compared individually, it was observed that the

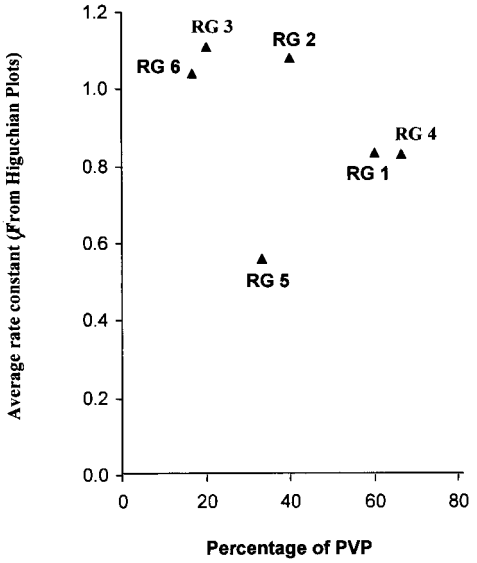

Figure 4. Average rate constant (from Higuchian plots) against percentage of PVP ($n = 5$).

formulation RG 5 had a much more satisfactory release profile towards zero-order kinetics along with the slowest release rate of drug of all the formulations. Thus, formulation RG 5 (PVP:EC, 1:2) was found to be the best choice of formulations studied for manufacturing transdermal patches of diltiazem hydrochloride with a polymeric combination of PVP and EC.

CONCLUSION

From this study, it can be reasonably concluded that diltiazem hydrochloride can be formulated into transdermal polymeric films to prolong its release characteristics and formulation RG 5 (PVP:EC, 1:2) was found to be best choice for manufacturing transdermal patches of diltiazem hydrochloride with a polymeric combination of PVP and EC. Again, it may be used for further pharmacokinetic and pharmacodynamic studies in suitable animal models.

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REFERENCES

1. Yanagimoto, G.; Hayasjo, T.; Seki, T.; Juni, K.; Sagibayashi, K.; Morimoto, Y. A new in-situ experimental method to evaluate the cutaneous disposition of flurbiprofen after topical application in hairless rats. Pharm. Pharmacol. Commun. **1998**, *9*, 261–265.
2. Singh, P.; Roberts, M.S. Skin permeability and local tissue concentrations of nonsterioidal antiinflammatory drugs after topical application. J. Pharmacol. Expt. Ther. **1994**, *268*, 144–151.
3. Mazzo, D.J.; Obetz, C.L.; Shuster, J. *Analytical Profiles of Drug Substances*; Brittain, H.G., Ed.; Academic Press: San Diego, 1994; 53.
4. Burger, G.T.; Cheryl Miller, L. Animal care and facilities. In *Principles and Methods of Toxicology*, 2nd Ed.; Wallace Hayes, A., Ed.; Raven Press Ltd.: New York, 1989; 521–552.
5. Chien, Y.W.; Valia, K.H. Development of a dynamic skin permeation system for long term studies. Drug Dev. Ind. Pharm. **1984**, *10*, 575–599.
6. Sarpotdar, P.P.; Gaskill, J.L.; Giannini, R.P. Effect of polyethelene glycol 400 on the penetration of drugs through human cadaver skin in vitro. J. Pharm. Sci. **1986**, *75*, 26–28.
7. The United States Pharmacopeia, 23rd Ed.; In *The United States Pharmacopeial Convention*; Rockville, MD, 1995; 1796.
8. Chien, Y.W. Development of transdermal drug delivery system. Drug. Dev. Ind. Pharm. **1987**, *13*, 589–651.
9. Tojo, K.; Valia, K.H.; Chotani, G.; Chien, Y.W. Longterm permeation kinetics of estradiol iv. A theoretical approach to simultaneous skin permeation and bioconversion of estradiol esters. Drug Dev. Ind. Pharm. **1985**, *11*, 1175–1193.
10. Chien, Y.W. *Controlled Drug Delivery Fundamentals and Application*, 2nd Ed.; Robinson, J.R., Lee, V.H.L., Eds.; Marcel Dekker, Inc.: New York, 1987; 39–55.
11. Rao, P.R.; Diwan, P.V. Formulation and in vitro evaluation of polymeric films of diltiazem hydrochloride and indomethacin for transdermal administration. Drug Dev. Ind. Pharm. **1998**, *24*, 327–336.
12. Bartek, M.J.; La Budde, J.A.; Maibach, H.I. Skin permeability in vivo: Comparison in rat, rabbit, pig and man. J. Invest. Dermatol. **1972**, *58*, 114–123.

13. Hunziker, N.; Feldmann, R.J.; Maibach, H.I. Animal models of percutaneous penetration: Comparison between Mexican hairless dogs and man. Dermatologica **1978**, *156*, 79–88.
14. Tragear, R.T. *Physical Functions of Skin*; Academic Press: New York, 1966; 23–46.
15. Mosquera, M.J.; Cal, S.; Souto, C.; Concheiro, A.; Martinez-Pacheco, R.; Gomez-Amoz, J.L. Effects of storage humidity on the mechanical, microstructural and drug release properties of hydroxypropyl cellulose-based hydrophilic matrix tablets. Drug Dev. Ind. Pharm. **1997**, *23*, 403–406.