

Optimization of Factors Affecting Iontophoretic Drug Delivery of Diltiazem Hydrochloride Using Factorial Design

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Abstract: Gel formulation of Diltiazem hydrochloride was prepared using hydroxy propyl methyl cellulose polymer and Dimethyl sulfoxide as a permeation enhancer. Ethanol was added as a co-solvent for proper mixing of Dimethyl sulfoxide in gel. Four factors related to iontophoretic delivery like drug concentration, permeation enhancer concentration, quantity of applied electric current and area of application were optimized using fractional factorial design (2^{4-1}). Twelve formulations of Diltiazem hydrochloride gel were prepared and evaluated for in-vitro drug release study using modified diffusion cell. Steady state flux (Jss) for all prepared formulations were calculated as a response and data was evaluated using Design-Expert® software. Based on optimization results final optimized formulation was prepared by using drug concentration of 2.56 % w/w, permeation enhancer concentration of 5% w/w, 0.49 mA current and 1.50cm² of application area and evaluated for in-vitro iontophoresis using diffusion cell. Steady state flux was found to be 296 µg/h cm² which was closer to the targeted theoretical Jss (300 µg/h cm²). Desired therapeutic concentration of Diltiazem hydrochloride was achieved by optimizing various factors affecting iontophoretic drug delivery. Iontophoretic drug delivery of Diltiazem hydrochloride using gel formulation is possible.

Keywords: Permeation enhancer, Iontophoresis, Factorial design, Steady state flux.

Introduction

Iontophoretic drug delivery through skin improves patient's convenience over parenteral route of drug administration. Additionally, drug delivery in a controlled manner is possible for iontophoretic drug delivery. Now a day iontophoretic drug delivery is useful to treat not only the local treatment but also used for controlled systematic drug delivery [1]. Treatment of cardiovascular disease using iontophoretic drug delivery is still a challenging field for development [2].

A calcium channel blocker - Diltiazem hydrochloride is commonly prescribed for the treatment of cardiovascular diseases [3]. But due to its first pass hepatic metabolism, shorter half life and protein binding its bioavailability is poor. Considering above limitation and physicochemical property of

Diltiazem hydrochloride is a strong candidate for transdermal drug delivery through skin. There are various studies reported for transdermal delivery of Diltiazem hydrochloride using film and gel formulation [4, 5].

Considering pharmacokinetic parameters of Diltiazem hydrochloride, $t_{1/2}$ = 4 hrs, V_d = 305 liters and C_{ss} = 40 ng/ml, desired steady state flux was 300 µg/h cm² [6]. Hence, designed iontophoresis formulation should have 300 µg/h cm² drug releases to achieve therapeutic drug concentration. In the present study a gel formulation of Diltiazem hydrochloride was prepared and various factors affecting iontophoretic delivery was optimized using fractional factorial design [7,8].

Material and Methods

Diltiazem hydrochloride film was prepared by using hydroxy propyl methyl cellulose as a polymer and Dimethyl sulfoxide as a permeation enhancer [9-11]. Polymer and permeation enhancer was selected based on primary study along with other polymers and permeation enhancers [12]. A Gel formulation of Diltiazem hydrochloride was prepared by adding hydroxy propyl methyl

cellulose polymer in cold water under continuous stirring. In the prepared gel drug solution in water, Methanol and Dimethyl sulfoxide was added and stirred continuously until it mix properly. Ethanol acts as a co-solvent for proper mixing of Dimethyl sulfoxide in gel [13]. Detail composition of different gel formulation prepared for optimization study is given in the TABLE 1.

Table 1: 2⁴⁻¹ factorial design for formulations of Diltiazem hydrochloride Gel

Batch	Quantity of applied electric current	Drug concentration	PE concentration	Radius of application area
	mA/cm ²	% w/w	% w/w	cm
1	0.10	1.00	1.00	0.80
2	0.30	2.00	3.00	1.15
3	0.50	1.00	1.00	1.50
4	0.10	3.00	1.00	1.20
5	0.30	2.00	3.00	1.15
6	0.50	3.00	1.00	0.80
7	0.10	1.00	5.00	1.50
8	0.30	2.00	3.00	1.15
9	0.50	1.00	5.00	0.80
10	0.10	3.00	5.00	0.80
11	0.30	2.00	3.00	1.15
12	0.50	3.00	5.00	1.50

Factorial Design

Factors affecting iontophoresis are physico-chemical property of drug substance, anatomy and physiology of skin and type of formulation. Factors related to drug and physiology of skin will remains constant and cannot be optimized for iontophoretic drug delivery [14, 15]. Only factors related to formulation will be optimized to get desired drug delivery through skin using iontophoresis.

A gel formulation of Diltiazem hydrochloride was prepared and various factors related to formulation like drug concentration, permeation enhancer concentration, quantity of applied electric current (mA) and area of application (Radius of application area) were optimized using fractional factorial design (2⁴⁻¹).

Independent variables and its quantity were optimized using Design-Expert® software. Following four independent variables were studied at two levels.

- I. Drug concentration.
- II. Quantity of applied electric current.
- III. Permeation enhancer concentration (PE)
- IV. Radius of application area.

To get significant model fitting from Design-Expert® four center points also studied. Effect of formulation variables of steady state flux (J_{ss}) was measured as a response. A possible experiment (Run) for 2⁴⁻¹ factorial design with four variables is given in the TABLE-2.

Table 2: Level of independent variables for fractional factorial design

Independent variables	Quantity of applied electric current	Drug concentration	PE concentration	Radius of application area
Units	mA/cm ²	% w/w	% w/w	cm
Lower level (-1)	0.10	1.00	1.00	0.80
Higher level (+1)	0.50	3.00	5.00	1.50
Center points (0)	0.30	2.00	3.00	1.15

As given in the TABLE 3 total twelve formulation of Diltiazem hydrochloride was

prepared and evaluated for iontophoretic drug delivery using diffusion cell.

Table 3: 2⁴⁻¹ factorial design with four variables in coded form

Experiment No.	Independent variables (coded formulation)			
	Quantity of applied electric current	Drug concentration	PE concentration	Radius of application area
1	-1.0	-1.0	-1.0	-1.0
2	+1.0	-1.0	-1.0	+1.0
3	-1.0	+1.0	-1.0	+1.0
4	+1.0	+1.0	-1.0	-1.0
5	-1.0	-1.0	+1.0	+1.0
6	+1.0	-1.0	+1.0	-1.0
7	-1.0	+1.0	+1.0	-1.0
8	+1.0	+1.0	+1.0	+1.0
Center points				
9	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00
11	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00

A steady state flux of drug was calculated by plotting amount of drug release verses time. Slop of the straight line was determined as a Steady state flux (Jss)[16, 17].

***In vitro* Evaluation of Drug Release**

In-vitro release profile of drug from the prepared formulations was determined using modified diffusion cell. Diffusion cell consists of a donor compartment, receptor compartment and reference compartment.

A porcine skin was used as a semipermeable membrane for diffusion study. Prepared gel formulation of Diltiazem hydrochloride was placed in the donor chamber on the porcine skin and above the gel formulation an electrode was placed [18]. Similarly for reference chamber a gel formulation without drug was placed. Both electrodes were attached to a DC current generator. Phosphate buffer solution (pH 7.4) was filled in receptor compartment [19, 20]. Sample (2

ml) was withdrawn from receptor compartment at every hour and analyzed using UV visible spectrophotometer. Sample withdrawn quantity was replaced by fresh buffer solution in receptor compartment. Amount of drug release from the formulation was measured up to 6 hours and drug release was calculated. Steady state drug release (Jss) calculated by calculating slop of the graph plot (cumulative amount of drug release Vs time)[21].

Results and Discussion

Total twelve formulation of Diltiazem hydrochloride was prepared and evaluated for in-vitro drug release using diffusion cell. Steady state flux for in vitro dissolution

profile was calculated and results for the same are given in TABLE 4.

Table 4: Result of Steady state flux for all formulations

Experiments	Factor				Response
	Quantity of applied electric current	Drug concentration	PE concentration	Radius of application area	Steady state flux (Jss)
Unit	mA	% w/w	% w/w	cm	mcg/cm ² h
1	0.10	1.00	1.00	0.80	169.4
2	0.30	2.00	3.00	1.15	248.7
3	0.50	3.00	1.00	0.80	263.0
4	0.10	3.00	5.00	0.80	265.1
5	0.30	2.00	3.00	1.15	224.4
6	0.50	1.00	5.00	0.80	262.1
7	0.10	3.00	1.00	1.50	219.2
8	0.30	2.00	3.00	1.15	234.1
9	0.50	1.00	1.00	1.50	216.2
10	0.10	1.00	5.00	1.50	216.9
11	0.30	2.00	3.00	1.15	247.3
12	0.50	3.00	5.00	1.50	314.6

Results of all twelve experiments were incorporated in to the Design-Expert®

software and summary of the outputs of the study is presented in the TABLE-5.

Table 5: Summary of factorial design study

2 Level fractional design with 12 runs and 4 center points								
Variable		Units	Min	Max	Code		Mean	SD
A	Quantity of Current	mA	0.1	0.5	-1 = 0.1	+1 = 0.5	0.3	0.17
B	Drug Conc.	%	1.0	3.0	-1 = 1.0	+1 = 3	2.0	0.85
C	PE Conc.	%	1.0	5.0	-1 = 1.0	+1 = 5	3.0	1.70
D	Radius	cm	0.8	1.5	-1 = 0.8	+1 = 1.5	1.15	0.29
Response		Units	Min	Max	Mean	SD	Ratio	Model
R	Jss	µg/cm ² h	169.461	314.6	240.1	35.85	1.85	Main effect

Evaluation of selected model with standardized effects and error term with

values and % contribution for selected response Jss is given in the Fig. 1.

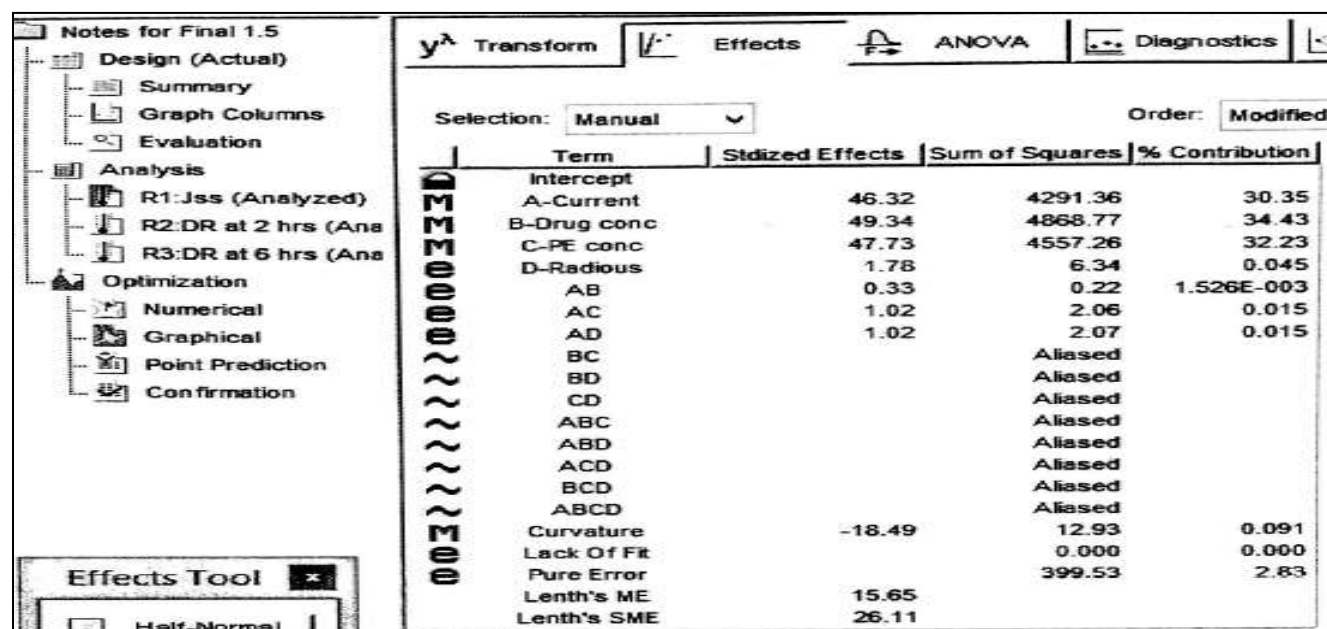


Figure 1: Standardized effects and error term for selected model

Normal plot of standardized effects and error

term for the selected model is given in the Fig. 2.

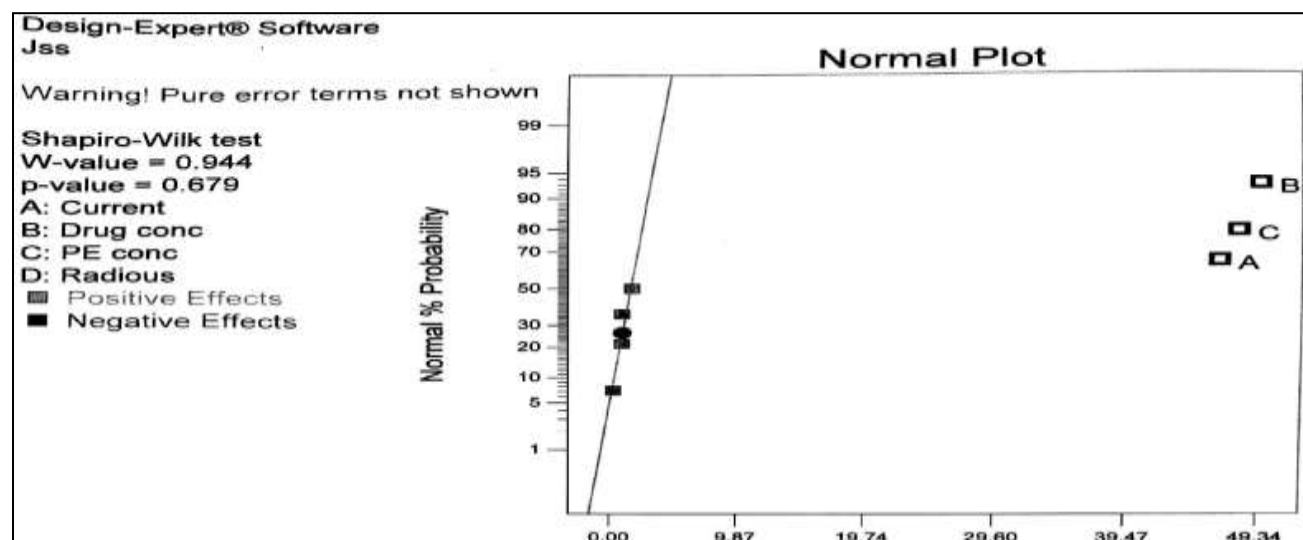


Figure 2: Normal plot of standardized effects and error term for the selected model

Value of standardized effect and Normal plot indicates that factors like quantity of current, drug concentration and permeation enhancer concentration have significant effect on steady state drug flux J_{ss} . Fourth independent factor radius of application area and combined effect of two independent variables was considered as an error term for selected model. Steady state flux was calculated as an amount of drug diffused due to iontophoresis per unit area, as a result area (Radius) remains constant for selected model. However, to get desired drug release for therapeutic effect, radius of application

area was incorporated in the study as an independent variable [22, 23].

The ANOVA results given in the fig. 3 indicate that F value of 86.15 implies the model is significant. There is only 0.01 % chance that a model is not significant. Value of Probability > F less than 0.05 indicates model term as a significant. In the present model Quantity of Current, drug concentration and permeation enhancer concentration are significant model term [4,24].

ANOVA for selected factorial model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	13717.40	3	4572.47	86.45	< 0.0001	significant
A-Current	4291.36	1	4291.36	81.13	< 0.0001	
B-Drug conc	4868.77	1	4868.77	92.05	< 0.0001	
C-PE conc	4557.26	1	4557.26	86.16	< 0.0001	
Residual	423.15	8	52.89			
Lack of Fit	23.62	5	4.72	0.035	0.9985	not significant
Pure Error	399.53	3	133.18			
Cor Total	14140.55	11				

Figure 3: ANOVA results for selected model

The “Lake of fit F-Value” of 0.04 implies the lack of Fit is not significant relative to the pure error. There is a 99.85 % chance that “Lake of fit F-Value” this large could occur due to noise. Non-significant lake of fit is desired for good model fit.

Finalized equations in term of coded and actual value based on factorial design are presented below:

Final equation in terms of coded factors

$$J_{ss} = + 240.14 + 23.16 * A + 24.67 * B + 23.87 * C \quad \dots (1)$$

Final equation in term of actual values

$$J_{ss} = +120.14 + 115.25 * \text{Current} + 24.67 * \text{Drug conc.} + 11.93 * \text{PE conc.} \dots (2)$$

Above equation also indicates that steady state flux (J_{ss}) is dependent on the quantity of applied current, drug concentration in gel formulation and permeation enhancer concentration.

Optimization of Formulation

To optimize all independent variables various formulation was suggested by Design-Expert® software. Various suggested results to achieve desired J_{ss} of 300 $\mu\text{g/h cm}^2$ is given in the fig. 4.

Constraints						
Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A:Current	is in range	0.1	0.5	1	1	3
B:Drug conc	is in range	1	3	1	1	3
C:PE conc	is in range	1	5	1	1	3
J_{ss}	is target = 300	169.461	314.638	1	1	5
Solutions						
Number	Current	Drug conc	PE conc	Radius*	J_{ss}	Desirability
1	0.49	2.56	4.99	1.50	299.999	1.000
2	0.46	2.80	4.78	1.50	300	1.000
3	0.46	2.91	4.62	1.50	299.999	1.000
4	0.50	2.74	4.56	1.50	300	1.000
5	0.49	2.71	4.74	1.50	300	1.000
6	0.46	2.84	4.84	1.50	300	1.000
7	0.48	2.85	4.47	1.50	300	1.000
8	0.40	2.99	4.95	1.50	300	1.000
9	0.42	2.92	4.97	1.50	300	1.000
10	0.45	2.81	4.84	1.50	300	1.000

Figure 4: Design Expert® suggested optimized formulations

There was number of solution suggested by Design-Expert® software for desired Jss. Out of that an optimized gel formulation of Diltiazem hydrochloride was prepared by using drug concentration of 2.56 % w/w, permeation enhancer concentration of 5% w/w, 0.49 mA of applied current and 1.50cm² of application area. Optimized formulation was evaluated for in-vitro iontophoresis using diffusion cell and steady state flux was found to be 296 µg/h cm² which is near to the targeted Jss of 300 µg/h cm². Hence, it is

possible to optimize formulation variables using Design-Expert® software [25].

Conclusion

Factorial design is a useful tool to optimize formulation for iontophoretic drug delivery of Diltiazem hydrochloride. Desired therapeutic concentration of Diltiazem hydrochloride was achieved by optimizing various factors affecting iontophoretic drug delivery. Iontophoretic drug delivery of Diltiazem hydrochloride using gel formulation is possible [26].

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