FRACTIONAL FACTORIAL DESIGN APPROACH FOR STUDYING IN VITRO TRANSDERMAL DELIVERY OF GRANISETRON USING ELECTROPORATION

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Abstract

Granisetron Hydrochloride is a 5-hydroxytryptamine 3 (5-HT_3) receptor antagonist used for the prevention and treatment of nausea and vomiting associated with cancer chemotherapy. Based on the hydrophilicity of this drug (pk_a=9.4) it is unlikely that passive diffusion across the skin could deliver therapeutic amounts from a large sized patch. This study was conducted to evaluate the feasibility of in vitro transdermal delivery of granisetron using electroporation across full thickness hairless rat skin. A 2^{4-1} fractional factorial design was used to determine the most important variables affecting the transdermal delivery of granisetron using electroporation. The variables studied and respective levels investigated were drug concentration (10, 20 mg/ml), voltage (155, 310 volt), pulse duration (12, 24 ms) and number of pulses (100, 200 pulses). Permeation profiles were used for the representation of data, where Q: the cumulative amount permeated per cm^2 (µg/cm^2) was plotted against time (min). The selected dependent variable (response) was Q_{120} which is the cumulative amount permeated per cm^2 after 2 hours. Design-expert version 7.0.0 was used for the statistical evaluation. The results of the analysis of variance (ANOVA) revealed that Q_{120} was significantly affected by the voltage, pulse duration and number of pulses. The drug concentration had a non significant effect on Q_{120}. A reduced polynomial regression equation which expresses the influence of process parameters on the response was obtained to enable navigation of the experimental space.

Keywords: Granisetron; Transdermal; Electroporation; Fractional factorial design.

Introduction

Control of chemotherapy-induced nausea and emesis remains an important issue in cancer therapy. The discovery of 5-hydroxytryptamine 3 (5-HT_3) receptors in afferent vagal nerve fibers and in neurons in the gastrointestinal tract suggested that chemotherapeutic agents such as cisplatin may induce the release of 5-HT_3 in the

small intestine, thereby initiating nausea and vomiting by stimulating the vagus nerve. Therefore, blockage of 5-HT_3 receptors in the small intestine by 5-HT_3 receptor antagonists might prevent the initiation of this reflex [(1)].

Some of 5-HT_3 receptor antagonists are available world wide in oral and intravenous dosage forms only and are used in the prevention and therapy of nausea and vomiting associated with cancer chemotherapy. However, they suffer from patient incompliance as well as being invasive in case of injections and susceptible for being vomited in case of tablets.

Granisetron (MW 348.9, $K_{o/w}$ 0.38, pK_a 9.4) is a selective 5-hydroxytryptamine 3 (5-HT_3) receptor antagonist shown to be effective in the treatment of nausea and vomiting induced by cancer chemotherapy. Intravenous infusion of 40 µg/kg body weight of granisetron is effective to prevent nausea and vomiting for repetitive chemotherapeutic regimens. Granisetron is very potent, hydrophilic in nature and has high first-pass hepatic metabolism [(2)].

Transdermal delivery offers a convenient alternative, particularly where nausea prevents administration of an oral dosage form [(3)]. Granisetron has a relatively small molecular weight and a little daily dose which make it suitable for transdermal delivery [(2)]. Yet, it is unlikely that passive diffusion across the skin could deliver therapeutic amounts of drug from reasonably sized patches because of its high hydrophillicity, which doesn't match with the lipophilic nature of the stratum corneum.

Electroporation is the physical process of inducing transient permeability of biological membranes by high voltage short pulses. This effect has been used in the laboratory for more than a decade as a research tool to facilitate cellular uptake of genetic material *in vitro*. More recently, electroporation has also been found effective for the intracellular delivery of molecules in living tissues, which led to a variety of medical applications. Some of these applications have already proceeded to clinical trials [(4)].

While iontophoresis utilizes a small amount of electric current (from 0.1 to 0.5 mA/cm^2) applied for a relatively long period (from minutes to hours) to push the drug through the skin, electroporation consists in the application of short (from 100 µs to less than 1s) high-voltage pulses (from about 100 to 1000 V) [(5)]. That is to say while iontophoresis acts primarily on the drug, electroporation acts on the skin with some driving force on the drug during a pulse.

Design of experiments has become a highly developed area. Factorial design is one of the experimental designs which enable excellent investigation of variables effects and interactions between variables. However, the number of runs $n = 2^k$ required by full factorial design increase geometrically as k is increased for e.g. k = 4, n = 16 etc. Moreover, a lot of calculated coefficients corresponding to interactions of second (and higher) order can be supposed to be insignificant [6]. Therefore, fractional factorial designs 2^{k-p} can be used as an efficient alternative. A fractional factorial design requires running only a fraction of experiments in the complete factorial design. They also help to identify factors and interactions that have significant effects [7].

A report was published on transdermal delivery of granisetron by radiofrequency-driven skin microchanneling [3]. Another paper was published to demonstrate the iontophoretic delivery of the same drug from solutions [2]. In our previous work the optimum conditions for iontophoretic delivery of granisetron from solutions across hairless rat skin were determined [8]. In another work a suitable gel formulation for the iontophoretic transdermal delivery of the same drug across hairless rat skin was optimized [9].

Thus, this work aims to study the feasibility of and to optimize the conditions for in vitro transdermal delivery of granisetron using electroporation.

Materials and Methods

Materials

Granisetron hydrochloride was obtained as a gift from Kahira Pharmaceuticals & Chemical Industries Company, Egypt; Potassium dihydrogen ortho-phosphate (Winlab, U.K.); Sodium hydroxide of analar grade (Adwic Co., Cairo, Egypt) and deionized water of resistivity ≈ 18 MΩ cm were used.

Equipment

Electrical balance (A&D Company limited); Spectrophotometer (UV-1601, Shimadzu, Japan); Magnetic stirrer (Thermolyne Corporation, USA) and Water deionizer (PUR1TE Still plus, USA)

Experimental design

Previous studies [10-12] showed that the different electrical factors (voltage, pulse duration and number of pulses) allowed control of the quantity of drug delivered by

electroporation. To check whether these electrical factors as well as drug concentration will affect granisetron permeation, a 2^{4-1}_{IV} fractional factorial design was applied [13]. The independent variables studied and respective levels investigated are shown in table 1.

This specific design, comprising 8 treatment combinations, is described as a 2^{4-1} design of resolution *IV* (4). Main effects are not confounded with two-way interactions, but only with three-way interactions. Two-way interactions in this design are confounded with each other. The generator of choice will be I = ABCD, to obtain the highest possible resolution (IV). Because the generator ABCD is positive, the fraction studied is the principal fraction. Using the defining relation, the aliase structures are shown in table 2. [13].

Voltages are expressed as applied values (transelectrodes), not as transdermal values [5, 14, 15]. Square wave pulses were selected to achieve reproducible constant pulse voltage and duration whatever the skin resistance and donor composition are [14, 16, 17]. The different variables levels for the eight experimental combinations are shown in table 3.

Each experiment was done in a duplicate using different skin samples giving rise to sixteen runs which resulted in fifteen degrees of freedom. Seven of these degrees of freedom were associated with the seven aliase structures and the remaining eight were due to error.

At this point the sixteen runs were performed in a random order to minimize the effect of systematic errors [18], according to the protocol stated later, and the data were collected for further treatment.

Preparation of skin membranes

Hairless rat skin [10,19] was used as a model skin membrane. Newly born albino rats (2-3 days age) were sacrificed. Full thickness skin was excised. The dermal surface was carefully cleaned to remove subcutaneous tissue without damaging the epidermal surface. The skin was soaked for 24 hours in phosphate buffer pH 7.4 before use. The skin was stored in the frozen state in phosphate buffer pH 7.4 and left to thaw at room temperature (25±1°C) before each experiment.

Transdermal permeation experiments

The permeation experiments was carried out using double open sided tube [20] as a part of the permeation assembly shown in figure 1. The donor vehicle was 5 ml of

the solution under study placed on the dorsal side of the skin. The available skin diffusion area was 5.107 cm^2. The receptor medium was 75 ml of phosphate buffer pH 7.4 stirred using a magnetic stirrer at 300 rpm. Experiments were carried out at room temperature (25±1°C). A pair of silver wires [16, 21] of diameter 1 mm and effective length of 10 mm) were connected to the outputs of the electroporation apparatus. The anode was placed in the donor site about 5 mm away from the dorsal. Pulses were given with a frequency of 1 Hz and zero time started just after the end of the pulses. Samples of 3 ml were withdrawn at predetermined intervals and compensated immediately with fresh receptor medium.

Experimental setup for the electroporation apparatus / device

Figures 2 and 3 show the experimental setup for custom made electroporation device used in the experiments. As illustrated in figure 2, the block diagram gives an idea about the different steps for generating high voltage (320V) square pulses. Figure 3 presents the detailed circuit diagram. The different parts can be described as:

- A monostable (555) multivibrator which is employed to generate precise prechosen time pulses. The monostable pulse generator has the usual configuration with an output pulse duration of 1.1 R_aC. The monostable has five preset values; .1 ms, 1.1 ms, 2.2 ms, 5.5 ms, 12 ms and 24 ms, from which we used 12ms and 24ms in our experiments. Sometimes the monostable circuit may mistrigger even with the control pin bypass capacitor. To prevent this from happening, a capacitor 0.001μF and a resistor 10kΩ were added to the input of the monostable to avoid the problems of mistriggering,
- The setup also contains a pulse counter to monitor the monostable pulses.
- An opto coupler 4N35 is used to isolate the high voltage side from the low voltage side.
- These pulses trigger an n-MOS transistor switch with 500V V_{CE} max (maximum collector to emitter voltage) and 0.5Ω saturation resistance. The n-MOS transistor switch (IRF 840) is interrupting a DC voltage of 155/310V. . It should be mentioned that the MOS switch is 4 way switch which is capable of handling 4 different signals at the same time.
- This 155/310 resulting from the rectification and filtering of the line voltage (with or without a 110V/220V Transformer).

- A digital storage oscilloscope tests before the beginning of the experiments for the duration and amplitude of the high voltage pulses.

Figure 4 presents one of the pulses with the horizontal and vertical axis adjusted to 10ms/div and 50V/div respectively. It is clear that the pulse has 24ms pulse width and 310V amplitude of the peak.

Quantification of granisetron

For granisetron quantification in receptor solution, samples were withdrawn at specified intervals from the receptor compartment and were analyzed spectrophotometrically for drug concentration at λ = 302 nm. Concentrations were derived from the calibration curve which was constructed and the absorbance was found to be linear within the concentration range of 4-60 µg/ml.

The drug concentration values were corrected for progressive dilution to obtain cumulative amount permeated using equation (1):

$$Q'_{t(n)} = V_r \cdot C_n + V_s \cdot \Sigma C_m \quad (1)$$

where, $Q'_{t(n)}$ is the current cumulative mass of drug transported across the membrane at time t, C_n represents the current concentration in the receptor medium, ΣC_m denotes the summed total of the previously measured concentrations [m = 1 to n – 1], V_r is the volume of the receptor medium and V_s corresponds to the volume of the sample removed for analysis [22].

Data treatment

The in vitro skin permeation data obtained were plotted as Q which is the cumulative amount of drug penetrated into the receptor compartment per unit area of skin membrane (µg/cm2) as a function of time (min) (15, 16).

The response (dependent variable) measured was Q_{120} (µg/cm^2), which is the cumulative amount permeated per cm^2 immediately after 2 hours. [15].

Statistical analysis

All data were statistically analysed using Design–Expert program version 7.0.0. [13] Means were compared by ANOVA-factorial and suitable regression models [23] were driven to enable navigation of the experimental space. Significance level was set at $P < 0.05$.

Results

Table 3 presents the 16 runs and the determined Q_{120}. The main effects and coefficients of the aliase structures were calculated and are shown in table 4.

The main effect estimate can be interpreted as the deviations of the mean of the response for the positive settings from the mean of the response for the negative settings for the respective aliase structure(table 4). The aliase coefficients shown in the same table could be used for the prediction of each response for new factor settings, via the linear equation:

$$Y_{pred} = b_0 + \Sigma b_j X_j + \Sigma b_{ij} X_i X_j \qquad (2)$$

where, Y_{pred} stands for the predicted response Q_{120}, i, j vary from 1 to the number of variables. Coefficient b_0 is the mean of responses of all the experiment. b_i represents the coefficient of the variable X_i, and it measures the change in the response y upon moving from the mean setting (coded value = 0) to the maximum settings (coded value = +1). Similarly b_{ij} are the coefficients of regression of the interactions of variables $X_i X_j$ [23]. So we observe that the coefficient terms are half the effects estimates [13].

After the estimation of the factors main effects, we used all aliase structures as model terms and ANOVA statistical analysis was used to determine the significant terms affecting the dependent variable of interest (Q_{120}).The details of the ANOVA calculations for the full model are not given and the p-value for each term is shown in table 4. The ANOVA data support the conclusion that, B, C, D, AB, AD are significant model terms.

For the full model analysis of variance, the R^2 statistics are as follows [24]:

- The ordinary $R^2 = 0.94282$ measures the proportion of total variability explained by the model.
- A potential problem with this statistic is that it always increases as factors are added to the model, even if these factors are not significant.
- The adjusted $R^2 = 0.89278$, which is a statistic that is adjusted for the "size" of the model; that is, the number of factors.
- The Prediction R^2 statistic = 0.77128 , indicates that the full model would be expected to explain about 77 % of the variability in new data.
- Adequate Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Our ratio of 12.699 indicates an adequate signal. This model can be used to navigate the design space.

Final equation in terms of Coded Factors for full model:

Q_{120} = 319.9+17.88 A+113.4B+84.88C+49.63D+37.38AB+26.63AC+69.38AD. (3)

Although the prediction R^2 of 0.7713 is in reasonable agreement with the adjusted R^2 of 0.8928, there are two insignificant terms in the full model in our model which are not needed to support hierarchy, so model reduction may improve the results.

Removing the 2 insignificant model terms (A and AC) led to a model with Prediction R^2 of 0.7702, ie. the percent of total variability explained by this model will be less than that explained by the full model. To find a better model we tried removing only 1 model term and we could obtain a model with a prediction R^2 of 0.7873. This reduced model is better than the full model and can explain 79 % of the variability associated with the Q_{120} response.

The insignificant model term A is the one with the least effect and will be removed in the reduced model. Also B, C and D are significant model terms. Then it is logic enough to think that the interactions CD, BD and BC are the interactions responsible for the aliased terms ℓ_{AB}, ℓ_{AC} and ℓ_{AD} respectively. This is an application of Ockham's razor (after William of Ockham), a scientific principle that when one is confronted with several different possible interpretations of a phenomenon, the simplest interpretation is usually the correct one [13]. The results of the statistical analysis of the chosen reduced model is as shown in table 6

The Model F-value of 20.79 implies that the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. This is also clear from the p-value [7] for the overall model which is very close to zero ($p < 0.0001$). B, C, D, BC, CD are significant model terms.

Furthermore, Validation of the regression model is necessary for testing its adequacy [7, 25]. This means how reliable the mathematical equation computed is for navigation of the experimental space. This can be done by testing the significance of the lack of fit. The residual sum of squares (SSR) of the model can be decomposed into the sum of squares corresponding to pure error (SSE) and the sum of squares corresponding to the lack of the fit (SSLOF). The model equation is considered to be adequate if Lack of Fit F-value is more than the tabulated value of F at a preselected value of the significance level [25]. For the reduced equation the "Lack of Fit F-value" of 1.42 implies the lack of fit is not significant relative to the pure error. Non-significant lack of fit is good.

Adequate precision for this model was calculated to be 13.273 indicating an adequate signal. Hence, this model can be used to navigate the design space.

Final equation in terms of coded factors for the reduced model:

$$Q_{120} = 319.88+113.38B+84.88C+49.63D+69.38BC+ 26.63BD+37.38CD \quad (4)$$

The reduced model equation with coded factors proves that we have 3 main factors affecting the response positively which are voltage, pulse duration and number of pulses, and 3 interactions. Two factors are said to interact with each other if the effect of one factor in the response is different at different levels of the other factor. In order to interpret the interactions, it is better to construct the interaction plots. Two of the three interactions are significant which are the positive interaction between voltage and pulse duration (BC) and the positive interaction between pulse duration and number of pulses (CD). The interaction plots for the significant terms are shown in figures 5 and 6. The lines are non-parallel to each other, so, an interaction exists between the factors. This implies that the change in the mean response from low to high level of a factor depends on the level of the other factor. Thus using this equation, the response Q_{120} can be represented by the contour plots shown in figures 7-9.

Discussion

Effect of electroporation on granisetron permeation

The results of all the permeation experiments showed that skin electroporation with any of the electrical protocols used is better than passive transport with no lag-times (figure 10). This indicates that granisetron molecules rapidly respond to electric pulses. The various values for Q_{120} depended on the pulsing protocols, showing that granisetron transport could be controlled by the electrical parameters [5].

There may be two possible mechanisms producing enhancement in transdermal granisetron delivery via electroporation. First, direct electrical repulsion similar to that seen in iontophoresis which plays an important role for the rapid onset of granisetron flux during the pulsing. Since granisetron is positively charged in the donor chamber electroosmosis may also be a contributing factor during the time the pulse is applied, though its contribution may be minimal due to the short pulse lengths. Secondly, electroporation enhanced passive diffusion may contribute to a

rise of granisetron flux since the permeability of the epidermis is increased dramatically due to electroporation-induced transient alteration of skin structure [26].

The increase in molecular transport by electroporation can be attributed to the creation of pores as well as by electrophoresis due to the local field. This electrophoresis is present only during pulsing. This may be the cause of the higher fluxes at the start of the experimental period for all the experiments [14]. Also rapid transport across the highly permeabilized skin may occur during pulsing [14,15].

Effect of drug concentration

Drug concentration appeared to have no significant effect on the permeation of granisetron over the experimental range tested in the present study, A finding which might be of economic advantage.

However, Chang et al. [27], reported that electroporation further enhanced the iontophoretic transdermal delivery of calcitonin only upon using higher drug concentration.

Effect of voltage

Changing voltage has proved to significantly affect the cumulative amount peremeated of granisetron. Voltage appeared to be the most important variable with the highest coefficient. The higher the voltage the higher the permeation is.

This result is consistent with known mechanisms for single bilayer electroporation, which demonstrated that the pore characteristics and sizes can be influenced significantly by pulsing voltage [28]. That is, the various applied voltages may alter the pore dimensions as well as pore size, which may in turn affect drug permeation rates [14].

These results are consistent with the results of many studies which showed that increasing the voltage increased the permeation. Sung et al [14] evaluated the transdermal permeation of nalbuphine and two of its prodrugs using different voltages (100, 300, 500). They found that the higher voltages resulted in higher total permeation amount. In a study conducted by Medi and Singh [16] a linear relationship ($R^2 = 0.97$) was found between electroporation voltage used and parathyroid hormone flux. Vanbever et al [29] found a linear relationship between the voltage and the cumulative metoprolol transported.

Yet, these results not in the same line of the findings of Jadoul et al., who reported a non significant difference in alniditan permeation upon changing the voltage of exponentially decaying pulses from 100 V to 200 V [12].

Effect of pulse duration

Pulse length could be the second most important parameter next to the voltage determining electroporative drug permeation [30]. This was the same in our study where its coefficient is the highest after that of voltage see equation 3. The increase in pulse duration enhanced the permeation of granisetron. This result is consistent with the results of many studies which showed that increasing the pulse duration or time constant increased the permeation.

Shamara et al. [19] found a fairly linear relationship ($r^2 = 0.94$) between terazosin hydrochloride delivered across hairless rat skin and the pulse lengths. Jadoul et al.[12] found a significant influence of time constant on alniditan transport. Sung et al [14] tested the influence of pulse duration on skin permeation of nalbuphine and its prodrugs by applying skins with twenty 500 V/100 ms and twenty 500 V/200 ms of pulses, the increase in pulse duration enhanced the permeation of the tested molecules. Vanbever et al [29] found a linear increment between the pulse duration and the cumulative metoprolol transported.

Many reports demonstrated that longer pulse duration may induce pores with larger sizes and thus enhance the permeation rates [28, 31, 32]. Other in vitro experiments show that the effective pore radius of the localized transport region (LTR) is proportional to pulse duration which is associated with Joule heating [32, 33].

Effect of number of pulses

As the number of pulses increased the amount permeated of granisetron increased. This can be explained by that more pulsing numbers may create larger size of pores [28, 32].

This is in the same line of findings of a study by Jadoul et al. [12] who reported that the application of twenty 300 V, 200 ms pulses resulted in a higher flux comparing to the application of ten 300 V/200 ms pulses for the delivery of nalbuphine and its produgs. In a study conducted by Hu et al [34] they found that fluxes of tetracaine increased with increasing the pulses number. This is in agreement with the findings by Jadoul and Préat [35] that increasing the number of pulses significantly enhanced domperidone permeation.

Significant interactions between B: voltage and C: pulse duration / C: pulse duration and D: number of pulses

Positive interaction occurred between B: voltage and C: pulse duration. Similar interaction was also found in alniditan permeation [12]. Another significant interaction was also found between C: pulse duration and D: number of pulses. These interactions may be due to that the two variables in each case acted synergistically to impair the barrier function of the stratum corneum, leading to higher granisetron permeation.

Conclusion

The fractional factorial design used led to the derivation of a reduced equation that could be used to navigate the experimental space. The most significant terms affecting Q_{120} were identified as voltage, pulse duration, number of pulses and the interaction between voltage and pulse duration. They all had a positive effect on Q_{120} and they account for about 86.59% of the experimental variance. Drug concentration did not affect granisetron permeation over the range used. The fluxes of all the experiments were much higher at the beginning of the experiments; this could be explained by the fact that granisetron electrophoresis contributes much to the permeation process. The maximum Q_{120} obtained with electroporation was 719 ± 120 µg/cm^2, which is much more higher than the values obtained from the passive diffusion (50± 2.10 µg/cm^2). This suggests that under the electrical protocols used in our studies electroporation is much more promising for granisetron delivery than passive diffusion.

Table 1: Variables studied and respective levels investigated

Variables	Levels investigated	
	Low (-)	**High (+)**
A: Drug Concentration (mg/ml)	10	20
B: Voltage (volt)	155	310
C: Pulse Duration (ms)	12	24
D: Number of Pulses	100	200

Table 2: Aliase structures for the 2^{4-1} factorial design

Alias Structures

ℓ_A = **A+BCD**

ℓ_B = **B+ACD**

ℓ_C = **C+ABD**

ℓ_D = **D+ABC**

ℓ_{AB} = **AB+CD**

ℓ_{AC} = **AC+BD**

ℓ_{AD} = **AD+BC**

Table 3: The variables levels and the response Q_{120} of the experimental combinations

Experiment	Treatment combination	Variables levels				Q_{120} (µg/cm^2)	
		A	B	C	D = ABC	Replicate 1	Replicate 2
1	*(1)*	-	-	-	-	192	183
2	*ad*	+	-	-	+	223	166
3	*bd*	-	+	-	+	338	262
4	*ab*	+	+	-	-	248	268
5	*cd*	-	-	+	+	239	290
6	*ac*	+	-	+	-	189	169
7	*bc*	-	+	+	-	520	392
8	*abcd*	+	+	+	+	634	804

Table 4: Effects, coefficients and sum of squares of all model terms and p-values for full model terms

Model Term	Main effect	Coefficent	p- value for full model terms
A-Drug Concentration	35.75	17.875	0.2681
B-Voltage	226.75	113.375	< 0.0001*
C-Pulse duration	169.75	84.875	0.0005*
D-Number of pulses	99.25	49.625	0.0108*
AB	74.75	37.375	0.0376*
AC	53.25	26.625	0.1142
AD	138.75	69.375	0.0017*

Table 5: ANOVA table for the reduced model

Source	Sum of squares	df	Mean square	F value	p-value
Model	471023.5	6	78503.917	20.788	< 0.0001*
B-Voltage	205662.25	1	205662.25	54.459	< 0.0001*
C-Pulse duration	115260.25	1	115260.25	30.521	0.0004*
D-Number of pulses	39402.25	1	39402.25	10.434	0.0103*
BC	77006.25	1	77006.25	20.391	0.0015*
BD	11342.25	1	11342.25	3.003	0.1171
CD	22350.25	1	22350.25	5.918	0.0378*
Residual	33988.25	9	3776.472		
Lack of Fit	5112.25	1	5112.25	1.416	0.2681
Pure Error	28876	8	3609.5		
Cor Total	505011.75	15			

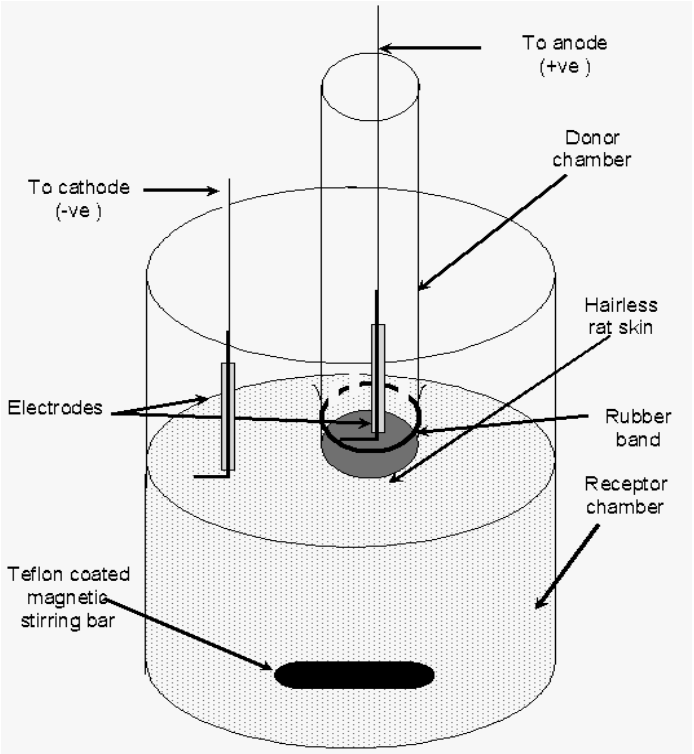


Figure (1) : Diagrammatic representation of the permeation cell.

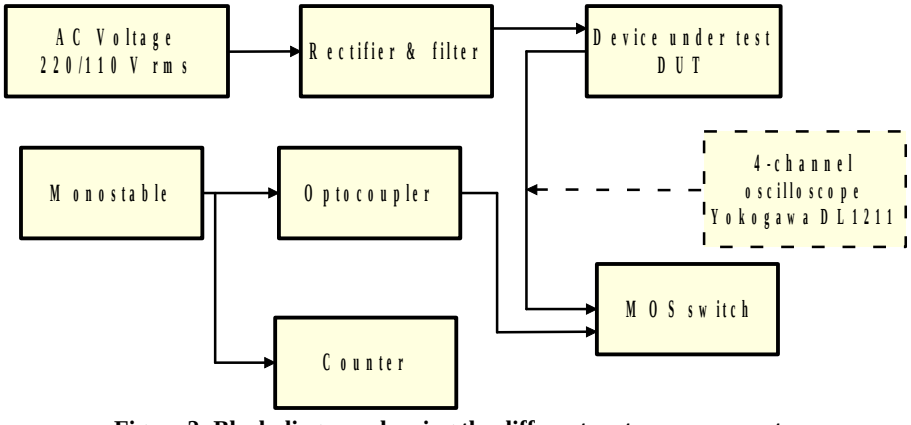


Figure 2: Block diagram showing the different system components

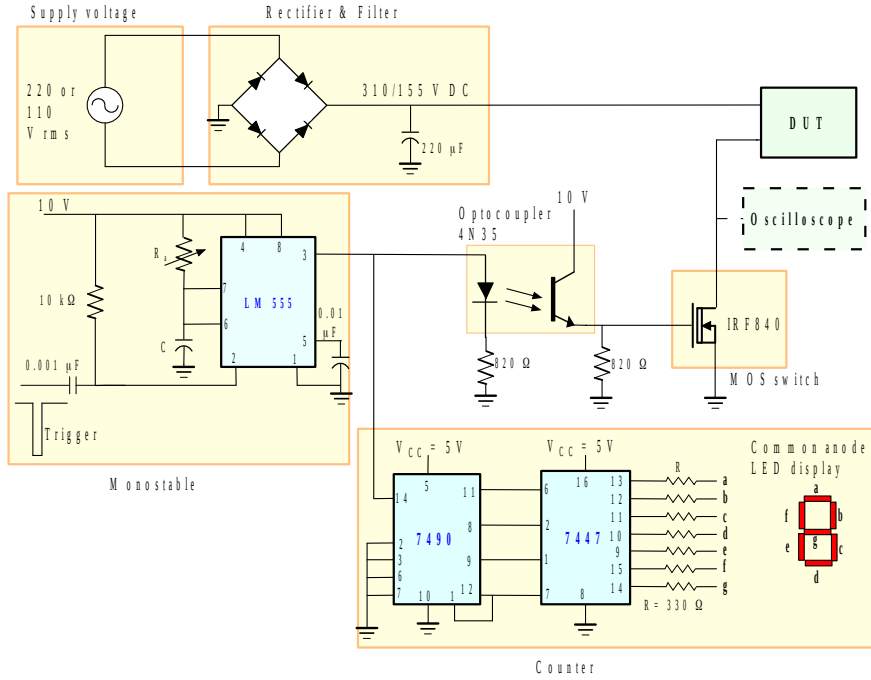


Figure 3: Detailed electrical circuit diagram.

Figure 4: Input Square wave pulse to the device under test as shown on the monitor of the oscilloscope

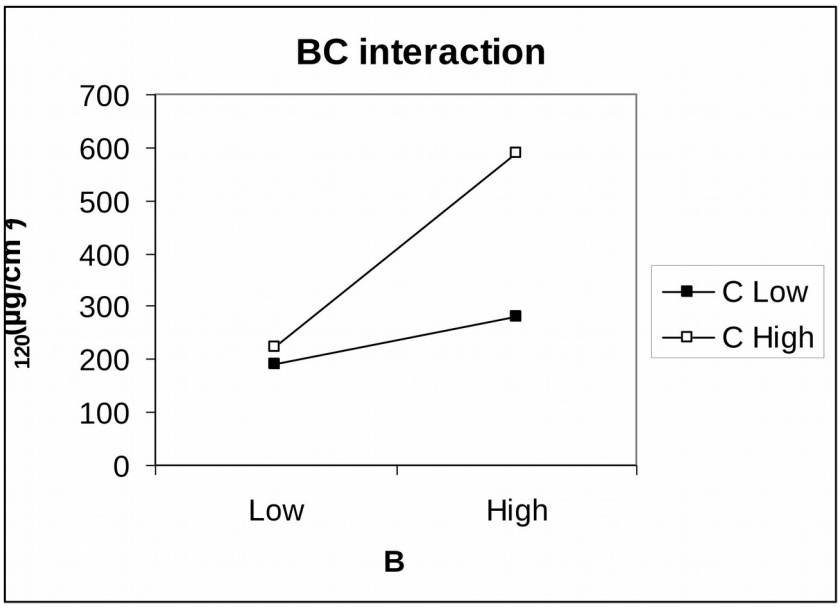
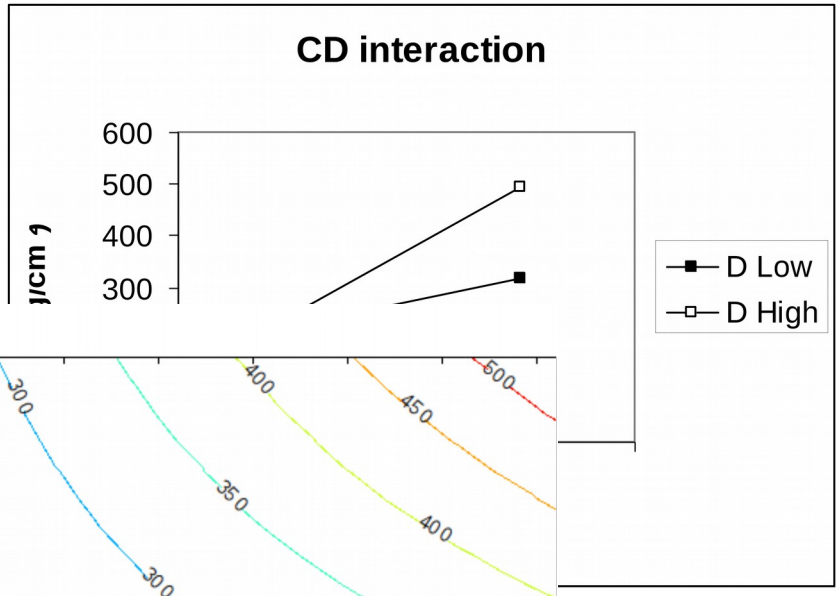
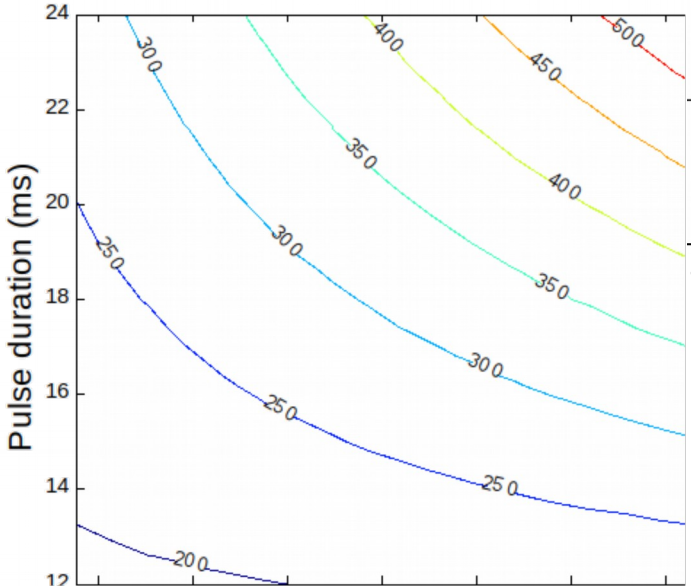


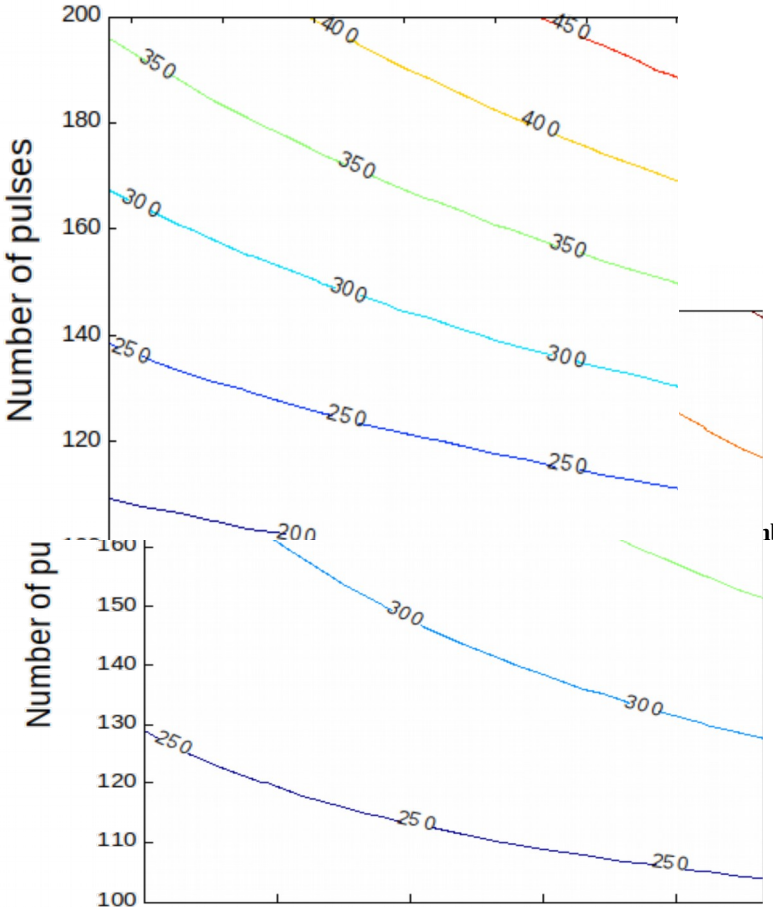
Figure 5: Interaction between B: voltage and C: pulse duration





and D: number of pulses.

Figure 7: Contour plot of Q_{120} between B: voltage and C: pulse duration at D: number of pulses = 150 (average number of pulses).



…ber of pulses, at C: pulse

Figure 9: Contour plot of Q120 between C: pulse duration and D: number of pulses at B: voltage = 232.5 volt (average voltage).

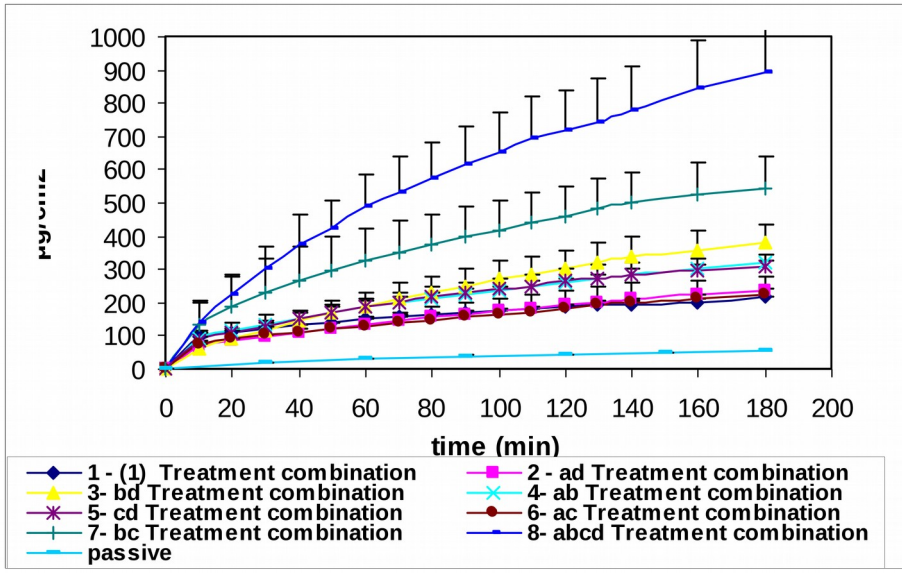


Figure 10: Cumulative amount of granisetron permeated per cm^2 across rat skin after passive or from different electroporation protocols.

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المــلخص العــربى

التصميم الأحصائى المعاملي الجزئي لدراسة توصيل عقار الجرانيسترون عبر الجلد معملياً بإستخدام التثيقب الكهربائى .

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عقار الجرانيسترون يعمل كمضاد انتقائى لمستقبلات خماسى هيدروكسى التريبتامين 5] [(5-HT_3) 3-hydroxytryptamine- وهـو يستخدم فى الوقاية والعلاج لحالات القىء والغثيان الناتجة عن العلاج الكيميائى والاشعاعى لمرضى السرطان ولعقار الجرانيسترون خاصيته المحبة للماء والتى لاتتوافق مع طبيعة الطبقة الخارجية من الجلد . ولهذا فإنه من غير المتوقع توصيل كميات علاجية من هذا العقار إلى الدم من لصقة علاجية مناسبة المساحة عن طريق النفاذية السلبية. وهذا يجعلنا مضطرين للبحث عن وسيلة فيزيائية مناسبة نستطيع من خلالها زيادة نفاذية عقار الجرانيسترون عبر الجلد. كان الهدف من هذا الدراسة هو إختبار إمكانية تحسين نفاذية عقار الجرانيسترون عبر الجلد معملياً باستخدام التثقيب الكهربائى electroporation

وقد أستخدم التصميم الإحصائى fractional factorial design 2^{4-1} لتحديد أهم المتغيرات المؤثرة على نفاذية عقار الجرانيسترون عبر الجلد بإستخدام التثيقب الكهربائى . وقد تم إختيار المتغيرات المدروسة ومستوياتها كالتالى : تركيز العقار (10،20 مللجم/مل), الجهد الكهربائى (155، 310 فولت) , مدة النبضة الزمنية (12، 24 مللى ثانية) , عدد النبضات (100، 200 نبضة) وتم رسم علاقة بيانية - بين كمية الدواء النافذ من خلال وحدة المساحة [Q ($\mu g/cm^2$)] والزمن .(min) وكان المتغير التابع هو كمية العقار المتراكم خلال 120 دقيقة من كل سم ^{2} [Q_{120} ($\mu g/cm^2$)]. وقد استخدم البرنامج الإحصائى Design-expert version 7.0.0 للتقييم الإحصائى. وأظهرت نتائج التحليل الأحصائى [ANOVA] أن Q_{120} قد تأثرت إحصائيا بشكل موجب مع تغير كلا من الجهد الكهربى والمدة الزمنية للنبضة وعدد النبضات. ولم يكن لتركيز العقار تأثيرا أحصائيا على Q_{120}. وتم الحصول على معادلة إنحدار مختصرة متعددة الحدود [Reduced polynomial regression equation] تمثل تأثير المتغير التابع Q_{120} بالمتغيرات المدروسة خلال المدى التجريبى .