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Formulation Development and Evalution of Transdermal Patch Og Anti-Diabetic Drug Pioglitazone

Mayank P. Patel*, M. M. Gupta[1]

1. Department of Pharmaceutics, Jaipur College of Pharmacy, Jaipur (Rajasthan) India
[Email: mayank.patel87@ymail.com]

In the present work, monolithic matrix transdermal systems containing Pioglitazone HCl were prepared using various ratios of the polymer blends of PVP K 30 and Eudragit NE 30 D with DMSO as a plasticizer. A 32 full factorial design was employed. The concentration of HPMC and ES were used as independent variables, while percentage drug release was selected as dependent variable. Physical evaluation was performed such as moisture content, moisture uptake, tensile strength, flatness and folding endurance. *In-vitro* diffusion studies were performed using Rat skin in a Franz's diffusion cell.The concentration of diffused drug was measured using UV-visible spectrophotometer at λ max 269 nm. The experimental results shows that the transdermal drug delivery system (TDDS) containing ES in higher proportion gives sustained the release of drug.

Keyword: PVP K-30, Eudragit NE 30 D, Pioglitazone HCl, Transdermal Delivery.

1. Introduction[1-3]

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 another. The difference between sustained release and controlled release is shown in **Fig. 1**.

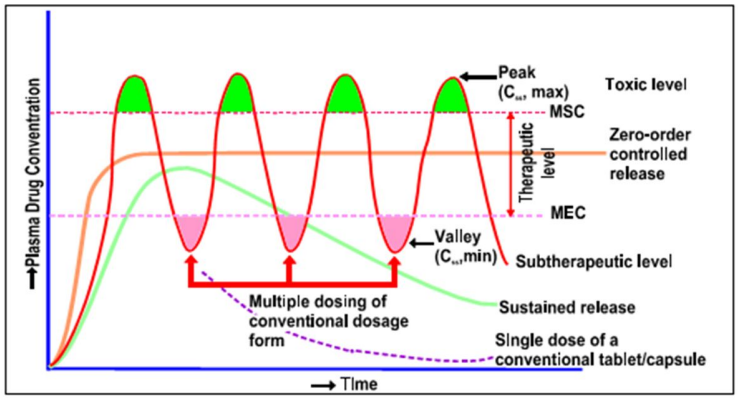


Fig 1: Comparative graphs of conventional, sustained and controlled release delivery systems

The classification of controlled drug delivery can be given as follows:

1. Rate-preprogrammed drug delivery systems
2. Activation-modulated drug delivery systems
3. Feedback-regulated drug delivery systems
4. Site-targeting drug delivery systems

Out of these classes first class contains new drug delivery systems as transdermal delivery, intra uterine delivery, ocular inserts, and sub dermal implants. The transdermal drug delivery has advantage to deliver medicines via skin to systemic circulation at a predetermined rate and maintain therapeutic concentration for prolong period of time.

2. Material And Method:

2.1 Evaluation of Transdermal Patch:

- Polymers Eudragit NE 30D (7%, 11% and 15%) and PVP K-30 (1%) were weighed accurately and dispersed with stirring in acetone.
- Drug (10mg) was weighed accurately and dissolved with stirring in varying amounts of DMSO (20%, 25%, and 30%).
- The polymeric dispersion was added to drug solution with gentle stirring, followed by addition of TEC (10%) to the solution.
- The solution was kept in a sonicator for 20 min.
- The solution was then gently poured into a glass ring mould (area 23.75 cm^2) placed over an aluminium foil and kept undisturbed for 24 hours, with an inverted funnel over it.
- On solvent evaporation, the film was further dried in a desiccator at 55°C for 3 hours and then packed in aluminium foil until use.

Table 1: evalution of weight, thickness and folding endurance of transdermal patch

Sr. No	Formulation Code	Weight (gm) *	Thickness (mm)*	Folding Endurance*
1	**A1**	0.97 ± 0.049	0.15 ± 0.008	335 ± 3.56
2	**A2**	0.98 ± 0.023	0.16 ± 0.012	340 ± 4.68
3	**A3**	0.98 ± 0.036	0.17 ± 0.008	350 ± 5.34
4	**A4**	0.99 ± 0.042	0.19 ± 0.013	530 ± 4.87
5	**A5**	1.10 ± 0.052	0.18 ± 0.006	540 ± 5.21
6	**A6**	0.99 ± 0.048	0.18 ± 0.014	530 ± 4.33
7	**A7**	1.12 ± 0.061	0.20 ± 0.014	730 ± 5.77
8	**A8**	1.13 ± 0.035	0.21 ± 0.009	740 ± 2.89
9	**A9**	1.14 ± 0.027	0.23 ± 0.015	740 ± 3.62

Table 2: % Moisture content and moisture uptake and drug content of formulations A1-A9

Sr. No	Formulation Code	% Moisture Content (%)*	% Moisture Uptake (%)*	% Drug Content*
1	**A1**	1.78 ± 0.011	3.63 ± 0.014	96.86 ± 0.21
2	**A2**	3.77 ± 0.011	5.55 ± 0.013	96.20 ± 0.23
3	**A3**	5.46 ± 0.013	6.25 ± 0.015	97.77 ± 0.20
4	**A4**	1.67 ± 0.012	3.32 ± 0.011	96.37 ± 0.20
5	**A5**	3.45 ± 0.012	5.33 ± 0.013	97.95 ± 0.21
6	**A6**	5.13 ± 0.011	6.12 ± 0.014	96.24 ± 0.22
7	**A7**	1.89 ± 0.015	3.53 ± 0.015	98.91 ± 0.18
8	**A8**	3.93 ± 0.014	5.50 ± 0.017	97.17 ± 0.24
9	**A9**	5.69 ± 0.015	6.28 ± 0.019	98.41 ± 0.22

* mean ± SD (n = 3)

2.2 *In vitro* dissolution study

- *In vitro* dissolution study using USP type-V apparatus (paddle over disk) was performed to find an estimate of approximate time taken for drug release, before proceeding over to the skin permeation study. As a change in concentration of permeation enhancer would not affect dissolution significantly, only batches A3, A6 and A9 having varying concentrations of polymer but highest concentration of permeation enhancer were taken up for this study.

The average absorbance found for placebo (in triplicate) was -0.005 ± 0.001. Thus, no interference could be accounted by the placebo. The results for the dissolution study of A3, A6 and A9 were graphically represented below.

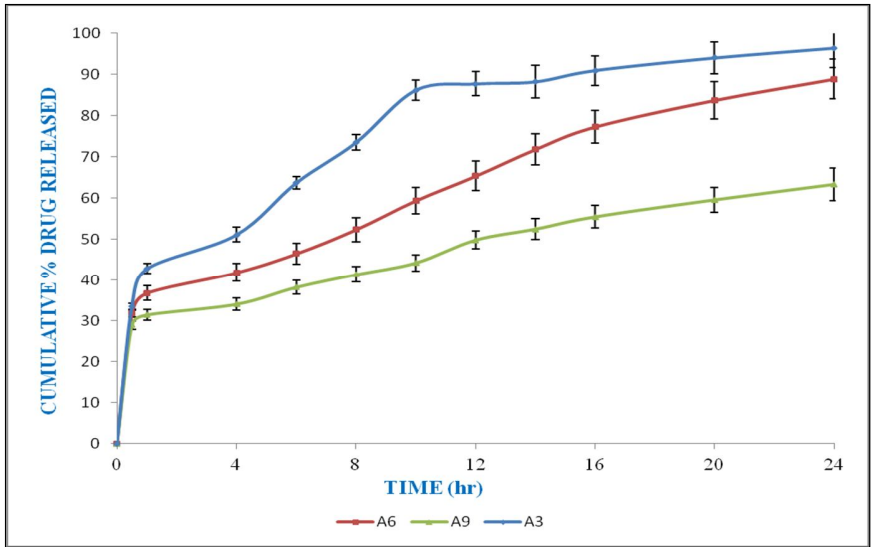


Fig 2: *In vitro* dissolution data for batches A3, A6 and A9

As the above figure shows, drug release was found to be maximum in A3 (96.42%) followed by A6 (88.93%) and A9 (63.37%), at the end of 24th hr. Thus the samples could be suitably used for further analysis (skin permeation study).

2.3 *Ex Vivo* Skin Permeation Study

- **Preparation of rat transdermal Skin**[4-7]

The hair of the test animals (Swiss albino mice) was carefully trimmed short with a pair of scissors and the full thickness skin was removed from the abdominal region. The epidermis was prepared surgically by the heat separation technique, which involved soaking of the entire abdominal skin in water at 60ºC for 45 sec, followed by careful removal of the epidermis. The epidermis was washed with water and used for permeability studies.

2.4 *In vitro* drug release

The *In vitro* skin permeation experiments for different formulations across rat skin were conducted using a modified Keshary-Chein cell, whereby a test tube of internal surface area 0.5024 cm2 was sealed at one end with the transdermal film of the same surface area, overlaid with the rat skin, affixed using an adhesive at its rim. The assembly was placed midway inside a beaker, filled with the buffer solution (100ml) and the solution was stirred (75 rpm) using magnetic stirrer and kept at a temperature of 32±0.5ºC. Samples were withdrawn periodically, analyzed spectrophotometrically at 269 nm after passing through 0.45µ nylon filter. Each withdrawal was followed by simultaneous replacement with a fresh lot of buffer.

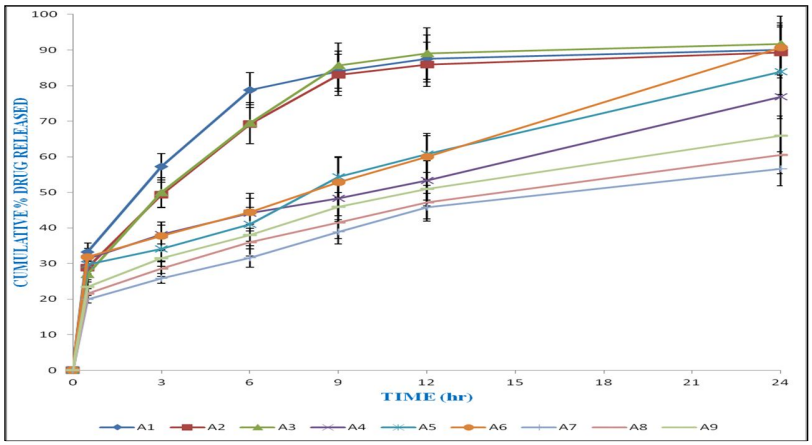


Fig 3: Skin permeation drug release data for batches A1-A9

Table 3: Regression coefficient (R^2) values of anti diabetic drug transdermal patches according to different kinetic models

Formulation	Zero order	First order	Higuchi	Hixon crowell	'n' values for Korsemeyer-Peppas
A1	0.8176	0.7627	0.8916	0.8874	0.4824
A2	0.9366	0.7931	0.9366	0.8270	0.5712
A3	0.9527	0.8207	0.9527	0.8249	0.6532
A4	0.8309	0.9969	0.8609	0.8583	0.6277
A5	0.9334	0.9769	0.9334	0.8922	0.5540
A6	0.9412	0.9168	0.9612	0.9164	0.7096
A7	0.9522	0.9210	0.9522	0.8697	0.5809
A8	0.9436	0.8907	0.9436	0.8940	0.6025
A9	0.9372	0.8713	0.9372	0.8319	0.5948

Though batches A1 (78.7%), A2 (81.1%) and A3 (85.6%) released the drug in an anomalous fashion in about 6, 8 and 9 hr respectively, batches A7, A8 and A9 released only 56.6%, 60.5% and 65.9% drug in a sustained manner by the end of 24 hr (**figure 18**). Batches A4, A5 and A6 also gave a sustained release of 76.8%, 83.96% and 90.7% by the end of 24 hr. The general trend shows that the higher polymer

concentration entrapped the drug more cohesively, thereby permitting its slower and sustained release by time-dependent polymer swelling. The drug release pattern shows that the formulations having highest polymer concentration (15%) gave a more steady release than those having 11% concentration. Moreover as the concentration of the permeation enhancer increased, the % drug released also increased, indicating enhanced extraction of skin proteins and lipids by the permeation enhancer.

Kinetic modeling was undertaken to study the pattern of drug release by fitting the data into zero order, first order, Higuchi's model, Hixon crowell cube root law and Korsemeyer-Peppas model. The slopes and the regression coefficient of determinations (R^2) are listed in **table 17**. The kinetics of the dissolution data were well fitted to zero order, Higuchi model and Korsemeyer-Peppas model. In case of the controlled or sustained release formulations, diffusion, swelling and erosion are the three most important rate controlling mechanisms. Formulation containing matrixing polymers show swelling as well as diffusion mechanism because the kinetic of swelling include relaxation of polymer chains

and imbibitions of water, causing the polymer to swell and changing it from a glassy to rubbery state. Hixon crowell law and Highuchi model was applied to test the release mechanism. R^2 values are higher for Highuchi model than Hixon crowell for all formulations. Hence, drug release from all batches follow **diffusion rate controlled** mechanism. The diffusion exponent 'n' is the indicative of mechanism of drug release from the formulation. For a matrixing drug delivery system, the n value of 0.45 is indicative of Fickian diffusion controlled drug release, n value between 0.45-0.85 signifies anomalous (non-Fickian) transport, n value of 0.85 indicates case II transport and n value greater than 0.85 indicates super case II transport. According to Korsemeyer-Peppas model, 'n' value for all 9 batches was ranges from 0.45 to 0.85. So, it indicates that all patches showed initial burst release followed by **non-fickian diffusion.** The drug release through the transdermal patches follows zero order kinetics with non-fickian diffusion.

3.2 *In vivo* skin irritation study[8]

Guidelines of the institutional animal ethics committee were followed for this experiment. The hair on the dorsal side of Wistar albino rats were removed by clipping a day before the experiment. The rats were divided into 3 groups (n=3). Group I served as control, group II received optimized transdermal film and group III received 0.8% (v/v) aqueous solution of formalin as a standard irritant. The films were placed over the skin with the help of adhesive tape. The films were removed after 24 hr and the skin was examined for any untoward reaction by visual scoring by the same investigator using modified method of Draize et al. The erythema scores were given from 0 to 4 depending on the degree of erythema as follows:

- no erythema: 0
- slight erythema (barely perceptible light pink): 1
- moderate erythema (dark pink): 2
- moderate to severe erythema (light red): 3
- severe erythema (extreme redness): 4.

Table 4: Draize score for skin irritation study

Rat No.	Control		Formulation (A6)		Formalin	
	Erythema	Edema	Erythema	Edema	Erythema	Edema
1	0	0	0	0	3	2
2	0	0	0	1	3	1
3	0	0	0	0	2	1
Average	0	0	0	0.33±0.56	2.67±0.58	1.33±0.58

Erythema scale: 0, none ; 1, slight ;2, well defined ;3, moderate ;4, scar formation
Edema scale: 0, none ; 1, slight ;2, well defined ;3, moderate ;4, severe

3. Result and Discussion

Skin irritation test of the transdermal formulation A6 showed a skin irritation score (erythema and edema) of 0.33 (**table 8**). According to Draize et al, compound producing score of 2 or less are considered negative (no skin irritation). Hence the developed
transdermal formulation could be accepted for topical use.

3.1 Stability study of the optimized formulation[9]

The stability study was carried out on the optimized formulation A6 as per ICH guidelines Q1C.

The stability study was performed at 40 ± 0.5 °C / 75 ± 5 % for 1 month.

The optimized formulations stored at 40 ± 0.5 °C / 75 ± 5 % RH were found stable.

- At the end of study, samples were analyzed for the folding endurance, % drug content, CPR and tensile strength.
- Folding endurance, tensile strength and drug content were found within range.

Table 5: Results of stability study

Batch code A6	Folding endurance	% Drug content	CPR (24 hr)	Tensile Strength
Initial	530 ± 4.33	96.24 ± 0.22	90.68	Highly elastic and remain intact
After 1 Month at 40 ± 0.5 °C/ 75 ± 5 %RH	540 ± 5.21	94.89 ± 0.18	89.92	Highly elastic and remain intact

3.2 Result and discussion:

After study of stabily study formulation A6 was given optimization folding endurance, tensile strength and drug content.

3.3 Factorial design[10-13]

To study all the possible combinations of all factors at all levels, a two-factor, three-level full factorial design was constructed and conducted in a fully randomized order. The design was analyzed using Design Expert 8.0.7 software. The dependent variables measured were % drug release at 10^{th} hr (Q10) and % drug release at 24^{th} hr (Q24). The responses of the 3^2 design are shown in **table 20.**, whereby X1 is Eudragit NE 30D concentrations and X2 is DMSO concentration.

Table 6: Composition and responses for 3^2 factorial design for anti diabetic drug

Batch code	variables		Response values	
	X1	X2	Q10	Q24
A1	-1	-1	84.98 ± 0.49	90.02 ± 0.35
A2	-1	0	84.00 ± 0.64	89.30 ± 0.29
A3	-1	+1	86.48 ± 0.39	91.74 ± 0.86
A4	0	-1	49.36 ± 0.55	71.81 ± 0.68
A5	0	0	57.89 ± 0.43	77.96 ± 0.81
A6	0	+1	54.68 ± 0.32	84.68 ± 0.45
A7	+1	-1	41.46 ± 0.41	56.64 ± 0.74
A8	+1	0	44.40 ± 0.70	60.49 ± 0.60
A9	+1	+1	47.18 ± 0.68	65.91 ± 0.33

* mean ± SD (n = 3)

3.4 Equations Relating Independent Variables and Responses

The equations representing the quantitative effect of the formulation variables on the % drug release are shown below.

ANOVA table showing coefficient and p value % drug release, i.e. Q10 and Q24, are shown in **table 7** and **table 8** respectively.

Table 7: Coefficient and p values for Q10

Parameters	Coefficients					
	b_0	b_1	b_2	b_{11}	b_{22}	b_{12}
	55.99	-20.4	2.08	10.13	-2.02	1.05
p Value	0.0004	0.0001	0.1245	0.0042	0.3057	0.4695
R^2	0.9901					

The equation representing the quantitative effect of independent variables Q10 is:

$Q10\ (Y) = 55.99 - 20.4\ (X_1) + 2.08\ (X_2) + 10.13\ X_1^2 - 2.02\ X_2^2 + 1.05\ (X_1X_2)$ ($P < 0.05$) ---------**6.1**

Table 8 : Coefficient and p values for Q24

Parameters	Coefficients					
	b_0	b_1	b_2	b_{11}	b_{22}	b_{12}
	77.74	-14.67	3.98	-2.59	0.76	1.9
p Value	0.0008	0.0010	0.0109	0.1436	0.6200	0.1556
R^2	0.9868					

The equation representing the quantitative effect of independent variables on Q24 is:

$Q24\ (Y) = 77.74 - 14.67\ (X_1) + 3.98\ (X_2) - 2.59\ (X_{11}) + 0.76\ (X_{22}) + 1.9\ (X_1X_2)$

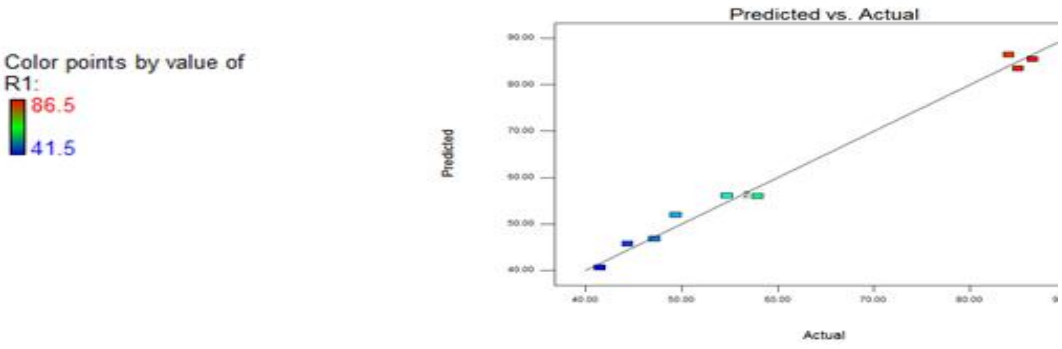
($P < 0.05$)---------**6.2**

Coefficients with one factor represent the effect of that particular factor, while the coefficients with more than one factor and those with second-order terms represent the interaction between those factors and the quadratic nature of the phenomena, respectively. A positive sign in front of the terms indicates a positive effect, while a negative sign indicates a negative effect of the factors. From the equations 6.1 and 6.2, it can be concluded that Eudragit NE 30D has a negative effect on % drug release while DMSO has a positive effect on % drug release.

3.5 Predicted v/s actual and surface plots for responses Q10 & Q24

The relationship between the independent variables and the response can be further explained by using predicted v/s actual plot and surface plots. The data were subjected to 3-D response surface methodology in Design expert 8.0.7 to determine the effect of polymers and permeation enhancer on dependent variable. **Figure 4** shows the predicted v/s actual plot and surface response plot for Q10. **Figure 5** shows the predicted v/s actual plot and surface response plot for Q24.

Here A is polymer (Eudragit NE 30D) while B represents DMSO. Response R1 represents Q10 while R2 refers to Q24.



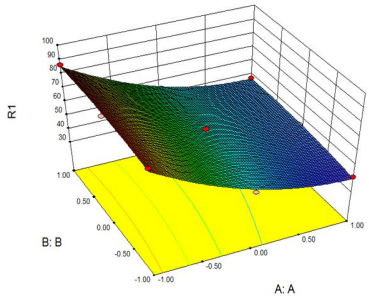


Fig 6: Effect of conc. change of polymer & permeation enhancer on Q10.

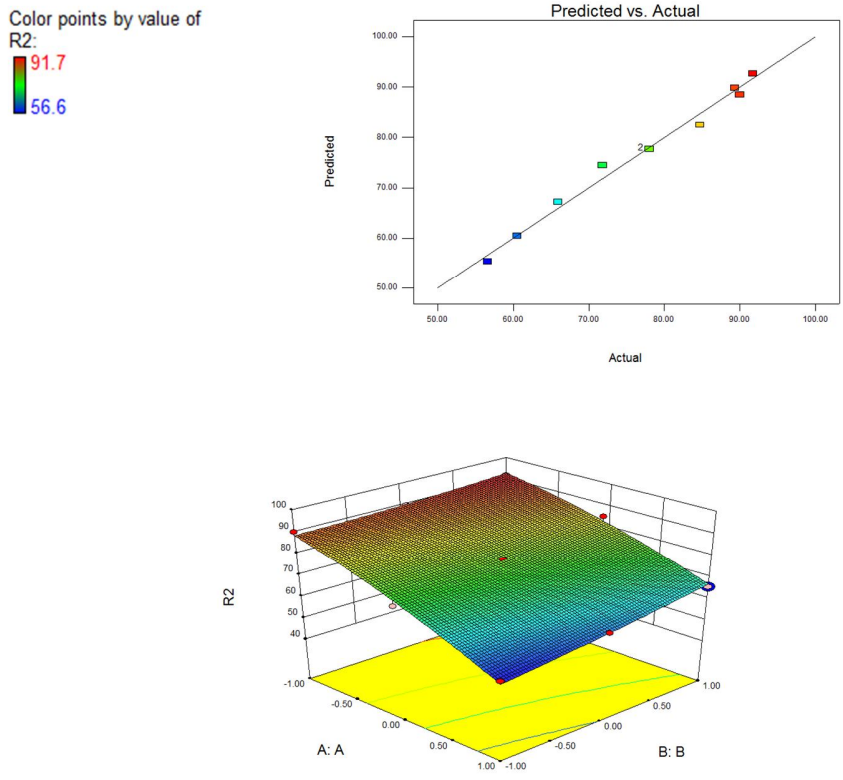


Fig 7: Effect of conc. change of polymer & permeation enhancer on Q24

3.6 Result and Discussion: The predicted v/s actual plot and surface response plot indicate that the addition of a higher amount of Eudragit NE 30D result in lower and slower % drug release while higher amount of DMSO result in a higher % drug release. The increase in amount of Eudragit NE 30D resulting in lower % drug release may be due to the hydrophobic nature of polymer. On the other hand, an increase in DMSO concentration resulting in higher % drug release may be due to the enhanced extraction of stratum corneal lipids and proteins.

3.7 Contour Plots for Responses Q10 & Q24

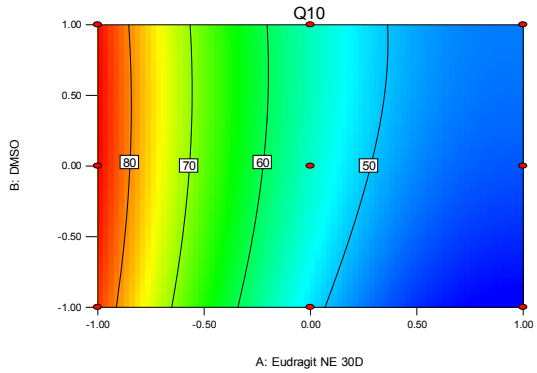


Fig 8: Contour Plot for Response Q10

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