FORMULATION AND CHARACTERIZATION OF TRANSDERMAL DRUG DELIVERY SYSTEMS OF AMILORIDE USING VARIOUS POLYMER

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ABSTRACT

The skin can be used as the site for drug administration for continuous transdermal drug infusion into the systemic circulation. For the continuous diffusion penetration of the drugs through the intact skin surface membrane-moderated systems, matrix dispersion type systems, adhesive diffusion controlled systems and micro reservoir systems have been developed. Various penetration enhancers are used for the drug diffusion through skin. In matrix dispersion type systems, the drug is dispersed in the solvent along with the polymers and solvent allowed to evaporate forming a homogeneous drug-polymer matrix.

Matrix type systems were developed in the present study. In the present work, an attempt has been made to develop a matrix-type transdermal therapeutic system comprising of Amiloride with different concentration of various polymers alone using solvent evaporation technique. The physicochemical compatibility of the drug and the polymers was studied by infrared spectroscopy. The results obtained showed no physical-chemical incompatibility between the drug and the polymers. F1 formulation has been selected as the best formulation among all the other formulations. The *in vitro* drug diffusion studies from the formulation were found to be sustained release. All the evaluation parameters obtained from the best formulation were found to be satisfactory. The data obtained from the *in vitro* release studies were fitted to various kinetic models like zero order, first order, Higuchi model and peppas model. From the kinetic data it was found that drug release follows peppas model release by diffusion technique from the polymer.

Keywords: Transdermal drug delivery, hydrophobic polymers and Amiloride.

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1. INTRODUCTION

Treatments of acute and chronic diseases have been accomplished by delivery of drugs to patients using various pharmaceutical dosage forms. These dosage forms are known to provide a prompt release of drug. But recently several technical advancements have been done and resulted in new techniques for drug delivery. These techniques are capable of controlling the rate of drug release.

The term controlled release has a meaning that goes beyond scope of sustained release. The release of drug ingredients from a controlled release drug delivery advances at a rate profile that is not only predictable kinetically, but also reproducible from one unit to other[1].the difference between sustained release and controlled release is shown by fig.1.

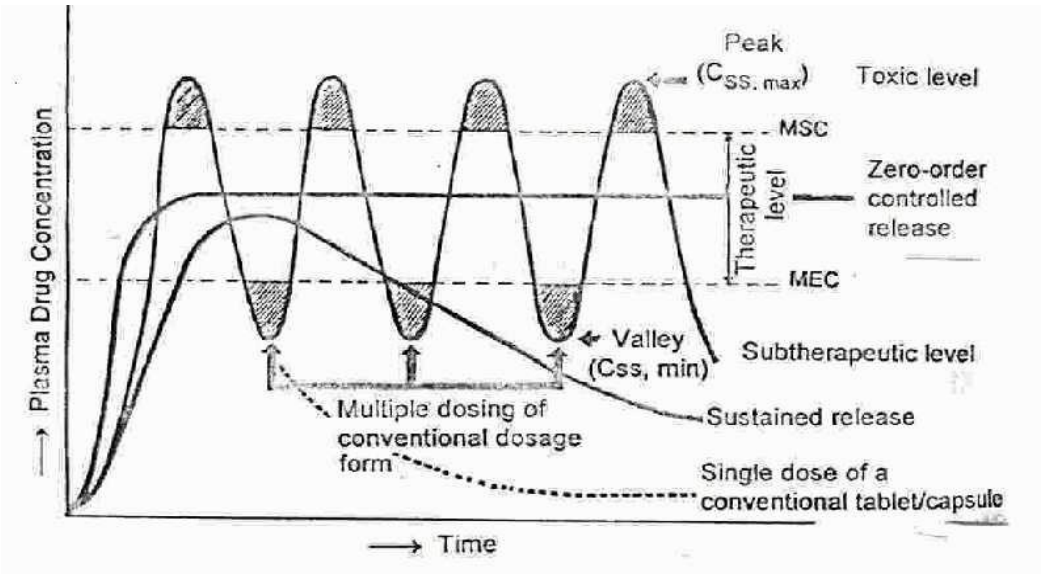

Figure1. 1: Comparative graphs of conventional, sustained- and controlled release delivery systems.

MATERIALS AND METHODS

The pure drug Ameloride was purchased from Chemo Pvt. Ltd, Mumbai, Eudragit RS 100, Eudragit RL 100, Propylene glycol, PEG 400 were purchased from Chemdyes corporation, Rajkot, India, HPMC K15M from Loba Chemie Pvt. Ltd., Mumbai, India. Ethanol was obtained from Shree Chalthan Vibhag Khandudyog Sahkari Mandli Ltd, Surat.

METHODOLOGY:

Analytical method development:

A.UV scan:

A 100mg of Amiloride was accurately weighed and was first dissolved in 35ml methanol solution. The solution was then diluted using phosphate buffer (pH- 7.4) to 100 ml. (stock solution-I). Take 10ml solution from stock solution 1 and volume make up to 100ml with phosphate buffer to get 100µg/ml concentrations (stock solution-II). Take 10 ml solution from stock II and volume make up to 100 ml with buffer to get 10µg/ml. 10µg/ml solution was scanned from 200-400nm.

B. Construction of calibration curve:

A 100mg of Amiloride was accurately weighed and was first dissolved in 35ml methanol solution. The solution was then diluted using phosphate buffer (pH-7.4) to 100 ml. (stock solution-I). Take 10ml solution from stock solution 1 and volume make up to 100ml with phosphate buffer to get 100 µg/ml concentrations (stock solution-II). It was further diluted with phosphate buffer pH – 7.4 to get solutions in concentration range of 5,10,15,20 and 25 µg /ml. The absorbance's of these solutions were determined spectrophotometrically at 285 nm.

Preformulation study

A. Colour, Odour, Taste and Appearance:

The drug sample was evaluated for its Colour, odour and appearance.

B. Melting point determination:

Melting point of the drug sample was determined by capillary method by using melting point apparatus.

C. Determination of solubility:

The solubility of Amiloride was determined by adding excess amount of drug in the solvent.

The solubility was determined in distilled water and phosphate buffer pH 7.4. The procedure can be detailed as follows.

Saturated solution of Amiloride prepared using 10 ml. of distilled water/ phosphate buffer pH 7.4 in 25 ml volumetric flasks in triplicate. Precaution was taken so that the drug remains in medium

in excess. Then by using mechanical shaker, the flasks were shaken for 48 hours. The sample withdrawn (1 ml after filtration) was diluted with appropriate medium and analyzed by using UV spectrophotometer at 285 nm and 283 nm for phosphate buffer and distilled water respectively.

Formulation of transdermal patches

Preparation of blank patches:

Polymers of single or in combination were accurately weighed and dissolved in respective solvent and then casted in a Petri-dish with mercury as the plain surface. The films were allowed to dry overnight at room temperature.

Formulation of drug incorporated transdermal patches:

The matrix-type transdermal patches containing Amiloride were prepared using different concentrations of Ethyl Cellulose, HPMC and Eudragit RSPO polymers. The polymers in different concentrations were dissolved in the respective solvents. Then the drug was added slowly in the polymeric solution and stirred on the magnetic stirrer to obtain a uniform solution. Dibutyl phthalate was used as plasticizers. Then the solution was poured on the Petri dish having surface area of 78 cm2 and dried at the room temperature. Then the patches were cut into 2x2 cm^2 patches. Drug incorporated for each 2x2 cm^2 patch. The formulation table is given in table no. 7.1.

Table 1: Formulation of Amiloride patches

INGREDIENTS	FORMULATION CHART								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Amiloride	5	5	5	5	5	5	5	5	5
Ethyl Cellulose	25	50	100	-	-	-	-	-	-
HPMC	-	-	-	25	50	100	-	-	-
Eudragit RSPO	-	-	-	-	-	-	25	50	100
PEG-400 (ml)	10	10	10	10	10	10	10	10	10
Chloroform	15	15	15	15	15	15	15	15	15
Dimethylsulphoxide (ml)	2	2	2	2	2	2	2	2	2
Dibutyl phthalate* (ml	7	7	7	7	7	7	7	7	7

Evaluation parameters of patches

Physical evaluations

a. Thickness

The thickness of patches was measured by digital Verniers calipers with least count 0.001mm. The thickness uniformity was measured at five different sites and average of five readings was taken with standard deviation.

b. Folding endurance

The folding endurance was measured manually for the prepared patches. A strip of patch (4x3 cm) was cut evenly and repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking gave the exact value of folding endurance.

c. Weight variation

The three disks of 2*1 cm^2 was cut and weighed on electronic balance for weight variation test. The test was done to check the uniformity of weight and thus check the batch- to- batch variation.

d. Drug content Determination

The prepared drug contained patches specified surface area (2 cm^2) were cut and dissolved in (5% of methanol contained) 100ml of pH 7.4 phosphate buffer, and vigorously shaked for 12hrs, and then sonicated for 15 minutes, centrifuged at 5000 rpm for 30 min. Filter the drug contained polymeric solution through 42 number whatmann filter paper, then 1ml of the filtrate was taken in a test tube and dilute it for five times with same solvent by using double beam Uv-Visible spectrophotometer to determined drug content at λmax 285 nm. Respected Placebo patch was taken as a blank solution.

Flatness: A transdermal patch should possess a smooth surface and should not constrict with time. This can be demonstrated with flatness study. For flatness determination, one strip is cut from the centre and two from each side of patches. The length of each strip is measured and

variation in length is measured by determining percent constriction. Zero percent constriction is equivalent to 100 percent flatness.

$$\% \text{ constriction} = I1 - I2 \text{ X } 100$$

I2 = Final length of each strip

I1 = Initial length of each strip

***In-vitro* drug diffussion study:**

The *in vitro* study of drug permeation through the semi permeable membrane was performed using a Franz type glass diffusion cell. The modified cell having higher capacity (25 ml) is used to maintain sink condition. This membrane was mounted between the donor and receptor compartment of a diffusion cell. The transdermal patch was placed on the membrane and covered with aluminum foil. The receptor compartment of the diffusion cell was filled with isotonic phosphate buffer of pH 7.4. The hydrodynamics in the receptor compartment were maintained by stirring with a magnetic bead at constant rpm and the temperature was maintained at 37 ± 0.5^0C. The diffusion was carried out for 12 h and 1 ml sample was withdrawn at an interval of 1 h. The receptor phase was replenished with an equal volume of phosphate buffer at each sample withdrawal. The samples were analyzed for drug content spectrophotometrically at 285 nm

Drug release kinetics:

Diffusion data of above two methods was fitted in Zero order, First order and Higuchi equations. The mechanism of drug release was determined by using Higuchi equation.

Zero-Order Kinetics:

Zero order as cumulative amount of Percentage drug released vs. time

$$C=K0t$$

Where K0 is the zero-order rate constant expressed in units of concentration/time and t is the time in hours. A graph of concentration vs time would yield a straight line with a slope equal to K0 and intercept the origin of the axes.

First order kinetics:

First order as log cumulative percentage of log (%) cumulative drug remaining vs time,

$$L o g C = L o g C o - k t / 2.303$$

Where C0 is the initial concentration of drug, k is the first order constant, and t is the time.

Higuchi model:

Higuchi's model as cumulative percentage of drug released vs square root of time

$$Q = K\, t\, 1/2$$

Where K is the constant reflecting the design variables of the system and t is the time in hours. Hence, drug release rate is proportional to the reciprocal of the square root of time.

Kors meyer Peppas equations:

Korsmeyer peppas equation used to determine the mechanism of drug release form the polymer matrix of the tablet. Log cumulative percentage of drug released VS Log time, and the exponent n was calculated through the slope of the straight line.

$$Mt/M\infty = Ktn$$

Where $Mt/M\infty$ is the fractional solute release, t is the release time, K is a kinetic constant characteristic of the drug/polymer system, and n is an exponent that characterizes the mechanism of release of tracers. For cylindrical matrix tablets, if the exponent $n = 0.45$, then the drug release mechanism is Fickian diffusion, and if $0.45 < n < 0.89$, then it is non-Fickian or anomalous diffusion. An exponent value of 0.89 is indicative of Case-II Transport or typical zero-order release.

Compatibility study

FTIR study:

The infrared spectrum of the pure Amiloride sample was recorded and the spectral analysis was done. The dry sample of drug was directly placed after mixing and triturating with dry potassium bromide.

RESULTS AND DISCUSSION

Initially the drug was tested by UV to know their significant absorption maximum which can be used for the diffusion study of the drug.

Analysis of drug:

A. UV scan:

The lambda max of Amiloride was found to be 285 nm.

B. construction of calibration curve:

Table 7.1: Standard graph of Amiloride

Concentration (µg/ml)	Absorbance (at 285 nm)
0	0
5	0.121
10	0.225
15	0.334
20	0.439
25	0.546

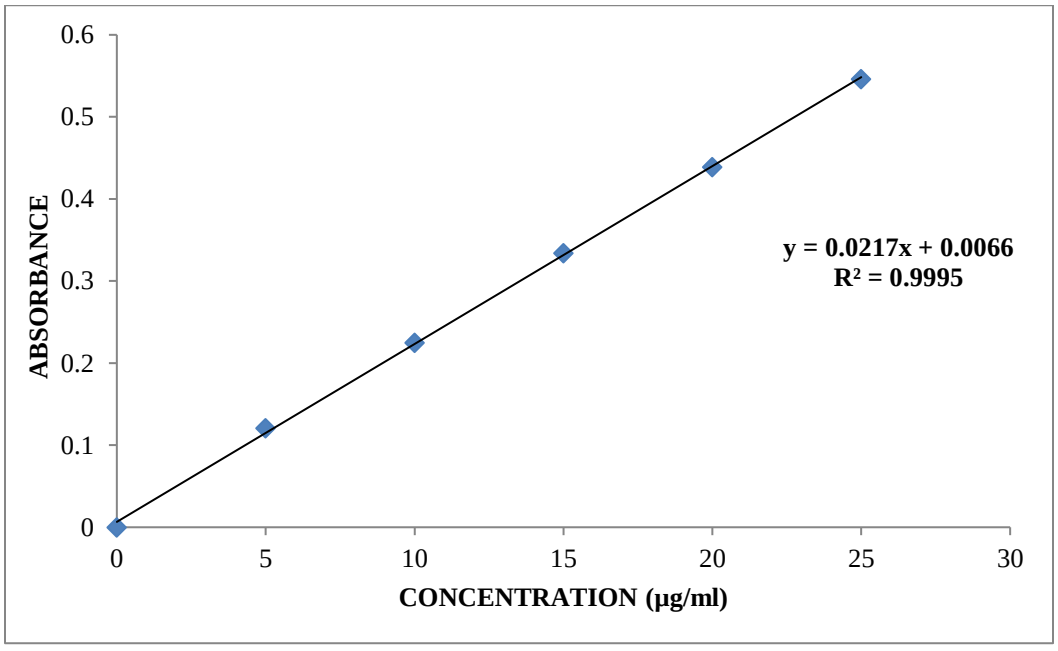

Figure 1: Standard calibration curve of Amiloride

Preformulation study

Totally, nine formulation trials were done with the aim to achieve the successful matrix type Amiloride transdermal patches. The blend trials prepared for the drug was evaluated for various physical parameters and content uniformity of drug by UV.

A. Colour, odour, taste and appearance

Table 2: Results of identification tests of drug

Parameter	Atenolol
Color	White
Odor	Odorless
Taste	Bitter
Appearance	A white powder

B. Melting point determination:

Table 3: Results of melting point determination tests of drug

Drug	Reported melting point
Amiloride	158-160 ^{0}c

C. Determination of solubility:

Table 4: Solubility Determination

solvent	Drug solubility(mg/ml)
Distilled water	4.93
Ph 7.4 phosphate buffer	78.3

Evaluation of Patch

The formulations F1 to F9 were varying in thickness when compared to other formulations which is due to the variation in the polymer concentration. Which shows the increase in polymer

concentration increases the thickness of patch. For all other formulations it was found to be in between 0.041±0.007 to 0.051±0.004 mm.

All formulations from F1 to F9 shows weight variation in between 70 ±9.58 to 79 ±6.85 mg.

Folding endurance from formulations F1 to F9 was found to be in between 81 ± 0.15 to 89 ± 2.15 which can withstand the folding of the skin.

All formulations showed % drug content from 95.1 ± 2.61 to 99.74 ± 1.57.

Table 5: Evaluation of patches

Formulation Code	Average weight(mg)	Thickness (mm)	Folding endurance	Flatness (%)	Appearance	% Drug Content
F1	75±1.05	0.046 ± 0.003	81 ± 0.15	100	Transparent	97.1 ± 2.10
F2	78 ±5.36	0.049±0.008	86 ± 1.39	99	Transparent	98.28 ± 0.45
F3	71 ±2.84	0.051±0.004	85 ± 2.26	100	Transparent	97.69 ± 2.21
F4	75 ±5.41	0.041±0.009	80 ± 1.84	100	Transparent	95.1 ± 2.61

F5	77 ±9.18	0.049±0.004	82 ± 3.10	99	Transparent	99.2 ± 3.87
F6	79 ±4.69	0.041±0.007	89 ± 2.15	100	Transparent	98.35 ± 0.59
F7	70 ±9.58	0.047±0.001	84 ± 2.36	99	Transparent	99.11 ± 2.34
F8	76 ±3.86	0.045±0.009	87 ± 2.04	100	Transparent	99.74 ± 1.57
F9	74 ±7.29	0.048±0.006	82 ± 2.96	100	Transparent	98.48 ± 0.44

***In vitro* diffusion study:**

All the formulation *in vitro* diffusion study was carried out by using Franz type diffusion cell under specific condition such as temp maintained at 32 ± 0.5 ^{o}C. The diffusion was carried out for 12 h and 5 ml sample was withdrawn at an interval of 1 h.

Table 6: *In vitro* drug permeation of Amiloride

Time (hr)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	4.22	7.04	2.81	3.68	4.56	2.17	5.14	7.29	4.98
2	13.57	14.07	10.37	7.29	10.32	9.74	8.66	11.63	9.35
3	16.78	22.00	15.20	13.04	16.44	16.54	12.73	16.13	12.70

4	20.09	28.75	23.03	20.61	21.80	22.20	17.65	23.80	17.74
5	28.77	30.42	30.43	24.68	29.08	29.44	23.22	29.10	22.88
6	36.28	39.25	38.17	29.30	35.44	35.87	30.49	35.54	29.18
7	54.93	48.77	43.39	36.94	51.36	42.76	36.73	40.81	33.99
8	66.75	56.42	46.45	45.22	67.97	50.62	44.30	48.21	41.40
9	73.37	60.38	54.91	57.35	76.35	58.26	53.10	67.06	47.78
10	79.12	76.86	60.38	74.73	82.15	62.79	66.08	78.10	54.20
11	83.69	86.19	64.99	89.11	95.64	72.08	79.99	82.64	60.21
12	98.29		69.51			81.32		86.78	65.52

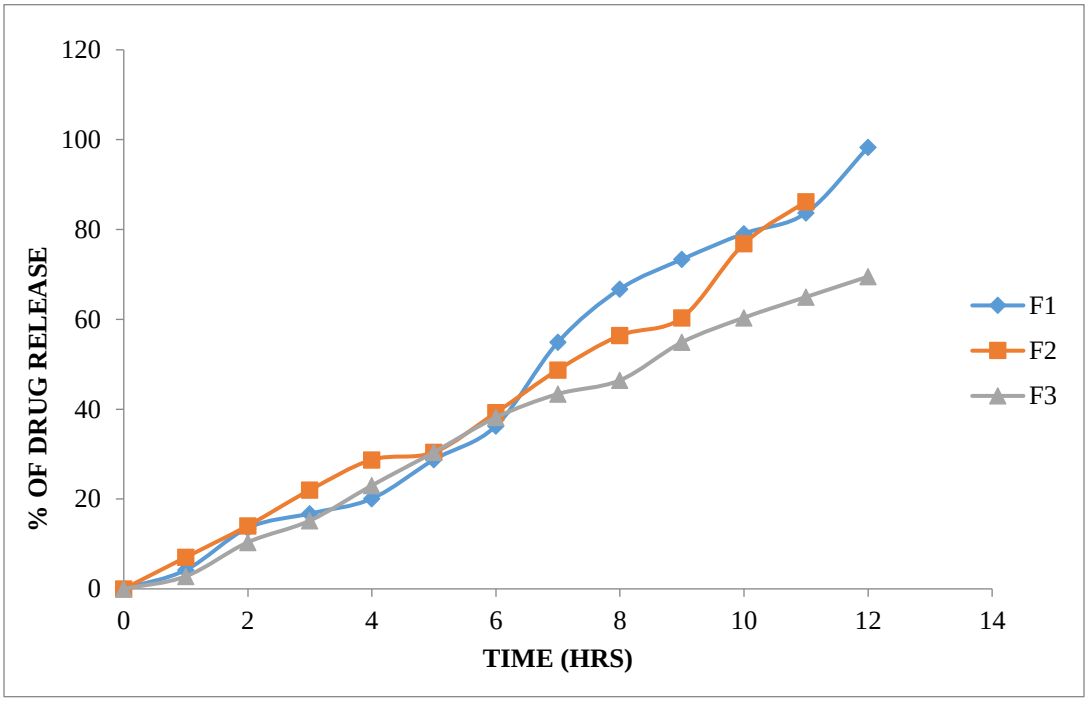

Figure:2 Cumulative % drug permeation of Atenolol patch (F1, F2 and F3)

The formulations F1 to F3 were prepared by different concentrations of Ethyl Cellulose (25, 50 and 100mg) in 2*2 cm^2patch, the drug release or drug permeation from the patch was dependence on the concentration of polymer in the matrix. At low polymer concentration the drug permeation is more within 12 hours it was total amount of drug was permeated. The 25 mg

concentration of polymer was showed maximum drug released at 12 hors 98.29%. Hence in that 3 formulation F1 formulations showed total drug release at desired time period.

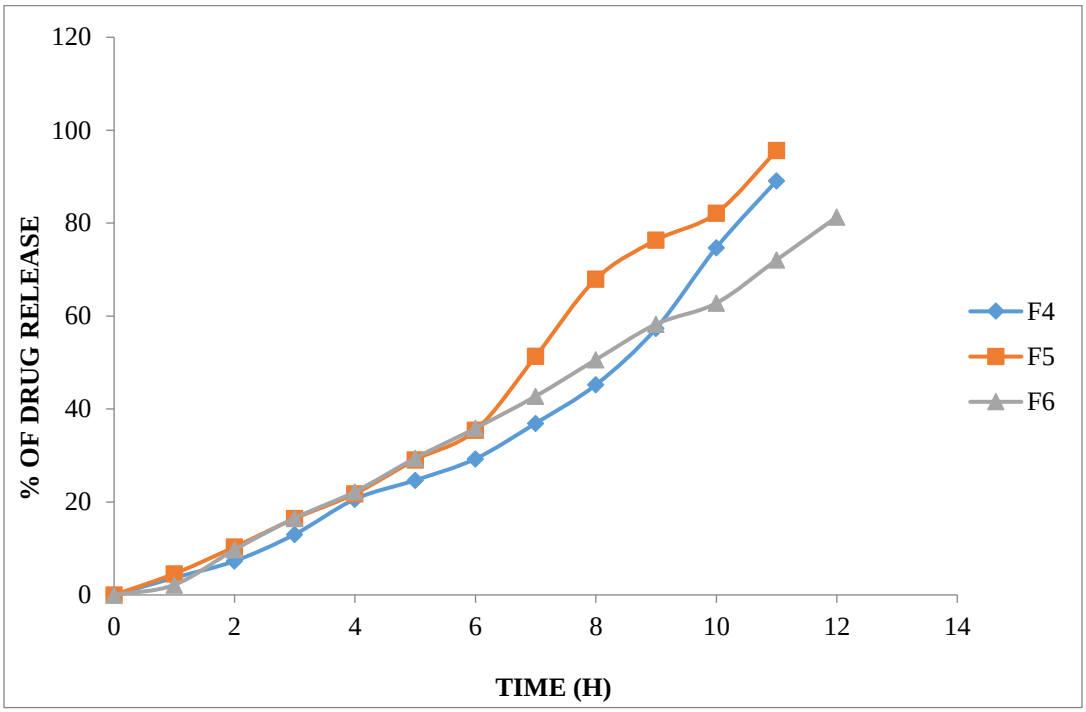

Figure:3 Cumulative % drug permeation of Atenolol patch (F4, F5 and F6)

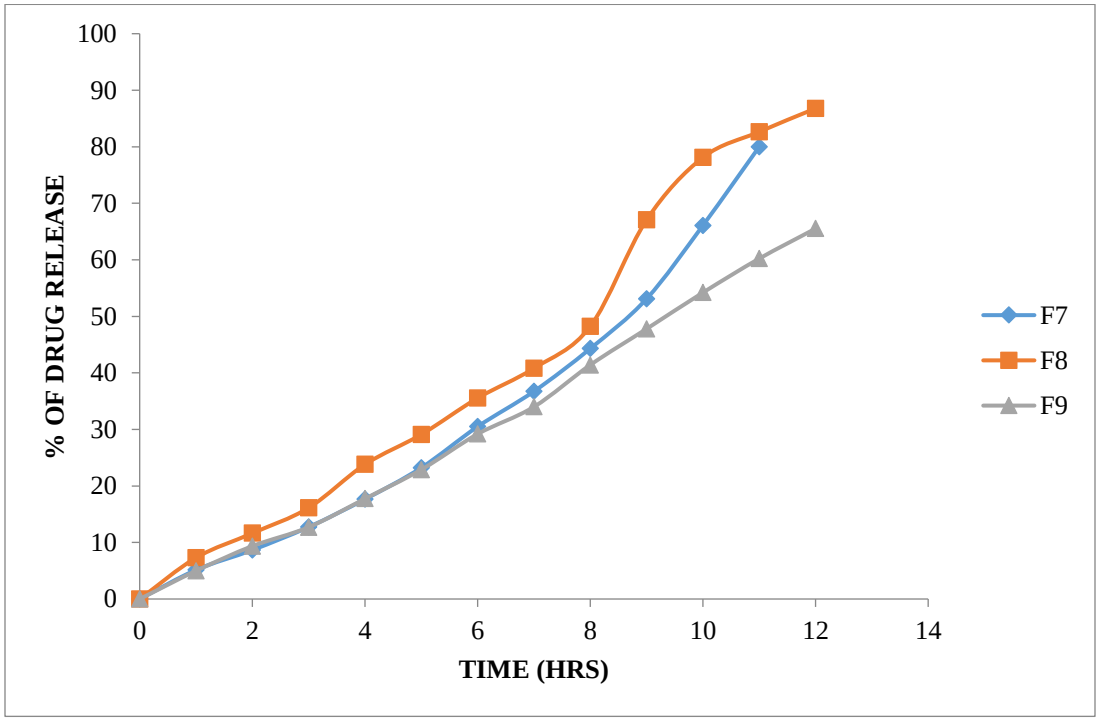

Figure:4 Cumulative % drug permeation of Atenolol patch (F7, F8 and F9)

The formulations F4 to F6 were prepared by different concentrations of HPMC (25, 50 and 100mg) in 2*2 cm^2patch the drug release or drug permeation from the patch was dependence on the concentration of polymer in the matrix. The 50mg (F5) concentration of polymer was showed maximum drug release 95.64 within 11 hours.

The 50mg (F8) concentration of polymer was showed maximum drug released at 12 hours 86.78%.

Among all 9 formulations F1 formulation showed good drug permeation from the patch. Among all *in vitro* evaluation parameters F1 formulation passed all evaluation parameters.

Kinetic models for Atenolol

Various models were tested for explaining the kinetics of drug release. To analyze the mechanism of the drug release rate kinetics of the dosage form, the obtained data were fitted into zero-order, first order, Higuchi, and Korsmeyer-Peppas release model.

Table: 8 Kinetics data of F1 Amiloride patch

CUMULATIVE (%) RELEASE Q	TIME (T)	ROOT (T)	LOG(%) RELEASE	LOG (T)	LOG (%) REMAIN	RELEASE RATE (CUMULATIVE % RELEASE / t)	1/CUM% RELEASE	PEPPAS log Q/100	% Drug Remaining	Q01/3	Qt1/3	Q01/3-Qt1/3
0	0	0	0	0	2.000	0	0	0	100	4.642	4.642	0.000
4.22	1	1.000	0.625	0.000	1.981	4.220	0.2370	-1.375	95.78	4.642	4.575	0.066
13.57	2	1.414	1.133	0.301	1.937	6.785	0.0737	-0.867	86.43	4.642	4.421	0.220
16.78	3	1.732	1.225	0.477	1.920	5.593	0.0596	-0.775	83.22	4.642	4.366	0.276
20.09	4	2.000	1.303	0.602	1.903	5.023	0.0498	-0.697	79.91	4.642	4.307	0.334
28.77	5	2.236	1.459	0.699	1.853	5.754	0.0348	-0.541	71.23	4.642	4.145	0.496
36.28	6	2.449	1.560	0.778	1.804	6.047	0.0276	-0.440	63.72	4.642	3.994	0.647
54.93	7	2.646	1.740	0.845	1.654	7.847	0.0182	-0.260	45.07	4.642	3.559	1.083
66.75	8	2.828	1.824	0.903	1.522	8.344	0.0150	-0.176	33.25	4.642	3.216	1.426
73.37	9	3.000	1.866	0.954	1.425	8.152	0.0136	-0.134	26.63	4.642	2.986	1.655
79.12	10	3.162	1.898	1.000	1.320	7.912	0.0126	-0.102	20.88	4.642	2.754	1.888
83.69	11	3.317	1.923	1.041	1.212	7.608	0.0119	-0.077	16.31	4.642	2.536	2.106
98.29	12	3.464	1.993	1.079	0.233	8.191	0.0102	-0.007	1.71	4.642	1.196	3.446

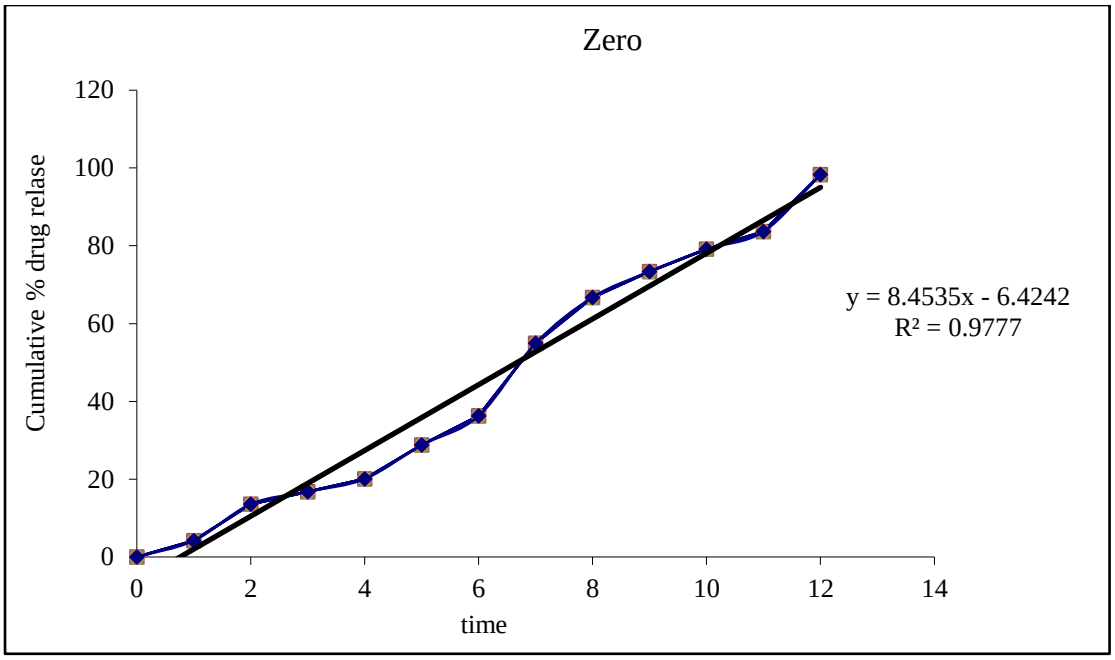

Figure: 5 Graph of Zero order kinetics

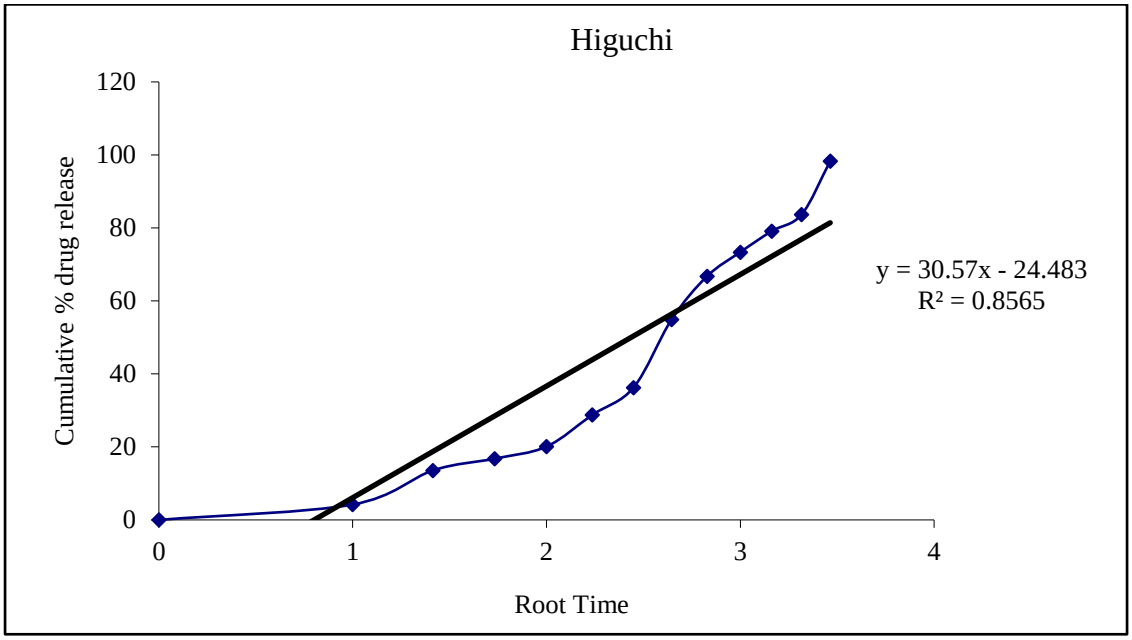

Figure: 6 Graph of Higuchi release kinetics

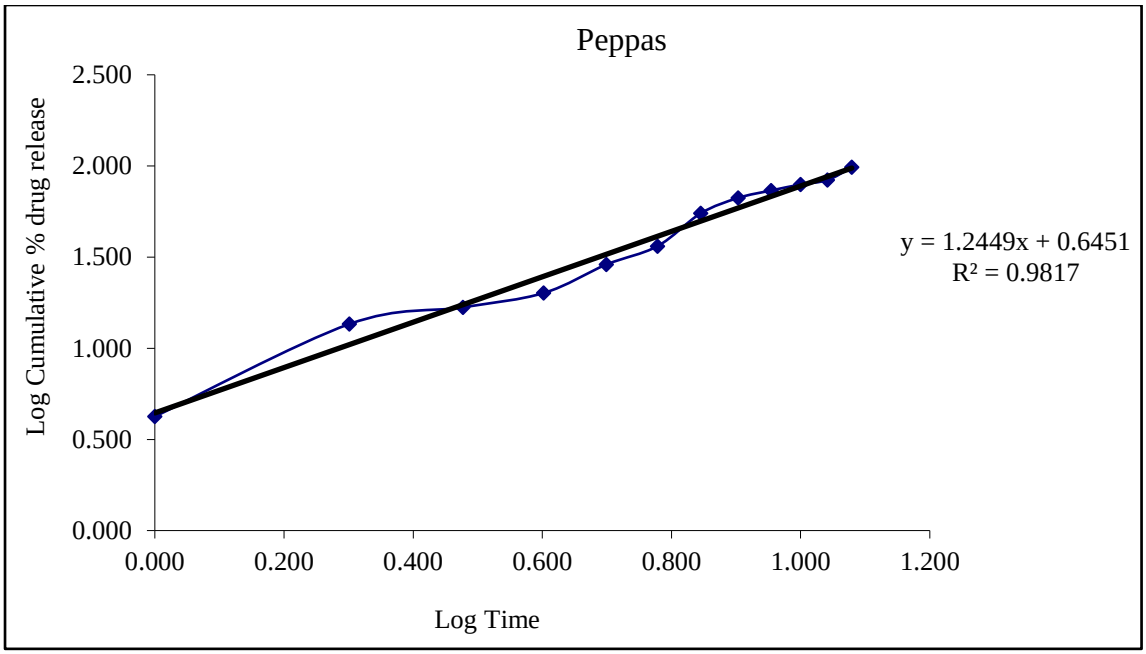

Figure :7 Graph of peppas release kinetics

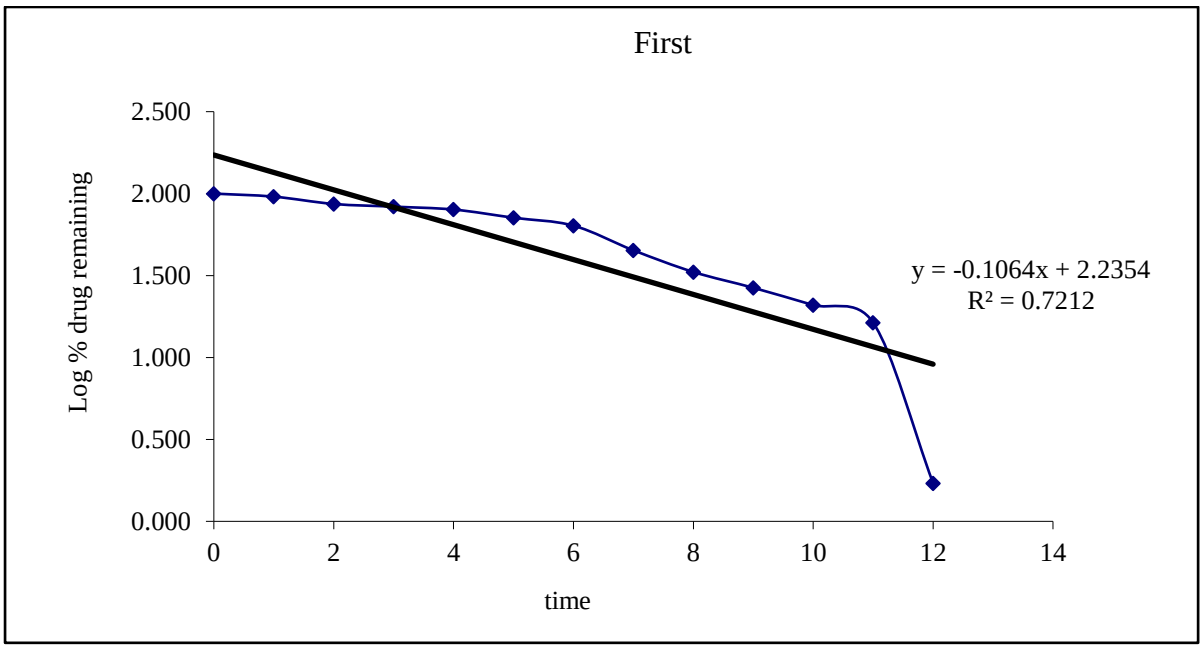

Figure: 8 Graph of First order release kinetics

From the above data the optimized formulation followed peppas model rule.

Compatibility studies:

IR SPECTROSCOPY:

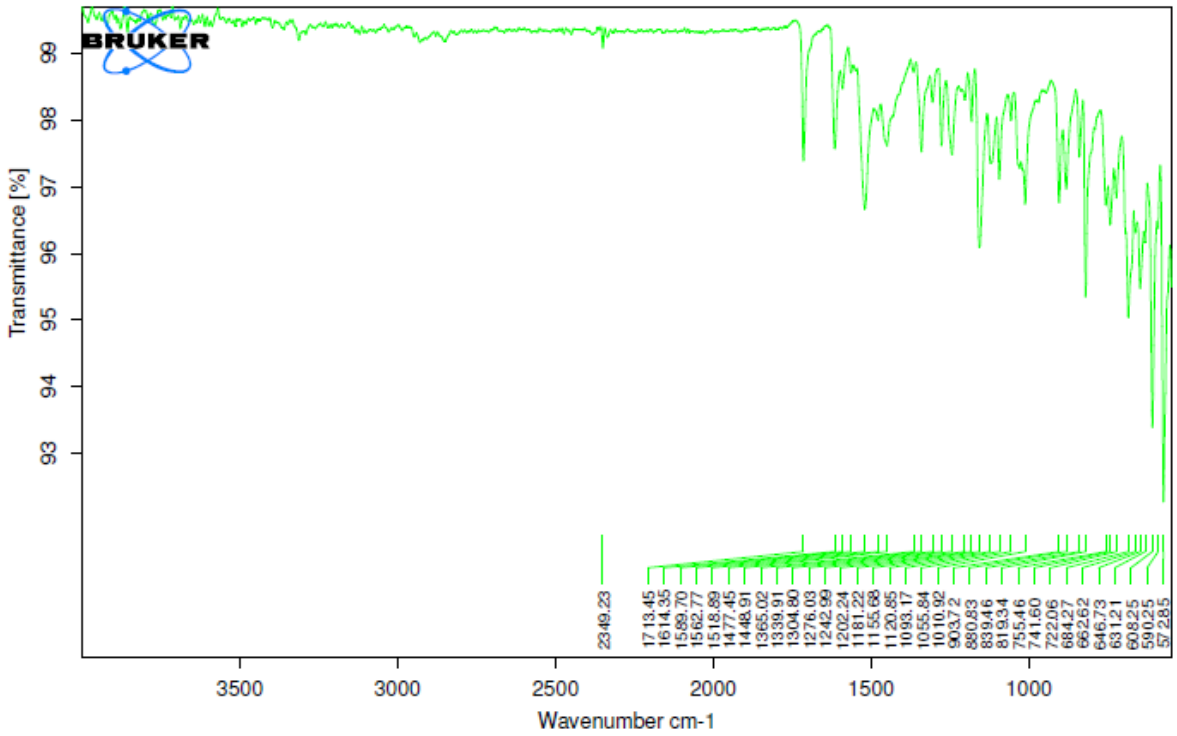

Figure 9: FTIR Spectrum of pure Amiloride drug

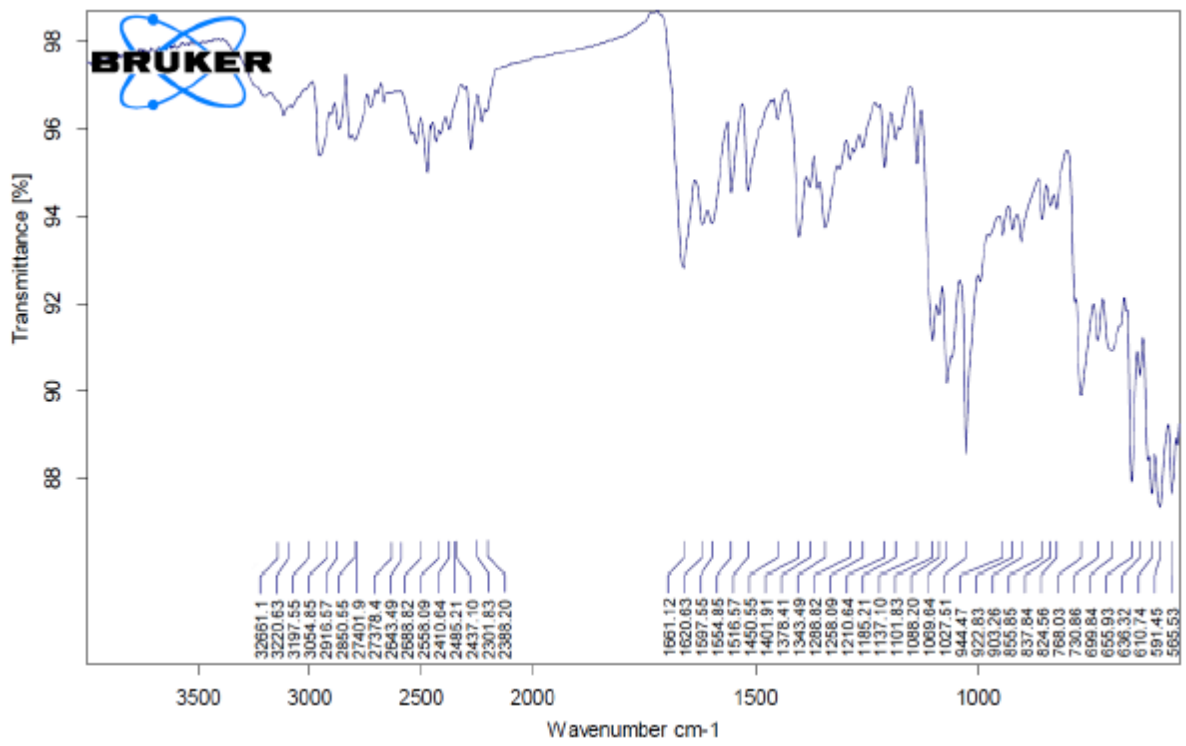

Figure 10: FTIR of Optimized formulation

The compatibility studies of the drug with excipients indicate no characteristic visual changes and no additional peaks were observed during FT-IR studies.

CONCLUSION

In the present investigation an attempt has been made to design and develop the formulation of Amiloride patches using different types of polymers by solvent evaporation technique and mercury substrate method. The drug used is the best studied for therapy in treating high blood pressure.

Amiloride was successfully formulated as controlled release transdermal patches, which prevents the frequency of administration and gives good patient compliance.

From the experimental results obtained, F1 formulation has been selected as the best formulation among all the other formulations. The *in vitro* drug diffusion studies from the formulation were found to be sustained release. All the evaluation parameters obtained from the best formulation were found to be satisfactory.

The data obtained from the *in vitro* release studies were fitted to various kinetic models like zero order, first order, Higuchi model and Pappas model.

From the kinetic data it was found that drug release follows peppas model release by diffusion technique from the polymer.

Based on the observations, it can be concluded that the attempt of formulation and evaluation of the Amiloride patches was found to be successful in the release of the drug for an extended period of 12hrs.

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