PREPARATION, *IN-VITRO* AND *IN-VIVO* CHARACTERIZATION OF TRANSDERMAL PATCH CONTAINING GLIBENCLAMIDE AND ATENOLOL: A COMBINATIONAL APPROACH

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ABSTRACT

A novel aspiration in treatment of chronic disease like diabetes associated with other non communicable disease risk factors, such as hypertension is to provide greater therapeutic effect, overcome the side effects by complex therapeutic regimen and to improve patient compliance upon administering combinational transdermal delivery of Glibenclamide (G) and Atenolol (A) which have not been tested literally. Hence, the present study was designed to develop a transdermal patch containing Glibenclamide and Atenolol using blends of different polymeric combinations such as Hydroxy propyl methyl cellulose (HPMC), Poly vinyl pyrolidone (PVP) and Carbopol (CP). The patches were subjected to physicochemical parameters, *in-vitro* and *in-vivo* drug release and *in-vitro* skin permeation studies. Good results were obtained in all the evaluated parameters. The drug release of all formulation follows zero order kinetics by diffusion mechanism of non fickian diffusion type. *In-vitro* transdermal permeation studies by using rat & goat skin and finally *in-vivo* studies by using rabbits were carried out for the optimized formulation (GA4 HPMC 1%, PVP 0.5%, CP 0.5%). The developed transdermal delivery system containing Glibenclamide & Atenolol might be a milestone in the combinational therapy of diabetes and hypertension.

Keywords: Transdermal patches, glibenclamide, atenolol, diabetes, hypertension.

INTRODUCTION

Many of the initial goals for transdermal drug delivery have been selectively achieved with currently marketed products, such as providing a convenient, painless method of drug delivery, improving patient compliance, reducing adverse delivery, reducing adverse effects and maintaining more consistent and prolonged blood levels than those achieved with oral or parenteral dosing. The technology was quickly accepted by patients and clinicians alike, and patches were viewed as a desirable platform for a variety of therapeutic uses, including motion sickness, hypertension, and angina, hormone therapy, smoking cessation and pain control (Robert 2006).

According to the American Heart Association, it is possible to say that diabetes mellitus is a cardiovascular disease (Grundy *et al.,* 1999). One reason for this description is that the major adverse outcomes of diabetes mellitus are a result of vascular complications, both, at the micro vascular and macro vascular levels (Grundy *et al.,* 1998). Furthermore, these vascular complications are augmented by the co-existence of hypertension (Epstein *et al.,* 1992). So, treatment should not only target lowering of blood glucose level, but should also focus on the correction of other non communicable disease risk factors, such as hypertension.

Patients with these conditions often take multiple medications. Some studies have suggested that patients fail to adhere to the medical regimen because of the number of medicines required (Kroenke *et al.,* 1991). The more medications prescribed, the fewer patients adhere to the full treatment regimen. In contrast, the total number of medications taken per day could be reduced if both the combinations were given in a single formulation.

Glibenclamide (G) is a popular anti-diabetic drug, belonging to the class of sulfonylurea. The drug is widely used for treating type II diabetes. The most frequently reported side effects are Gastric disturbances like nausea, vomiting, anorexia and increased appetite after oral therapy. Since these drugs are usually intended to take for a long period, patient compliance is very important (Srinivas mutalik *et al.,* 2005).

Atenolol (A), a β-blocker, is prescribed widely in diverse cardiovascular diseases, such as hypertension, angina pectoris, arrhythmias, and myocardial infarction. Administration of conventional tablets of atenolol has been reported to exhibit fluctuations in the plasma drug levels, resulting in either manifestation of side effects or reduction in drug concentration at the receptor site

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(Bhupinder Singh *et al.,* 2006). Diabetic patients may experience greater cardio protection with β-blockers than do nondiabetic patients **(**Vivian Fonseca *et al.,* 2008).

In this work it is designed to develop 24 hours transdermal therapeutic system of G and A with the following objectives to overcome gastrointestinal incompatibility and cardiac adverse effects, to avoid hepatic first pass metabolism (Clemett *et al.,* 2000) to reduce the frequency of administration, overcome the side effects, simplify treatment regimen and to obtain greater therapeutic efficacy to improve patient compliance.

MATERIALS AND METHODS

Glibenclamide obtained from Sri Raghavendra Chemicals and Suppliers, Bangalore. Atenolol obtained from Indian Drugs, Hyderabad. Hydroxy Propyl methyl cellulose (HPMC K4M), PVP K30, Carbopol (934P) obtained from Indian Drugs, Hyderabad. All other chemicals used for this study were of analytical grade.

Determination of partition coefficient

The partition co-efficient of the drugs was determined using n-octanol: water system. The n-octanol- water partition coefficient serves as a parameter of lipophilicity. n- Octanol and water were presaturated with each other for at least 24 h before the experiment. An accurately weighed quantity of each drug was dissolved in 10 ml of the n-octanol phase and shaken at 37°C for 24 h against 10 ml aqueous phase in a sealed container. The separated n-octanol phase was assayed by UV spectroscopy to determine its residual concentration and hence the amount partitioned into the aqueous phase (Marin *et al.,* 1998, McDaid *et al.,* 1996). The partition coefficient was expressed as the concentration of drug in the n-octanol phase (% w/v) divided by the concentration in the aqueous phase.

Drug–excipient interaction study

The pure drug, Glibenclamide and Atenolol and a mixture of it with the polymers, HPMC, PVP and, CP were mixed separately with IR grade KBr in the ratio of 100:1 and corresponding pellets were prepared by applying pressure in a hydraulic press (Jagmohan *et al.,* 2003). The pellets were scanned over a wave number range of 4000-400cm^{-1} in Thermo Nicolet USA, FTIR instrument.

Fabrication of transdermal patches

Transdermal patch composed of different polymers containing combination of Glibenclamide and Atenolol were prepared by Solvent Casting technique. Firstly, drugs were dissolved in ethanol. Base materials were added into the solution and swelled in ambient temperature (Yanli Gao *et al.,* 2009, Samanta *et al.,* 2002). Permeation enhancers and plasticizer were added to the solution, and then agitated in a sonicator. This was casted on a glass surface containing ring, it was covered by funnel to control evaporation of solvent and allowed to dry at room temperature over night. The films were separated and the backing membrane used was aluminium foil and the formulations were stored in desiccator. After being dried, the single-layer patch was obtained. The composition of patches was mentioned in table 1.

Physico chemical evaluation of the prepared films

Thickness and weight variation

The thickness of the patch at three different points was determined using thickness gauge and the patches were then weighed individually using digital balance to determine the weight of each patch taken out from the casted film. The patches were subjected to weight variation by individually weighing ten randomly selected patches. Such determinations were carried out for each formulation (Mundada *et al.,* 2009).

Drug content

Films of specified area were cut and the pieces were taken into a 100 ml volumetric flask containing phosphate buffer (pH 7.4), and the flask was sonicated for 8 h (Mazzo *et al.,* 1994). A blank was prepared in the same manner using a drug-free placebo patch of same dimensions. The solution was then filtered using a 0.45-µm filter and the drug content was analyzed at 229 nm and 275 nm respectively by UV spectrophotometer.

Table 1: Composition of transdermal patches using glibenclamide and atenolol.

Formulation Code	HPMC (%)	PVP (%)	CP (%)
GA1	1	1	-
GA2	1	-	1
GA3	-	1	1
GA4	1	0.5	0.5
GA5	0.5	1	0.5
GA6	0.5	0.5	1
GA7	0.7	0.7	0.6

Drug loaded in each film: Glibenclamide: 10 mg, Atenolol: 25mg; Plasticizers: Propylene Glycol (30% w/w of polymer), backing membrane: Aluminium foil.

Folding endurance test

Folding endurance test was carried out by folding the patch at the same point a number of times till it broke (Ubaidulla *et al.,* 2007). The test was carried out to check the efficiency of the plasticizer and the strength of the film prepared using varying ratios of the polymers. The test was carried out in triplicate.

Percentage Moisture Loss

Accurately weighed films of each formulation were kept in a desiccator and exposed to an atmosphere of 98% relative humidity (containing anhydrous calcium chloride) at room temperature and weighed after 3 days (Kusum Devi *et al.,* 2003). The test was carried out in triplicate. The percentage of moisture loss was calculated as the difference between initial and final weight with respect to initial weight.

Percentage moisture uptake

Accurately weighed films of each formulation were kept in a desiccator which is maintained at 79.5% relative humidity (saturated solution of aluminium chloride) at room temperature and weighed after 3 days (Biswajit Mukherjee *et al.,* 2005). The test was carried out in triplicate. The percentage of moisture uptake was calculated as the difference between final and initial weight with respect to initial weight.

Water absorption capacity

Three film units of each formulation were kept in an atmosphere of relative humidity RH = 82% for one week and the difference in weight of the film was taken as the water absorption capacity for that film (Udupa *et al.,* 1992).

Water vapor transmission rate

For water vapor transmission studies glass vials of equal diameter were used as transmission cell (Kulkurni Raghavendra *et al.,* 2000). These transmission cells were washed thoroughly and dried in an oven. About 1 gm of anhydrous calcium chloride was taken in the cell and the polymer film was fixed over the brim with the help of the solvent. The cell were accurately weighed and kept in a closed desiccator containing saturated solution of potassium chloride to maintain a humidity of 84% RH. The cells were taken out and weighed after 1, 2, 3, 4, 5, 6 and 7th day. Water vapor transmission rate usually expressed as the number of grams of moisture gain/hours/ sq.cm.

W V T = WL/S

Where, W is water vapor transmitted in mg, L is thickness of the film in mm, S is exposed surface area in cm^2.

***In vitro* drug release studies**

The *in-vitro* release studies were carried out by using Chein apparatus. The receptor compartment was maintained at 37±1°C by means of a water bath, circulator, and a jacket surrounding the cell. The cells were filled with freshly prepared phosphate buffer pH 7.4. The solution in the receptor compartment was continuously stirred at 60 rpm by means of Teflon coated magnetic stirrer, in order to avoid diffusion layer effects. The Commercial Semi-permeable membrane were mounted between the donor and receptor compartment and secured in place by means of a clamp. The combination patch was placed on one side of the semi-permeable membrane (Ji-Hui Zhao *et al.,* 2007, Yanli Gao *et al.,* 2000). Aliquots of 1ml were removed from the receptor compartment by means of a syringe and replaced immediately with the same volume of buffer solution kept at 37± 1°C. Test samples were taken from the medium at predetermined time intervals over a period of 24 hours and the samples were analyzed for Glibenclamide and Atenolol content by UV spectrophotometer at 229 nm and 275 nm respectively (Vlassios Andronis *et al.,* 1995).

***In-vitro* Transdermal permeation**

a) Rat skin

The hairs of the male Wistar albino rat were cleared by using scissors. After cleaning the skin with Phosphate buffer pH 7.4, animal was sacrificed by excessive ether inhalation. An incision was made on the flank of the animal and the skin was separated. The prepared skin was washed with Phosphate buffer pH 7.4 and used (Yanli Gao *et al.,* 2009).

b) Goat skin

Skin was obtained from slaughtered goat. The skin was removed carefully and separated from the underlying cartilage with a scalpel. After separating the full thickness skin, the fat adhering to the dermis side was removed using a scalpel and isopropyl alcohol.

The transdermal permeation was performed in Chein Diffusion cell. The cells were filled with freshly prepared phosphate buffer pH 7.4. While placing the patch, the donor compartment contains patch on stratum corneum side of skin and dermis side was facing receptor compartment (Ke *et al.,* 2005). Receptor compartment contains phosphate buffer pH 7.4 and samples were withdrawn at regular time intervals and replaced the same with receptor fluid. The samples were analyzed at 229 nm and 275 nm against blank by UV spectrophotometer (Srinivas Mutalik *et al*., 2006).

***In-vivo* studies**

Primary Skin Irritation Test

The dorsal part of rabbit was carefully shaved, and patch was applied on the shaved skin for 7 days. After the patch was removed, conditions of the dorsal skin were observed and are evaluated most often by modification described by Draize and his colleagues in 1944, which is based on scoring method. Scores as assigned from 0 to 4 based on the severity of erythema or oedema formation. The safety of the patch decreases with increase in scoring.

***In-vivo* drug release study**

Selection of animals

Rabbit's (crytolagus cuniculus) of male sex 10-12 weeks old weighing 1-2 kg were selected. They were kept with husk bedding and were fed with standard rodent pellet diet and water. Light & dark cycles with 12 hours light and 12 hours dark were maintained. The temperature and relative humidity conditions were 28 ± 2^{o}C and 60 ± 15% respectively. The protocols for all animal studies were approved by Institutional Ethical Committee (1220/a/08/CPCSEA/ANCP/06).

Method

A set of healthy rabbits were selected. They were checked to ensure that they were free from disease. The dorsal surface of the selected rabbits was cleaned and hair was removed. The dose of Glibenclamide and Atenolol was calculated according to the body weight i.e., 0.32mg and 3.2mg respectively (Jayaprakash *et al.,* 2010). The patch GA4 (HPMC 1%, PVP 0.5%, CP 0.5%) was placed on the dorsal surface. At specific time interval the patch was removed from the rabbit carefully and analyzed for remaining drug content. Initial drug content was determined before placing the film. The remaining drug content was subtracted from the initial drug content of the film. The value obtained denotes the amount of drug in diffused from the patch into the body (Chakkapan *et al.,* 1994).

Amount of drug released at any time interval = Initial drug content before placing the film -
Remaining Drug content after removal of the film

***In-vitro In-vivo* correlation**

In-vitro and *in-vivo* correlation was carried out for the therapeutic efficacy of a pharmaceutical formulation. It is governed by the factors related to both *in-vitro* and *in-vivo* characteristics of the drug. Percent *in-vitro* release on X-axis was plotted against *in-vivo* drug release on Y-axis for the same period of time.

RESULTS

In the present work efforts have been made to prepare Combinational transdermal drug delivery system of Glibenclamide and Atenolol using various blends of polymers such as Hydroxy Propyl methyl cellulose, Carbopol and Poly vinyl pyrrolidone. Permeation enhancer used was Propylene glycol.

The observed partition coefficients of Glibenclamide and Atenolol using n-octanol/water found to give log K values 2.34 and 2.54.

The physicochemical compatibility of the drugs and the polymer was established through FTIR studies. In the physical mixture of Glibenclamide and atenolol with Hydroxy propyl methyl cellulose, Carbopol and Poly vinyl pyrrolidone the major peaks of Glibenclamide and Atenolol were 1714, 1638 (C =O Stretching), 1415 (CH2

Table 2: Physico-chemical evaluation of Transdermal films

Formulation Code	Thickness (mm) ± SD	Weight Uniformity ± SD	Drug Content (%)		Folding Endurance ± SD	Percentage Moisture Uptake ± SD
			G	A		
GA1	0.30± 0.01	182.7±0.51	99.40	99.22	251 ±1.0	5.2±0.015
GA2	0.35± 0.04	162.3±0.32	99.65	99.51	230± 2.0	12.21±0.02
GA3	0.32± 0.02	172.4±0.20	98.63	98.36	253 ±2.0	4.26±0.015
GA4	0.33± 0.04	186.2±0.32	98.36	99.35	272 ±2.0	10.43±0.01
GA5	0.36± 0.01	192.3±0.58	98.26	98.64	286 ±2.0	6.32±0.012
GA6	0.38± 0.04	210.2±0.42	98.36	99.48	220±1.0	9.34±0.032
GA7	0.31±0.02	182.4±0.24	99.54	98.65	270 ±1.0	7.38±0.024

Table 3: Physico-chemical evaluation of Transdermal films

Formulation Code	Percentage Moisture Loss ± SD	Water Absorption Capacity ± SD	Water Vapour Transmission Rate (mg/cm^2/hr) ± SD
GA1	10.12±0.024	3.2±0.015	9.987x10^{-6}
GA2	4.12±0.015	10.21±0.02	3.588x10^{-6}
GA3	7.21±0.01	3.92±0.12	5.166x10^{-6}
GA4	4.92±0.021	12.24±0.01	8.287x10^{-6}
GA5	11.26±0.032	5.5±0.015	8.468x10^{-6}
GA6	9.20±0.015	8.21±0.02	5.214x10^{-5}
GA7	4.26±0.014	6.24±0.04	7.641x10^{-6}

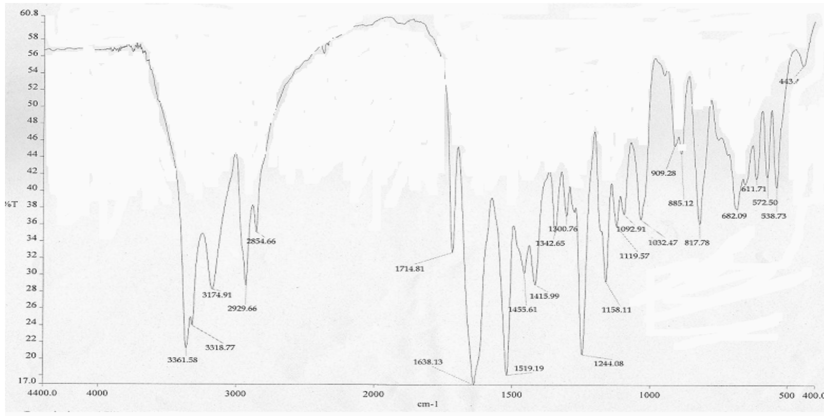

Fig. 1: IR Spectra of Pure Glibenclamide and Atenolol

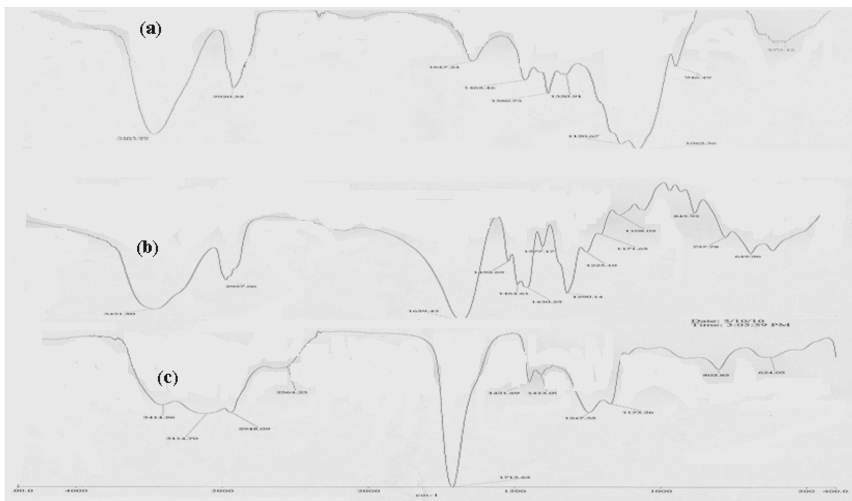

Fig. 2: IR Spectra of (a) HPMC K4M (b) PVP K30 (c) Carbopol 934P

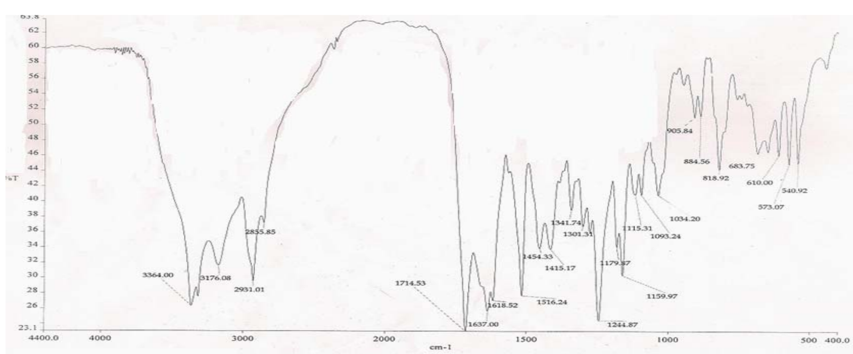

Fig. 3: IR spectra of Glibenclamide and Atenolol with polymers like HPMC K4M, CP 934P and PVP K30.

Bending), 1342, 1300 (SO2 Asymmetric Stretching), 1244, 1158 (C – N Stretching) wave numbers. However, some additional peaks were observed with physical mixtures, which could be due to the presence of polymers which are shown in figs. 1-3.

Physico-chemical evaluation data of table 2 and 3 indicates that thickness of the patches varied from 0.30 ± 0.01 to 0.38 ± 0.04. Folding endurance values of matrix films was found within 230-286 numbers of folds, indicating good strength and elasticity.The percentage Moisture uptake in the formulation GA2 (1% HPMC, 1% CP) has shown the highest value of moisture absorption 12.21±0.02. The formulation GA5 (0.5% HPMC, 1% PVP, 0.5% CP) shows higher value of Moisture loss 11.26±0.032 and formulation GA2 (1% HPMC, 1% CP) shows low value of 4.12 ± 0.015.

The high water absorption capacity was found in GA4 (1% HPMC, 0.5% PVP, 0.5% CP) as 12.24±0.01. The formulation GA1 (1% HPMC, 1% PVP) has shown maximum water vapor transmission of 9.987 X 10-6 among all the patches and GA6 has less water vapor transmission of 3.588 X 10-6.

Table 4 indicates the cumulative percentage drug release of various formulations. The cumulative percentage of drug released in 24 h was found to be the highest for Formulation GA4 (1%HPMC, 0.5%PVP, 0.5%CP) which has shown the drug release of 96.24% and 97.21% respectively. The in-vitro drug release plot which is shown in figure 4 indicated that the drug release followed zero order kinetics, which was envinced from the regression value of the above mentioned plot.

Table 4: Data for *in vitro* drug release for all formulations

Formulation code	Time (h)	Cumulative percentage release of drug (%)	
		G	A
GA1	20	99.1	99.58
GA2	19	98.68	99.6
GA3	19	99.9	98.9
GA4	24	96.24	97.21
GA5	24	92.32	93.25
GA6	22	98.21	97.6
GA7	22	99.34	98.42

The Higuchi's plot has shown the regression value of 0.994 and 0.995 respectively. In order to confirm this fact, Peppa's plot was drawn which has shown slope value of 0.686 and 0.590 respectively. Hence formulation GA4 was selected as the optimized formulation by virtue of its drug release kinetics.

In-vitro transdermal permeation study was carried out in rat skin, the formulation GA4 (HPMC 1%, PVP 0.5%, CP 0.5%) showed drug diffusion for 24 hours up to the extent of 93.24% and 94.26% respectively. The studies, which were carried out with goat skin showed drug diffusion of 92.92% and 93.64% respectively and these were mentioned in table 5.

Table 5: Data for *in-vitro* Transdermal permeation and *in-vivo* drug release GA4 (1%HPMC, 0.5% PVP, 0.5%CP)

Time (h)	*In-vitro* Transdermal permeation				*In-vivo* drug release	
	Rat skin		Goat skin			
	G	A	G	A	G	A
1	9.25	10.32	6.00	4.30	10.25	12.25
6	29.36	32.21	25.33	32.89	32.12	31.25
12	54.69	50.24	43.00	52.56	51.26	53.65
18	78.23	71.54	64.84	73.40	71.87	73.68
24	93.24	94.26	92.92	93.64	90.62	91.25

The result obtained from the primary skin irritation studies revealed that neither the adhesive nor the drugs Glibenclamide and Atenolol caused any noticeable irritation on the rabbit skin throughout the study.

In-vivo study was carried out in rabbit, at the end of 24th hour the in-vivo drug release showed 90.62 % and 91.25% respectively and values were mentioned in table 5. The results which are mentioned in table 6 indicated that the in-vitro and in-vivo techniques correlation was very good. The release pattern has followed the predicted zero order kinetics in biological systems also which are shown in figs. 5-6.

Table 6: Data for *in vitro* and *in vivo* correlation GA4 (1%HPMC, 0.5% PVP, 0.5%CP)

Time (h)	*In-vitro* drug release		*In-vivo* drug release	
	G	A	G	A
1	10.12	14.12	10.25	12.25
6	35.12	38.45	32.12	31.25
12	58.32	57.45	51.26	53.65
18	79.21	79.10	71.87	73.68
24	96.24	97.21	90.62	91.25

DISCUSSION

Transdermal drug delivery is an alternative route for the delivery of systemically acting drugs. This route has advantages of avoidance of first pass metabolism, predictable and extended duration of activity, minimizing under able side effects, utility of short half- life drugs, improving physiological and pharmacological response, avoiding the fluctuation in drug levels, inter and intra patient valuations, and most importantly, it provides patient compliance.

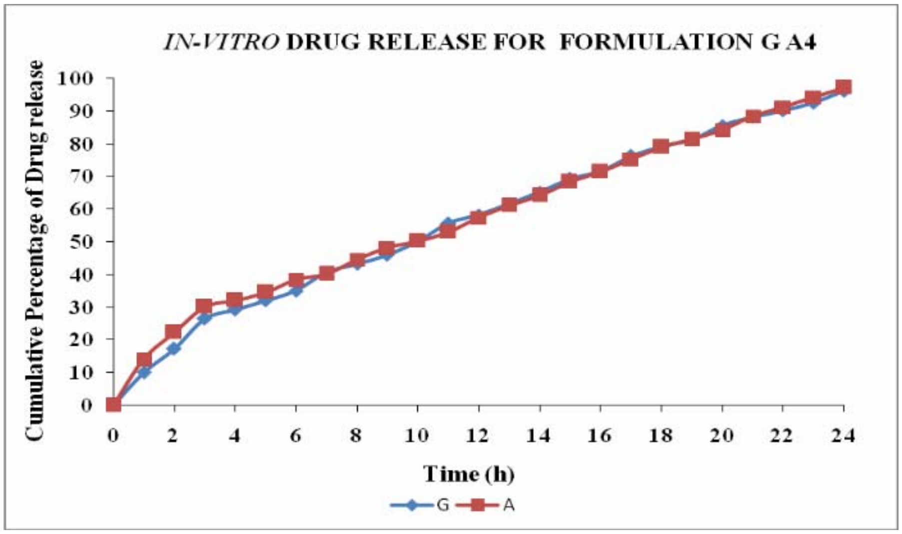

Fig. 4: *In-vitro* drug release of formulation GA4.

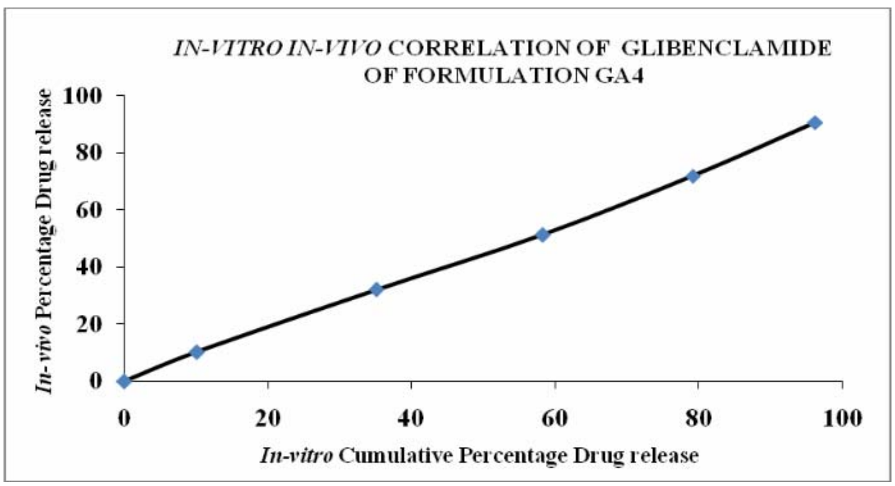

Fig. 5: *In-vitro in-vivo* Correlation of Glibenclamide of formulation GA4

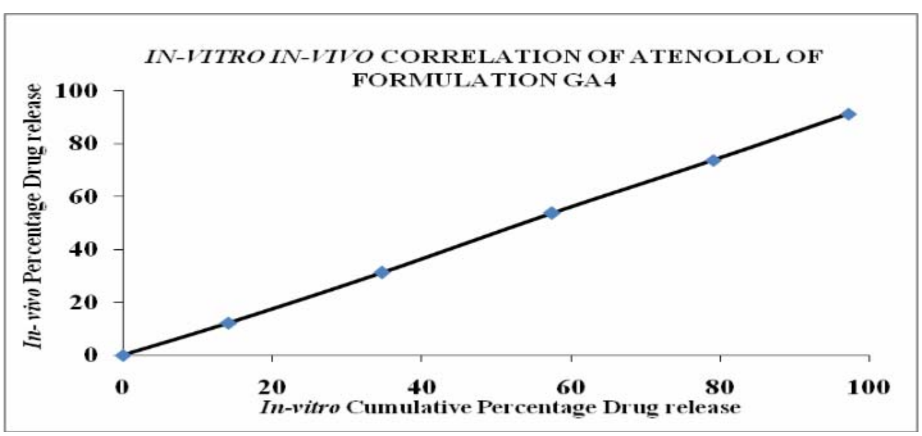

Fig. 6: *In-vitro in-vivo* Correlation of Atenolol of formulation GA4.

The Values of Partition coefficient of Glibenclamide and Atenolol in n-octanol/water system was found to be favorable for the transdermal drug delivery system.

The Spectra of IR analysis indicated that there was no chemical interaction between Glibenclamide and Atenolol and other excipients. It is already well known that the common polymers such as HPMC, PVP and CP are popular in controlled/sustained release matrix type patches because of their compatibility with a number of drugs.

The thicknesses of all batches indicate the physical uniformity of prepared patches. The drug content analysis and the weight uniformity of the prepared formulation have shown that the process adopted for casting the films in this investigation is capable of giving films with uniform drug content and with minimum intra batch variability. Folding endurance test results indicated that the patches would maintain the integrity with general skin folding when applied.

The percentage Moisture uptake in the formulation GA2 (1% HPMC, 1% CP) has shown the highest value which may be due to higher polydispersity index and solubility parameter of HPMC, CP as compared to those of PVP. The formulation GA5 (0.5% HPMC, 1% PVP, 0.5% CP) shows higher value of Moisture loss which may be due to presence of higher concentration of PVP. The high water absorption capacity was found in GA4 (1% HPMC, 0.5% PVP, 0.5% CP) which again revealed its high hydrophilicity than other polymers.

The formulation GA1 (1% HPMC, 1% PVP) has shown maximum water vapor transmission among all the patches this may be due to the presence of more PVP. All formulations were permeable to water vapor.

In vitro tests are very useful in the quality control of finished TDDS. The in-vitro release plots of all other formulations were suggestive of zero order release and are diffusion mediated which was confirmed from the regression value of Higuchi's plot. All the formulations undergo non-fickian type of release which is confirmed from the slope values obtained from the Peppa's plot. Based on the drug release the optimized formulation selected for further study was GA4 (1% HPMC, 0.5% PVP, 0.5% CP).

Release of the drug from transdermal patches is controlled by the chemical properties of the drug and delivery form, as well as physiological and physicochemical properties of the biological membrane. In vitro permeation studies are predictive of in vivo performance of a drug. In this study, different formulations released variable amounts of Glibenclamide and Atenolol through rat skin and Goat skin in the in vitro fluid. The variation among the used biological membrane could be attributed to the fat content and thickness of the membrane used. As the goat skin has more fat deposition and the thickness compared with rat skin, it might have hampered the drug release through the membrane.

The primary skin irritation studies revealed that neither the adhesive nor the drugs Glibenclamide and Atenolol caused any noticeable irritation on the rabbit skin throughout the study.

In-vivo study was carried out in rabbit revealed that the consistence in-vitro release pattern of the formulation GA4 was reproducible even in biological environment. The results indicated that the in-vitro and in-vivo techniques correlation was very good. They are well correlated, so the release pattern has followed the predicted zero order kinetics in biological systems also.

In conclusion formulation GA4 (1%HPMC, 0.5% PVP, 0.5%CP) has achieved the targets of present study such as controlled release, prolonged zero order release, reduced frequency of administration, greater therapeutic effect, overcome the side effects, simplify the treatment regimen and thus may improve patient compliance.

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