RESEARCH ARTICLE

Preparation of transdermal monolithic systems of indapamide by solvent casting method and the use of vegetable oils as permeation enhancer

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Transdermal drug delivery systems of indapamide have been formulated by using solvent casting method. Monolithic systems were prepared by using hydroxy propyl methyl cellulose (HPMC) and ethyl cellulose (EC) polymers by incorporating glycerine and dibutyl phthalate as plasticizers, respectively. All the patches were uniform with respect to physicochemical and scanning electron microscopy (SEM) evaluation. The *in vitro* drug release studies indicated that HPMC containing films have shown better release than that of EC containing films without any permeation enhancer. A total of eight monolithic systems were prepared by using a drug polymer ratio of 1:4 and incorporated different vegetable oils as permeation enhancers in different concentrations. The prepared systems released the drug in the following order: F3 > F4 > F7 > F5 > F8 > F6 > F1 > F2. The various permeation parameters such as flux, permeability coefficient, enhancement ratio and diffusion rate constants were determined for all the formulations. The maximum flux of 9.08×10^2 mg/cm^2 h was observed with HPMC monolithic system containing 30% w/w olive oil. A significant improvement of flux was observed in the following order: olive oil > linseed oil > sunflower oil > cottonseed oil > coconut oil > castor oil. Further improvement of flux was observed, when 30% w/w olive oil was applied directly onto the skin prior to the studies. The *in vitro* release studies revealed that the release was sustained up to 24 h and it follows zero-order kinetics. All the films were found to be stable at 37°C and 45°C with respect to their physical parameters and drug content.

Key words: EC, HPMC, linseed oil, olive oil, permeation enhancers, sunflower oil, transdermal

INTRODUCTION

There has been increased interest and challenges in the delivery of an active ingredient through the skin. Transdermal drug delivery systems are a class of novel drug delivery systems, which are gaining worldwide accolade, as evidenced by the numerous scientific documents being published. Indapamide is a long-acting hypertensive with both diuretic and vasodilative actions and is defined by the 1999 WHO/ISH Hypertension Guidelines and JNC VII as a first-line drug for the treatment of hypertension. This anti-hypertensive action is maximal at a dose of 2.5 mg/day, and the diuretic effect is slight, usually without clinical manifestation. At higher doses, the diuretic effect becomes more prominent. The extra-renal anti-hypertensive action of 2.5 mg/day is demonstrated as a reduction in vascular hyperactivity and a reduction in total peripheral and arteriolar resistance. Indapamide is marketed as immediate release pharmaceutical formulations containing 1.25 and 2.5 mg active substance per dose and as sustained-release coated tablets of 1.5 mg per dose. However, the oral delivery of this drug has certain disadvantages such as frequent administration and adverse drug reactions. Additionally, since indapamide is usually intended to be taken for a long period, patient compliance is also very important. Transdermal drug delivery offers many advantages such as reduced side effects, less frequent administration to produce the desired constant plasma concentrations associated with improved patient compliance, elimination of the first-pass effect, sustained drug delivery and interruption of treatment when necessary.[1,2]

MATERIALS AND METHODS

Materials

Indapamide was received as a gift sample from Sun Pharmaceuticals Ltd., Baroda, India. HPMC and EC were supplied by Reliance Cellulose Products Limited, Hyderabad, India. Other materials used in the study were of analytical grade.

Method of Preparation of Monolithic Transdermal Systems

The HPMC and EC films were prepared by solvent casting method using mercury substrate and evaluated for various parameters.[3] Monolithic transdermal systems of HPMC and EC were prepared according to the formulae shown in Table 1. The drug: polymer ratio was used in all the formulations. The solutions were stirred for 20 min using a magnetic stirrer. Glycerine and dibutyl phthalate were

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Table 1: Detailed formula of different monolithic transdermal systems containing indapamide

Ingredients (mg/ml)	Formulation codes								
	F1	F2	F3	F4	F5	F6	F7	F8	*F9
Indapamide I(mg)	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
HPMC(mg)	200	-	200	200	200	200	200	200	200
EC (mg)	-	200	-	-	-	-	-	-	-
Dibutyl phthalate(ml)	-	0.067	-	-	-	-	-	-	-
Glycerine (ml)	0.055	-	0.055	0.055	0.055	0.055	0.055	0.055	0.055
Olive oil (ml)	-	-	0.016	-	-	-	-	-	0.0165
Linseed oil (ml)	-	-	-	0.016	-	-	-	-	-
Castor oil (ml)	-	-	-	-	0.016	-	-	-	-
Sunflower oil(ml)	-	-	-	-	-	0.016	-	-	-
Coconut oil (ml)	-	-	-	-	-	-	0.015	-	-
Cottonseed oil (ml)	-	-	-	-	-	-	-	0.016	-
Alcohol (ml)	2	2	2	2	2	2	2	2	2
Dichloromethane (ml)	1	1	1	1	1	1	1	1	1
Chloroform (ml)	2	2	2	2	2	2	2	2	2

*Permeation enhancer was applied directly on to the skin 10 min before experiment. The above formula gave a total area of 19.63 cm^2 for each

used as plasticizers for HPMC and EC films, respectively. A specific quantity of the drug was dissolved in alcohol and then added to respective polymer solution. The solutions were stirred and poured within a circular piece of glass with a diameter of 5 cm placed on the surface of mercury contained in a petridish. The rate of evaporation of the solvent was controlled by inverting cut funnel over the petridish. After 24 h, the dried films were taken out and stored in a desiccator. Films F3, F4, F5, F6, F7 and F8 contained olive oil, linseed oil, castor oil sunflower oil, coconut oil and cottonseed oil, respectively, in different concentrations as permeation enhancers. The films were prepared by incorporating them along with a plasticizer.[4] In all the cases, 30% w/w concentration of permeation enhancer has shown a good release; hence 30% w/w concentration of permeation enhancer alone was used in the further studies.

Physicochemical Evaluation

Physical appearance, weight uniformity and thickness uniformity

All the transdermal films were visually inspected for colour, clarity, flexibility and smoothness. For weight uniformity, the dried films were weighed on Afcoset digital balance. The average of five observations was taken in each batch. Film thickness was measured by a screw gauge at five different random points on the film.[5]

Water Vapour Absorption and Transmission Studies

The water vapour absorption (WVA) and water vapour transmission (WVT) were calculated by taking the differences in the weight of the film before and after the study at regular intervals of 24 h for a total period of seven days. For the determination of water vapour absorption studies of polymer films, 3.14 cm^2 areas was taken and weighed accurately and then placed on a wire gauge, which was kept in a desiccator containing a saturated solution of potassium bromide (200 ml). The humidity was found to be 84% RH. The films were taken out and weighed after 1, 2, 3, 4, 5, 6, and 7 days of storage.[6] Glass vials of equal diameter were used as transmission cells. Approximately 1 gm of fused calcium chloride was taken in the cells and film of area equivalent to brim of vial (1.36 cm^{-2}) was fixed with the help of an adhesive. Then, all the cells were weighed accurately and kept in a saturated solution of potassium bromide (200ml). The humidity was found to be 84% RH. The cells were taken out and weighed after 1, 2, 3, 4, 5, 6, and 7 days of storage.[7]

Drug Content Uniformity

Transdermal films with an area of 1 cm^2 was cut into small pieces and transferred into 100 ml buffer (pH 7.4) and shaken for 4 h to extract the drug.[8] Finally, suitable dilutions were made using a buffer with a pH of 7.4, and the absorbance was measured by HPLC with reference to a calibration curve after appropriate dilution with methanol when necessary. The column was maintained at 40°C and the flow rate was 1 ml/min while the UV detector was set at 240 nm.

Folding Endurance

Folding endurance 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 was the folding endurance value [Table 2]

***In Vitro* Permeation Across the Rat Abdominal Skin**

Preparation of the rat skin

The Swiss albino rats with a weight range of 170-190 gm were decapitated. The abdominal skin of excised hairless rat skin was separated along the epidermal junction, and it was kept in the water bath, which was maintained at 60°C for 50 s. The heat-treated skin was cleared of its subcutaneous fatty substances and immediately kept in normal saline solution for flattening and smoothening.[9]

Permeation Studies

Vertically assembled Keshary-Chien diffusion cells having

Table 2: Physical parameters and drug content of the transdermal systems.

Batch codes	Physical appearance	*Weight (mg)	*Thickness (mm)	**Drug content	WVA rate constant (gm/24 h/cm²)	WVT rate constant (gm/24 h/cm²)	Folding endurance
F1	++	253.4(0.41)	0.154 (0.0049)	96.97 (0.38)	1.76×10^{-4}	4.432×10^{-3}	80 ± 5
F2	++	283.1(0.42)	0.144 (0.0048)	98.96 (0.16)	1.96×10^{-4}	4.526×10^{-3}	98 ± 1.8
F3	++	257.9(0.36)	0.149 (0.0057)	97.30 (0.37)	1.09×10^{-4}	2.298×10^{-3}	124 ± 1.4
F4	++	259.6(0.32)	0.147 (0.0043)	97.58 (0.21)	2.01×10^{-4}	4.386×10^{-3}	104 ± 1.6
F5	++	262.3 (0.46)	0.151 (0.0051)	97.03 (0.33)	2.04×10^{-4}	4.359×10^{-3}	51 ± 2.5
F6	++	258.8(0.34)	0.158 (0.0054)	97.65 (0.26)	1.93×10^{-4}	4.009×10^{-3}	121 ± 4
F7	++	264.7(0.38)	0.163 (0.0063)	98.13 (0.25)	1.99×10^{-4}	4.114×10^{-3}	86 ± 1.9
F8	++	259.6(0.40)	0.154 (0.0049)	97.18 (0.18)	1.98×10^{-4}	4.508×10^{-3}	92 ± 2.4
F9	++	253.4(0.41)	0.154 (0.0049)	96.97 (0.38)	2.00×10^{-4}	4.368×10^{-3}	88 ± 2.8

The figure inside the parenthesis denotes the standard deviation values; * Average of five observations; ** Average of three observations; ++ Satisfactory

Table 3: Diffusion rate, permeability coefficient, flux, enhancement ratio and permeability rate of transdermal monolithic systems. Comparative kinetic values of drug release from transdermal monolithic systems

Batch codes	Diffusion rate mg/h	Permeability coefficient cm/h	Flux mg/cm².h rate mg/h·cm	Enhancement ratio	Permeability	Zero order equation		Higuchi's equation	
						N	R	N	R
F1	0.189	6.49×10^{-3}	5.50×10^{-2}	-	9.35×10^{-4}	2.22	0.9971	10.63	0.92
F2	0.132	4.42×10^{-3}	3.84×10^{-2}	-	6.51×10^{-4}	1.52	0.9975	7.55	0.96
F3	0.311	10.7×10^{-3}	9.08×10^{-2}	1.65	15.45×10^{-4}	3.66	0.9963	18.29	0.96
F4	0.290	9.9×10^{-3}	8.45×10^{-2}	1.54	14.37×10^{-4}	3.39	0.9977	16.89	0.96
F5	0.220	7.6×10^{-3}	6.42×10^{-2}	1.17	10.91×10^{-4}	2.59	0.9909	13.17	0.98
F6	0.277	9.4×10^{-3}	8.07×10^{-2}	1.47	13.72×10^{-4}	3.24	0.9932	16.35	0.978
F7	0.253	8.7×10^{-3}	7.45×10^{-2}	1.35	12.66×10^{-4}	2.97	0.9909	15.11	0.9792
F8	0.273	9.3×10^{-3}	7.88×10^{-2}	1.43	13.40×10^{-4}	3.18	0.9945	15.99	0.9742
F9	0.325	11.2×10^{-3}	9.47×10^{-2}	1.72	16.10×10^{-4}	3.83	0.9829	19.76	0.9865

a down stream volume of 50 ml was used. The obtained skin was mounted on the diffusion cell, and the receiver compartment was filled with 50 ml phosphate buffer of pH 7.4 and the temperature was maintained at 37°C. The samples were withdrawn every hour (replaced with 1 ml of fresh buffer to maintain sink condition) and their concentrations were measured by HPLC.[10]

Stability Studies

All the films (F1-F8) were exposed to two selected temperatures of 37°C and 45°C in two different hot air ovens. Transdermal films with an area of 19.63 cm^2 were kept in the oven for a period of 4 weeks. The film sample with an area of 1 cm^2 was cut from each formulation, and it was analyzed for the drug content at the end of every week. The average of triplicate readings was taken.[11-13]

SEM (Scanning Electron Microscopy)

The morphology of the selected film F3 was characterized by scanning electron microscopy to determine the drug distribution in the film at magnification of 2.51 Kx.[14]

RESULTS AND DISCUSSION

A total of 9 formulations were prepared using HPMC and EC polymers as per formulae given in Table 1.All the films were evaluated for their physical parameters, and they were found to be flexible, smooth and transparent. They were also found to be uniform in their weight and thickness with low SD values, as shown in Table 2. The rate of WVA from all monolithic systems was determined and they followed the order: F4 > F3 > F8 > F6 > F7 > F1 > F5 > F2. A graph was constructed by taking WVA versus time in days, which showed that there was an increase in WVA at initial days (1-3 days); further, it was found that a steady state was maintained. Among all the formulations, HPMC containing films showed good WVA than that of EC films. Later, the WVT studies for all monolithic formulations were conducted, which indicates that all the formulations from F1 to F8 were permeable to water vapour. The order of rate of WVT from all monolithic systems was as follows: F1 > F7 > F3 > F8 > F4 > F6 > F5 > F2 [Table 2]. It was observed that there was a gradual increase in WVT for first 7 days at regular intervals of 24 h and it followed zero-order kinetics. Among all the formulations, HPMC containing films showed good WVT than that of EC films. The results revealed that the drug content was almost uniform in all the films with low SD values. The purpose of this study was to investigate the *in vitro* release profile of indapamide from two different polymers and then permeation enhancers were added to the polymeric system, which showed better release. The influence of permeation enhancers on drug release was studied when it is applied directly on skin 10 min before the actual studies. The *in vitro* release of drug across rat skin from HPMC and EC films showed only 53.63% (F1) and 36.50% (F2) at the end of 24 h, respectively [Table 3, Fig. 1].

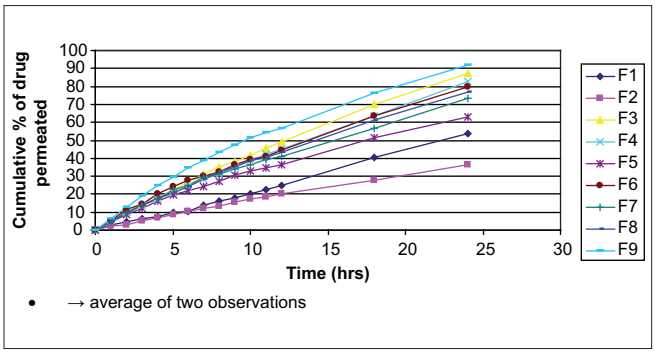

Figure 1: *In vitro* drug release across rat abdominal skin for monolithic systems

The flux was calculated from the slope of linear graph, and it was found to be 5.50×10^{-2} and 3.84×10^{-2} mg/cm^2·h, diffusion rate constant was 0.189 and 0.132 mg/h respectively. A poor drug release might be attributed to a tough barrier: the stratum corneum of skin contributes to low diffusivity. It was evident from the above result that there was a lower flux and lower diffusion rate through the rat skin. Hence, a permeation enhancer must be incorporated in the system. The HPMC film gave better release than EC film; therefore, the HPMC film was selected for incorporation of various vegetable oils as permeation enhancers. In the later studies, the effect of permeation enhancer on the release of the drug from different monolithic systems was conducted. Six vegetable oils were selected and used in various concentrations of 10, 20 and 30% w/w. Among various concentrations, 30% w/w concentration showed good release from F1 in all the cases. Hence, the same concentration was used in the subsequent experiments.

To know the mechanism of drug release, the data was subjected to various kinetic studies. (1) Cumulative % of drug permeated versus time. (2) Cumulative % of drug permeated versus square root of time. The kinetic values of drug release were determined using P-STAT package. The diffusion rate and permeability coefficient were calculated and tabulated [Table 3].

In Vitro Drug Release from Monolithic Systems

The drug release from HPMC (F1) and EC films (F2) without any permeation enhancer are found to 53.63% and 36.50%, respectively. The results indicated that HPMC film has shown better release than that of EC film, which may attributed to high water vapour permeability of HPMC film and hydrophobic nature of EC. The flux, permeability coefficient, permeability rate and diffusion rate were high for HPMC film than that of EC film. Hence it was decided to incorporate permeation enhancers in the HPMC monolithic system for better release. The drug release profiles of F3, F4, F5, F6, F7 and F8 were 87.47, 82.41, 63.08, 79.84, 73.34 and 76.84%, respectively at the end of 24 h. Among the systems, film containing 30% w/w olive oil in HPMC polymer (F3) has shown maximum release than that of systems containing other vegetable oils as permeation enhancers. The flux, diffusion rate, permeability coefficient and permeability rate were compared. The results indicated that F3 exhibited good flux and permeation than that of other systems, which may be due to high percentage of oleic acid (83.5%) present in olive oil.[15-17] The selected permeation enhancer (olive oil, 30% w/w) was applied directly onto the skin 10 min before the study (F9) and was compared with the release shown by F3 in which the permeation enhancer was added in the formulation itself. 92.1% release was observed in case of F9 as compared to 87.47% in case of F3. This might be due to the reason that in F3, the permeation enhancer has to pass through the monolithic system and then contact the stratum corneum of skin, where it temporarily disturbs the lipid bilayer of the skin. However, in F9, the permeation enhancer was in direct contact with skin. The drug release profiles of all monolithic systems were fairly linear with their correlation coefficients between 0.9829 and 0.9977. To know the mechanism of drug release from all the monolithic systems, the data was plotted according to Higuchi's equation. The plots were linear with their correlation coefficients between 0.9279 and 0.9865. The results confirmed that, the mechanism of drug release all the monolithic systems was diffusion controlled. In order to get linear plots, the data was subjected to the regression analysis. Lastly, the enhancement ratio was calculated by dividing the flux of formulation with permeation enhancer by flux of formulation without permeation enhancer. The order of enhancement ratio was found to be: F9 > F3 > F4 > F6 > F8 > F7 > F5. They were observed for the changes in colour, appearance, flexibility and drug content at a regular interval of one week for one month. All the films were stable at 37°C and at 45°C with respect to their physical parameters and drug content [Figs. 2 and 3]. [Fig. 4] showed that the drug was uniformly distributed in the selected formulation (F3).

CONCLUSION

Thin, flexible, smooth and transparent films were obtained with HPMC and EC polymers using glycerine and dibutyl phthalate as plasticizers. Thickness, weight and drug contents of all the formulations remained uniform with low SD values. All the systems were permeable to water vapour at 84% RH and followed zero-order kinetics. All the monolithic systems containing HPMC polymer showed good release than that of EC systems. The monolithic systems were found to be stable at 37°C and 45°C. SEM

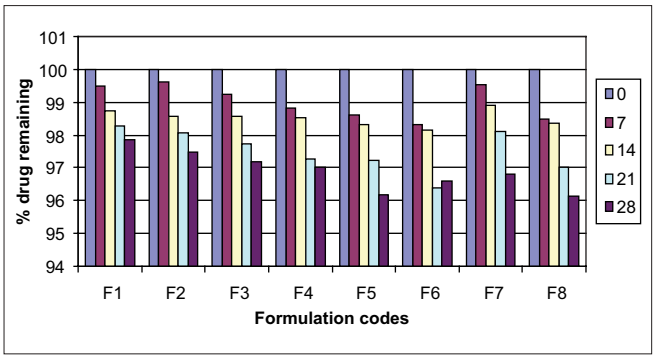

Figure 2: Stability studies of monolithic systems at 37°C

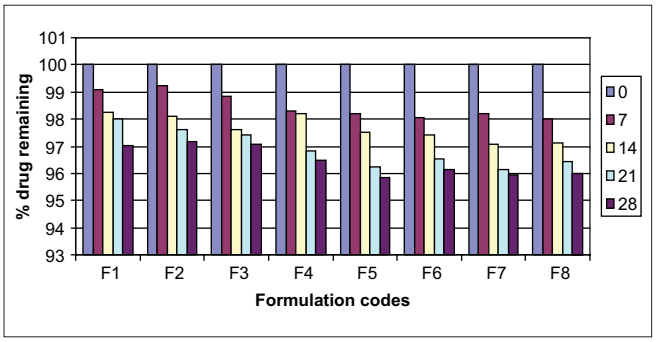

Figure 3: Stability studies of monolithic systems at 45°C

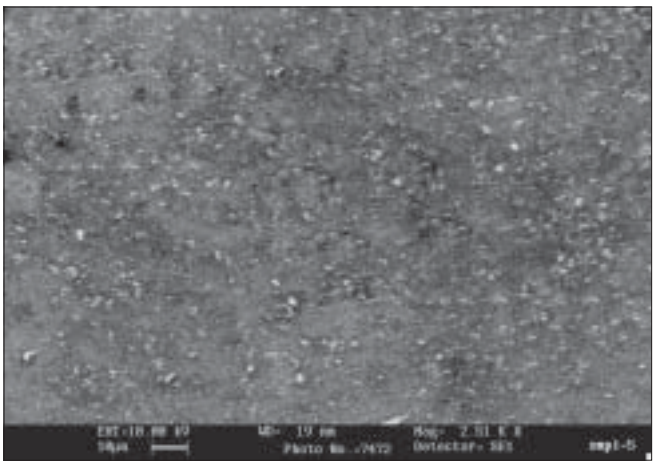

Figure 4: SEM photograph of F3 formulation

studies confirmed that there was uniform distribution of drug in the selected film. Studies have shown promising results; hence, there is a scope for further pharmacodynamic and pharmacokinetic evaluation.

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