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Original article

OPTIMISATION OF TRANSDERMAL GEL FORMULATIONS OF TOLTERODINE TARTRATE BY EXPERIMENTAL DESIGN

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Abstract

The aim of this study was to evaluate the permeation enhancing effect of cosolvents on transdermal gel formulation of Tolterodine Tartrate (TT) using experimental design technique. The two factors chosen for Taguchi robust design were the concentrations of ethanol and propylene glycol. The influence of cosolvents on the invitro penetration of TT through a synthetic membrane and abdominal rat skin from carbopol gels were investigated using Keshary-chein type diffusion cells. Penetration through the synthetic membrane was well described by the Higuchi model where as when using rat skin, the penetration rate was controlled by the membrane(skin). The permeation rate of TT significantly increased in proportion to the ethanol and propylene glycol concentration showing cosolvent action. In conclusion, a transdermal TT gel was formulated successfully using the technique of Taguchi robust design and these results were useful in finding the optimum formulation for transdermal drug release. The optimized ratios of cosolvents were ethanol (60%) and propylene glycol (10%).

Key words: *Transdermal gel, Tolterodine Tartrate, Taguchi robust design, Cosolvents, Carbopol.*

Tolterodin Tartaratın Transdermal Jel Formülasyonlarının Faktöriyel Tasarım İle Optimizayonu

Bu çalışmanın amacı, Tolterodin Tartarat (TT)' ın transdermal jel formülasyonlarında kosolvanların permeasyon artırıcı etkilerinin deney tasarımı tekniği ile değerlendirilmesidir. Taguchi dayanıklılık tasarımı için seçilen iki faktör propilen glikol ve etanol konsantrasyonudur. TT'nin karbopol jellerden sentetik membran ve abdominal sıçan derisine in vitro penetrasyonu üzerine kosolvanların etkisi Keshary-chein tipi difüzyon hücreleri ile değerlendirilmiştir. Sentetik membrandan penetrasyon Higuchi Modeli ile iyi tanımlanırken, sıçan derisinden penetrasyon membran (deri) tarafından kontrol edilmiştir. TT'nin permeasyon hızı kosolvan etkisi gösteren etanol ve propilen glikol konsantrasyonu ile orantılı olarak önemli derecede artmıştır. Sonuç olarak, transdermal TT jeli Taguchi dayanıklılık tasarımı ile başarılı bir şekilde fromüle edilmiştir ve bu sonuçlar transdermal ilaç salımı için optimum formülasyonun bulunmasında faydalıdır. Kosolvanların optimize edilmiş oranları etanol için % 60 ve propilen glikol için %10'dur.

Anahtar Kelimeler: *Transdermal jel, Tolterodin tartarat, Taguchi dayanıklılık tasarımı, Kosolvanlar, Karbopol.*

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INTRODUCTION

Tolterodine tartrate (TT); a synthetic tertiary amine antimuscarinic agent is a genitourinary antispasmodic. It decreases contraction of the detrusor muscle of normal and overactive urinary bladder. It has been shown to exhibit functional selectivity for urinary bladder over secretory (e.g., Salivary) glands. It is used for the management of symptoms associated with both neurogenic and nonneurogenic overactive bladder (1).

TT is available as conventional tablets and extended release capsules. However its oral administration is limited on account of its dose-related adverse side effects, including dry mouth, tachycardia, dizziness and gastrointestinal obstructive disorder. TT is extensively metabolized following oral administration, and its major metabolite acts similarly as the parent substance on receptors, which restricts its application in patients with liver cirrhosis (2). This originates the need for an alternative route of administration, which can bypass the hepatic first-pass metabolism. Transdermal route could be an alternative route for these patients, because it bypasses first-pass metabolism, minimizes the gastrointestinal side effects, increases patient compliance, maintains a constant drug level in plasma and makes it possible to interrupt or terminate treatment when necessary. Its physico-chemical properties like molecular weight (475.6), half-life (1.9-3.7), log P value (1.51 at a pH of 7.4), low dose (2mg twice daily) and aqueous solubility (12g/L at room temperature) make it an ideal drug candidate for transdermal delivery (3). One limitation to transdermal drug delivery is that the drug must be capable of passing through the skin which forms a major barrier to most exogenous substances, including drugs. The primary approach to overcome this barrier, for drug penetration is the skillful selection of vehicles and penetration enhancers, which facilitate penetration by reversibly altering the structure of the skin (4).

Taguchi method is a combination of mathematical and statistical techniques used in an empirical study which is economical for characterizing a complicated process. It uses fewer experiments to study all levels of input parameters and determines the experimental condition having the least variability as the optimum condition. The variability of a property can be expressed by signal to noise (S/N) ratio where the experimental condition having the maximum S/N ratio is considered as the optimal condition, as the variability of characteristics is in inverse proportion to the S/N ratio (5).

In this study, TT was formulated as gel and the effect of co-solvents (ethanol and propylene glycol) was tested in order to select the one with the most promising properties and use it as the base for the testing of penetration enhancers in our further studies. For optimization of co-solvents ratio Taguchi robust design was used.

EXPERIMENTAL

Materials

Tolterodine tartrate (TT) was obtained as a gift sample from RA Chem Ltd (Hyderabad, India). Acrypol-974 was purchased from Corel Pharma Ltd (Ahmedabad, India). Ethanol, propylene glycol and triethanolamine were purchased from S. D. Fine-Chem. Ltd. (India).

Preparation of gel

Gel dosage forms of TT were prepared using a serial mixture of distilled water, propylene glycol and ethanol as the vehicle and a gelling agent of Acrypol 974 at a concentration of 2% (w/w). After complete hydration of Acrypol 974 by the vehicle, drug (0.07%) was added. Later triethanolamine was added and mixed completely, and then vehicle was added to give a total weight of 100g. Concentration of propylene glycol and ethanol were added according to the Taguchi L9 orthogonal array experimental design.

Experimental design

Taguchi L9 Orthogonal array experimental design (5) was constructed which involves the selection of parameters and the choice of responses. The two factors (independent variables), their corresponding levels and the responses (dependent variables) are shown in Table1. MINITAB 16 software (Minitab Inc., PA, U.S.A) was used for the generation and evaluation of the statistical experimental design.

Table 1. Factors and their corresponding levels implemented for the construction of Taguchi L9 orthogonal array experimental design.

Factors		Levels 1	2	3
A	Ethanol (%)	20	40	60
B	Propylene glycol (%)	0	5	10
Response : Amount of (TT) permeated at 24 h (Q_{24},μg/cm^2) and Flux (J_{ss}, μg/cm^2/hr).				

Physico-chemical properties

Gel formulations were tested for various physicochemical properties. pH measurement of the gels were done by using a calibrated digital pH meter (Systronics) by dropping the electrode into gel formulation. The homogeneity was tested by visual observations. The spreadability of the gel formulations were determined by measuring the spreading diameter of 500 mg of the gel between two glass plates after 1 min. The mass of the upper plate was standardized at 125g. The spreadability was calculated by using the formula, S=m.l/t, where, S is the spreadability, m is the weight tied to the upper slide, l is length of the glass slide and t is the time taken. The viscosity was measured using brookefield programmable DVIII+ rheometers with spindle RV-2 at 10 rpm. Drug content was measured by dissolving 1000 mg of gel in 100 ml of phosphate buffer saline 7.4 with 0.5% methanol. The volumetric flasks were kept for sonication for 30 min to mix it properly. The solution was passed through the whatmann filter paper no.42 and filtered and appropriate dilutions were done and the drug content was measured spectrophotometrically against corresponding placebo formulations at 280 nm.

In vitro diffusion studies

Diffusion studies of the formulations were performed using Keshary-chein diffusion cell. The cell was locally fabricated and volume of receptor compartment was 20ml. The dialysis membrane was mounted between the donor and receptor compartments. 1000 mg of gel formulation was applied uniformly on the dialysis membrane and the compartment clamped together. The receptor compartment was filled with phosphate buffer saline pH 7.4 and the hydrodynamics in the receptor compartment was maintained by stirring with a magnetic bead. At pre-determined time intervals 1 ml of samples were withdrawn and an equal volume of buffer was replaced. The samples were analysed after appropriate dilution for drug content spectrophotometrically at 280 nm.

TT release rate, *k*, was determined from the slope of the amount of drug released per unit area versus the square root of time (6).

Ex vivo permeation studies

The experimental study was approved by the Institutional Animal Ethical Committee (IAEC). A male Wistar rat (180-200 g) was sacrificed by excessive ether anesthesia and the hair was removed from the ventral portion using an animal hair clipper (Aesuclap, Germany). After harvesting the full thickness skin, the fat adhering on the dermis side was removed using a

scalpel and isopropyl alcohol. Finally, the skin was washed in tap water and stored at -20°C in aluminum foil packing. The skin was used within a week.

Phosphate-buffered saline (pH 7.4) was sonicated for 30 min and was placed in the receptor compartment of jacketed Keshary-Chein diffusion cells, having an area of 4.9 cm^2. The receptor fluids were thermostated at 37± 0.5°C and stirred at 600 rpm. The thawed skin piece was mounted on the diffusion cell with the stratum corneum side facing the donor compartment and was equilibrated for 1 h. The gel formulation (1000 µl) was applied using a positive pressure pipette to the donor compartment and the hydrodynamics in the receptor compartment was maintained by stirring with a magnetic bead. The samples were periodically withdrawn from the receptor compartment, up to 24 hr and analyzed by UV-VIS double beam spectrophotometer (Chemito Spectrascan UV2600, India) at 280nm (3).

Skin irritation studies

The institutional animal ethical committee approved the experimental protocol. A primary skin irritation test was performed since skin is the vital organ through which the drug is transported. The test was carried out on three healthy rabbits weighing between 1.5-2 kg. The test was conducted on an unbraided skin of rabbits. Before placing the formulations, the unbraided skin was cleaned with rectified spirit. The control formulation was placed on the left dorsal surface of each rabbit, where as the test formulation (with drug) was placed on the right dorsal surface of the same rabbits and the other rabbit was kept as control. The formulations were removed after 24 h and the skin was examined for erythema/oedema.

Histopathological studies

Histopathological studies were performed on rats after approval by the Institutional animal ethical committee. Formulations control, aqueous gel and BT9 formulation were applied to the dorsal portion of rats. After 6 hours of application, the rats were killed by cervical dislocation; the skin was removed, cut into small pieces, fixed by immersion in 50% neutral formalin solution in saline for 24h, then subjected to histological processing and examination. The samples were subjected to routine fixation by dehydration and rehydration with graded alcohols, paraffin block processing and stained with haematoxylin-eosin. Microscopic evaluation using dark-light microscope was performed by a blinded assessor.

Data treatment

The cumulative amount (µg/cm^2) of TT permeated was plotted against time and the flux (J_{ss},µg/cm^2/h) was calculated from the slope of straight-line portion of the curve. Lag time (L) was determined from the X-intercept of the regression line. The apparent permeability coefficient (P,cm/h) was obtained by dividing J_{ss} by the donor concentration (C_d) according to Fick's First Law of diffusion (4). Means, standard deviation (S.D), coefficient of variation (%CV), and linear regression analyses were calculated using Microsoft Excel 2007. The data were subjected to one-way ANOVA and t-test at a significance level of $P \leq 0.05$ using Sigma plot software (Sigma plot 10, USA).

RESULTS AND DISCUSSION

Tolterodine tartrate is available as tablets and its drawback is adverse effects by active metabolite. Its physico-chemical properties make it suitable for transdermal delivery as an alternative approach but the main barrier is stratum corneum. To overcome this cosolvents as permeation enhancer has been selected.

Taguchi L9 orthogonal array experimental design was used, for formulation of TT transdermal gel. The factors (independent variables) and the corresponding levels were (Table 1): concentration of ethanol (20%,40%,60%) and concentration of propylene glycol

(0%,5%,10% v/v). The response (dependent variable) was the cumulative amount of TT per unit area after 24 h (Q_{24}), and flux through rat skin. Using the combinations of the two factors three levels, formulations were prepared as shown in Table 2.

Table 2. Taguchi L9 orthogonal array design for formulation of TT transdermal gel.

Formulation code	Factors		Responses	
	A (concentration of ethanol)	B (concentration of propylene glycol)	Q_{24}(μg/cm^2)	FLUX (μg/cm^2/hr)
BT1	1	1	41.90±3.26	1.59±0.08
BT2	1	2	46.18±4.75	2.06±0.16
BT3	1	3	53.48±2.52	2.37±0.09
BT4	2	1	43.41±2.91	1.89±0.08
BT5	2	2	61.94±1.20	2.70±0.01
BT6	2	3	62.55±2.37	2.68±0.07
BT7	3	1	49.15±2.90	1.97±0.07
BT8	3	2	69.09±5.45	2.71±0.15
BT9	3	3	91.89±2.30	3.86±0.13

Physico-chemical characteristics of the gel formulations are shown in table 3. From the results, it is evident that all the gel formulations showed good homogeneity. The pH values of all formulations were between 6.8 to 7.4. The drug content was in the range of 0.67 to 0.71 mg. The result of spreadability varies from 6.3 to 8.3 g.cm/sec indicating the gels can spread easily. Viscosity in hydroalcoholic gels depends on the polymer-alcohol complex formed which becomes hydrophilic, the proportion of water for expanding the complex (7) and gelling agent added.

With regard to BT4, BT5, and BT6 there is increase in viscosity with increase in propylene glycol when compared to reverse effect with other formulations. Propylene glycol is non-polar, so with increase in its content viscosity should be decreased as the quantity of water for expanding and gelling decreases. The increase in viscosity of BT4,BT5 and BT6 can be suggested due to 40% ethanol as an optimum concentration for hydroalcoholic mixture which is also used in vesicular carriers (ethosomes). Complex formation and quantity of gelling agent added have played a major role in viscosity of the formulations.

The viscosity of the formulations ranged between 31,780 to 41,480 cps which is in the optimum viscosity range of Acrypol 974, hence significant effect on permeation was not seen.

The results showed there was no significant effect of co-solvents on physico-chemical properties except viscosity.

Table 3. Physico-chemical properties of TT transdermal gels

Formulation code	Homogeneity	pH	Drug content (mg)	Spreada-bility (g.cm/sec)	Viscosity (cps)
BT1	++	7.1	0.67	8.3	40320
BT2	++	7.4	0.69	7.3	38780
BT3	++	6.9	0.7	6.9	35890
BT4	++	7.3	0.68	7.3	33670
BT5	++	7.0	0.71	8.3	36780
BT6	++	6.9	0.67	6.3	39480
BT7	++	7.1	0.71	7.9	41480
BT8	++	7.3	0.69	6.6	37890
BT9	++	6.8	0.68	6.3	31780

The transport behaviour of TT across the dialysis membrane or abdominal rat skin was investigated from a gel dosage form prepared by gelling a solvent mixture of ethanol, propylene glycol and water with carbopol (Table 2). The release profiles of TT from these gels through the dialysis membrane are presented in Figure 1 and Figure 2. When the amounts of drug released were plotted against the square root of time, a linear relationship was obtained for each formulation (r >0.9), showing that the release of TT from the gels was well described by the Higuchi model, where the rate controlling step is the process of diffusion through the gel matrix. This relationship exists for the formulations both in which the drug is fully dissolved and in which the drug is present as a suspension form in gel matrix (8). Hence, the membrane has no significant effects and the properties of the formulation control the release of the drug (6).

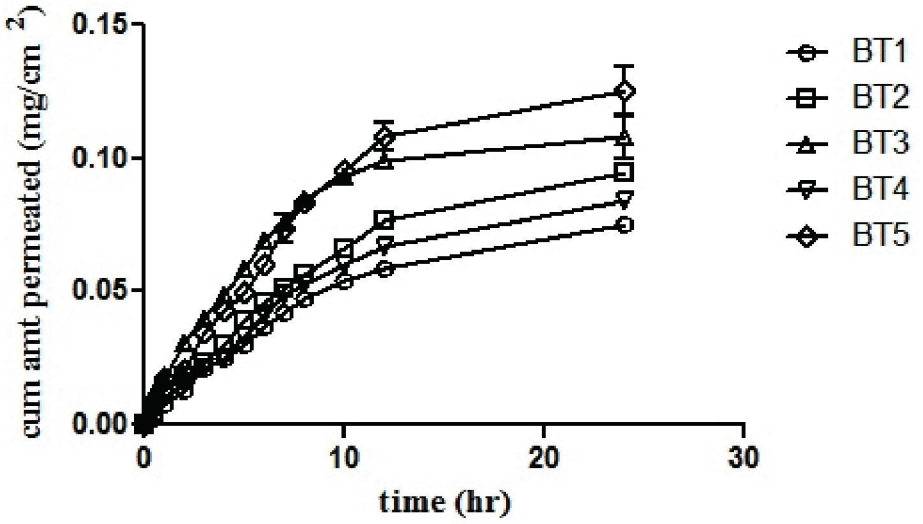

Figure 1. Release profiles of TT across dialysis membrane from formulations BT1- BT5. Each point represents the mean±S.E. of three to six experiments.

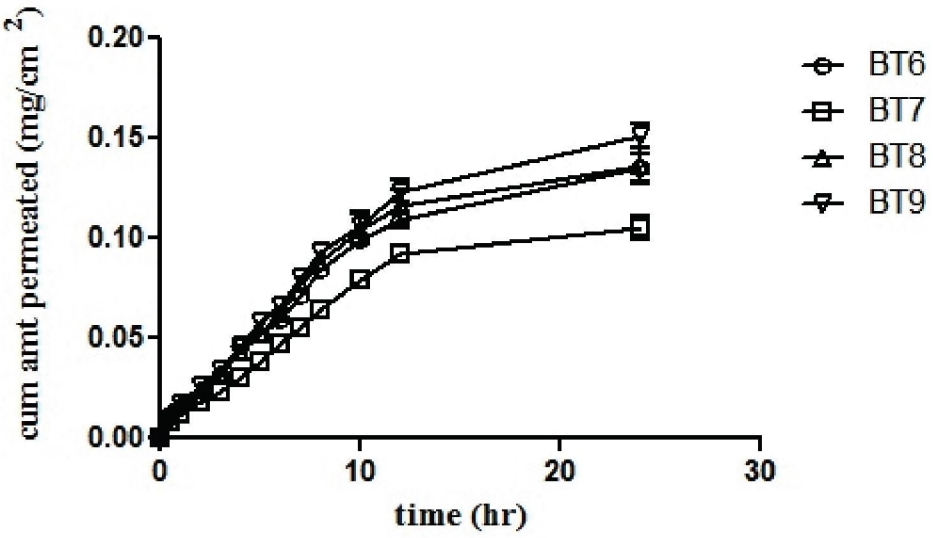

Figure 2. Release profiles of TT across dialysis membrane from formulations BT6 - BT9. Each point represents the mean±S.E. of three to six experiments.

The release rates of TT from these formulations are listed in Table 4. Formulation BT9 containing 60% ethanol and 10% propylene glycol, showed the highest release rate, indicating that the cosolvents provided fully solubilized drug in the vehicle. Cosolvents may also modify the structure of the skin, thus altering the penetration rate of drugs.

When using rat skin as the barrier, the penetration rate is controlled by the skin membrane. The permeation profiles of these gel formulations are presented in Figure 3 and Figure 4. The permeation parameters cumulative amount (Q_{24}), steady-state flux (J_{ss}), lag time (L) and permeability coefficient (P) are summarized in Table 2 and Table 4 respectively.

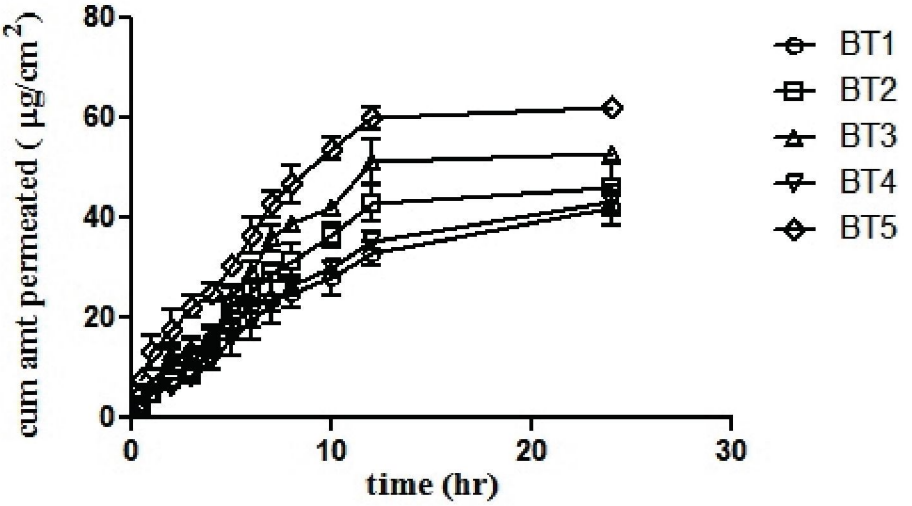

Figure 3. Permeation profiles of TT across rat abdominal skin from formulations BT1- BT5. Each point represents the mean±S.E. of three to six experiments.

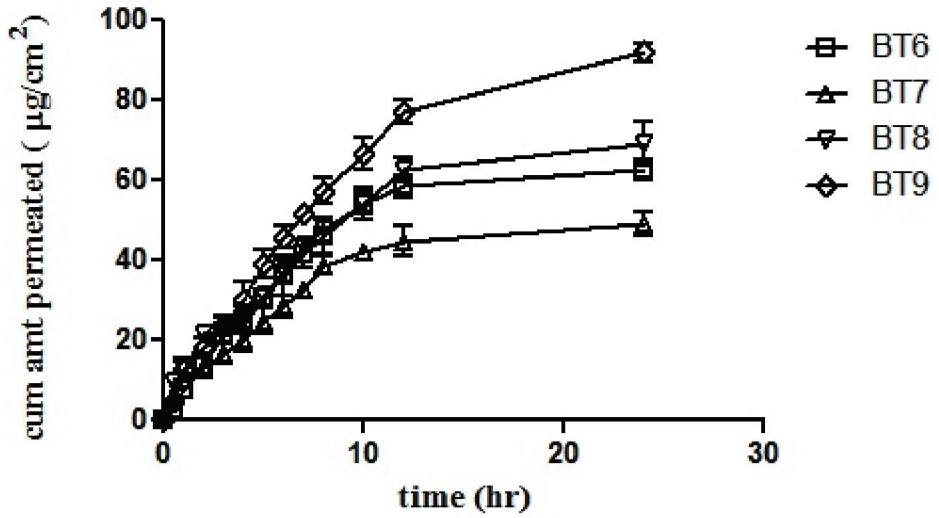

Figure 4. Permeation profiles of TT across rat abdominal skin from formulations BT6 - BT9. Each point represents the mean±S.E. of three to six experiments.

Table 4. TT release rates across dialysis membrane and skin permeation parameters.

Formulation code	Release rate ($mg/cm^2/hr^{-1/2}$)× 10^{-02}	Permeability coefficient (cm/h)×10^{-03}	Lag time (h)
BT1	1.70±0.01	2.28±0.12	1.10±0.20
BT2	2.13±0.06	2.95±0.24	1.76±0.25
BT3	2.60±0.10	3.39±0.14	1.56±0.20
BT4	1.93±0.06	2.70±0.13	2.30±0.20
BT5	3.00±0.17	3.86±0.02	1.33±0.20
BT6	3.17±0.12	3.84±0.11	1.23±0.37
BT7	2.50±0.10	2.81±0.11	1.63±0.15
BT8	3.20±0.20	3.88±0.22	2.10±0.10
BT9	3.46±0.12	5.51±0.19	0.93±0.15

The formulations were optimized using Taguchi L9 orthogonal array experimental design. The factors and responses for optimization are shown in Table 2. The data were analyzed to identify the factors that affect statistically significant the responses by S/N ratio plots.

Effect of concentration of ethanol on skin permeation of TT

The main effects plot for SN ratios which was analyzed from Taguchi design, is shown in Figure 5 (A), 6 (A) for response variables, cumulative amount permeated in 24hrs (Q_{24}) and flux (J_{ss}). It can be seen that the influence of concentration of ethanol on Q_{24} and flux (J_{ss}) were significant ($p< 0.05$). Ethanol exerts its permeation enhancing activity through various mechanisms, as a solvent it can increase the solubility of the drug in the vehicle. On permeation into the stratum corneum it can alter the solubility properties of the tissue with a consequent improvement for drug partitioning into the membrane (9). Additionally rapid permeation of ethanol, or evaporative loss of this volatile solvent from the donor phase modifies the thermodynamic activity of the drug within the formulation (10). Such effect is most apparent when applying a finite dose of a formulation onto the skin surface before evaporation of the alcohol; as ethanol is lost, drug concentration can increase beyond saturated solubility providing a supersaturated state with a greater driving force for permeation. A further potential mechanism of action, is 'solvent drag' where ethanol may carry the permeant into the tissue as it traverses (9,10).

As can be seen, a linear relationship between concentration of ethanol and permeation rate exists. This can be explained as ethanol enhances skin permeation by physical perturbation of the lipoidal barrier region in the stratum corneum at low concentrations and at higher concentrations the effect comes by conformational changes within the keratinized protein component and partial extraction of the stratum corneum lipid (11).

Effect of concentration of propylene glycol on skin permeation of TT

From the SN Ratio Plots shown in Figure 5(B) and Figure 6(B) the effect of concentration of propylene glycol on Q_{24} and J_{ss} were significant. The maximum permeation was obtained with 10% propylene glycol. The proposed mechanism of propylene glycol by biochemical investigations is it acts by solvating the α-keratin structures of the cells, by disrupting the lamellar lipid structure and/or by interacting with the polar head group regions of the lipids by replacing bound water, resulting in a slight shortening of the mean alkyl chain length in the bilayers (11).

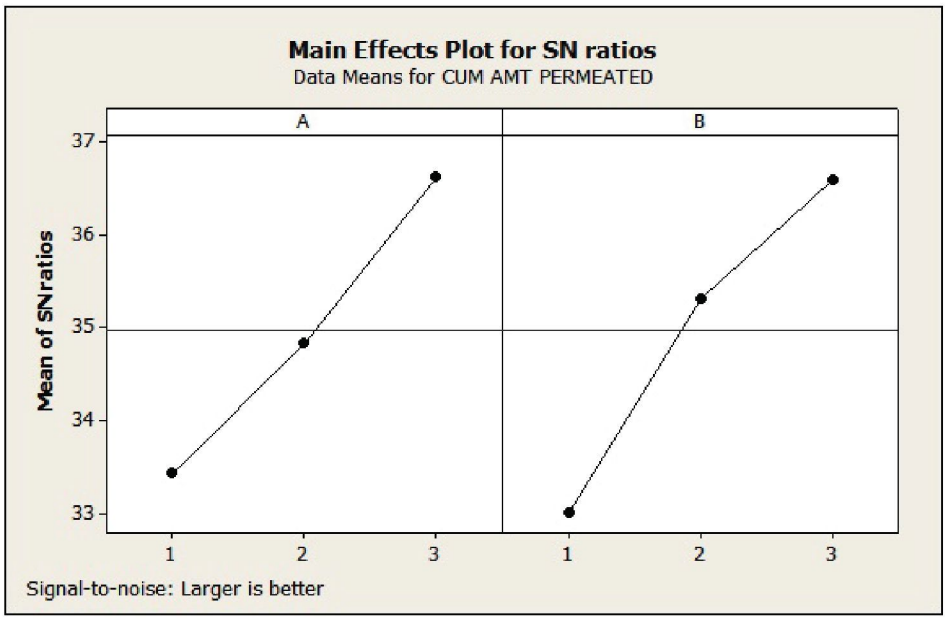

Figure 5. The main effects plot for SN Ratios of A) concentration of ethanol B) concentration of propylene glycol for cumulative amount permeated

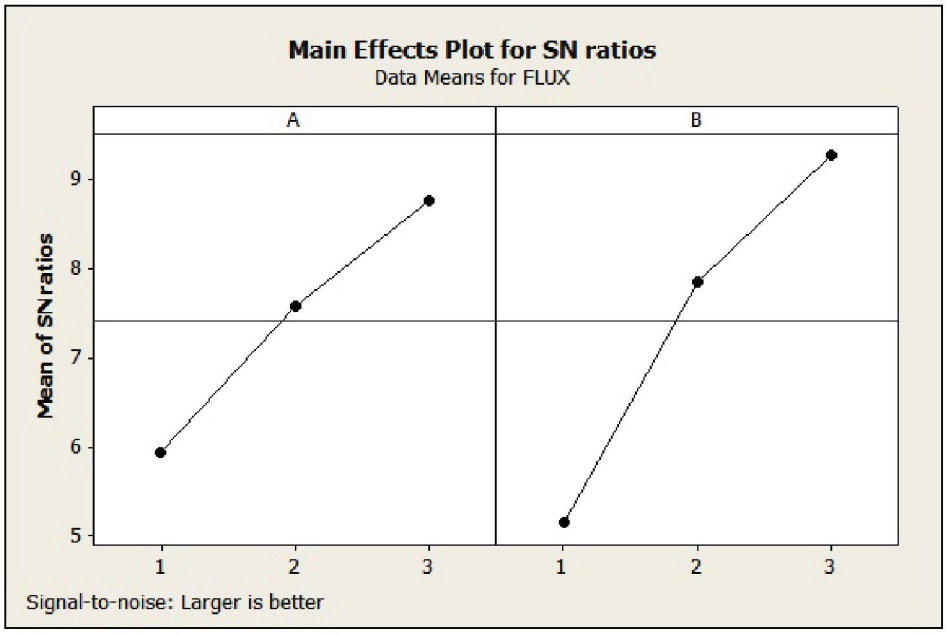

Figure 6. The main effects plot for SN Ratios A) concentration of ethanol B) concentration of propylene glycol for flux.

Cosolvency is one of the most widely studied formulation strategies to facilitate drug transport across the skin. The proposed mechanism for such systems include: (a) change in the thermodynamic activity (e.g., by increasing the degree of saturation in the solvent) (b) specific interaction with the stratum corneum, either by increasing the drug solubility in the stratum corneum (i.e., facilitate partitioning of drug from the vehicle into the skin) or by altering the various transport pathways (i.e., the polar and nonpolar pathways) in the stratum corneum (12).

Formulation BT9 containing 60% ethanol and 10% propylene glycol showed maximum permeation of TT as with ethanol, propylene glycol permeates well through human stratum corneum (9). This combination of water, ethanol and propylene glycol was found to enhance transdermal permeation of naloxone (13) and aspirin (10).

Skin irritation studies were performed on rabbits according to the institutional ethics committee. No signs of skin-irritation such as redness or rash or scratching by the animals were observed which indicates no signs of toxicity.

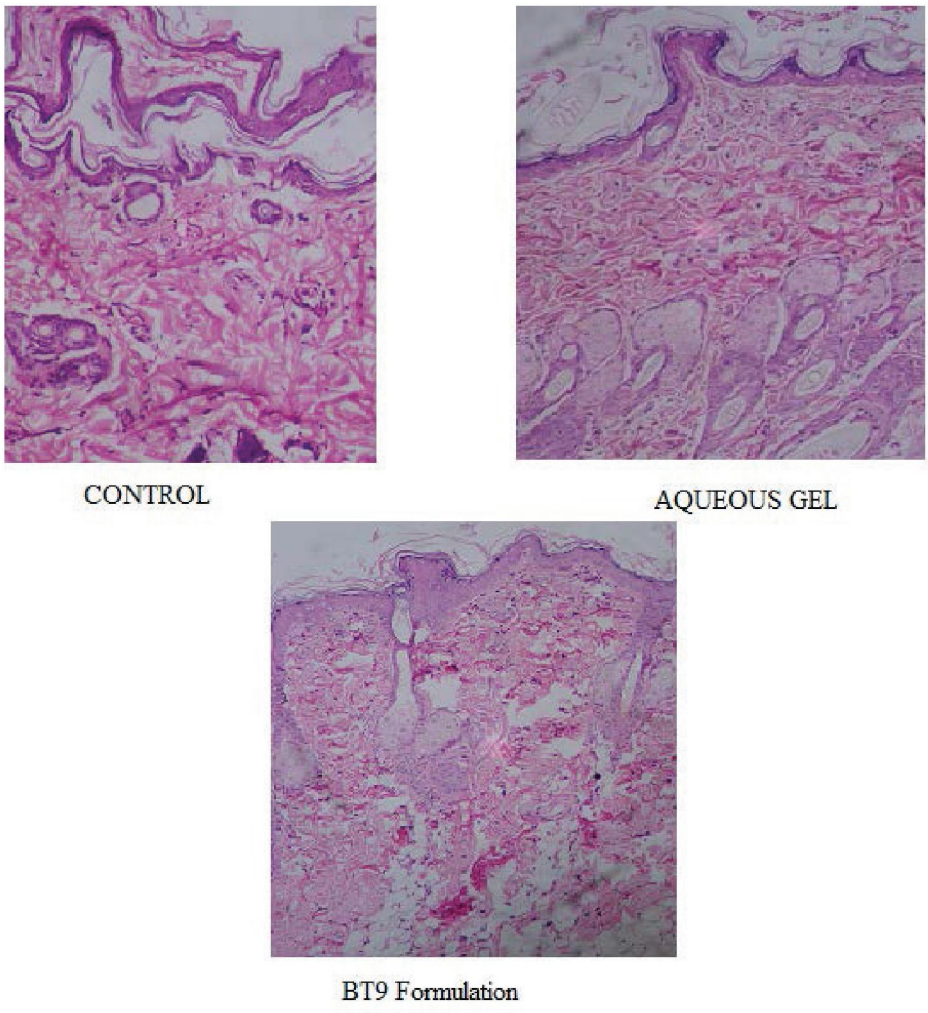

Figure 7. Histological sections of a) control b) aqueous gel and c) BT9 Formulation under 200x magnification.

Haematoxyllin and Eosin (H&E)sections of treated skin under 200x magnification showed slightly fatty change and moderate to severe congestion in dermis of skin treated with aqueous gel. H&E section of skin treated with BT9 Formulation showed moderate to severe degeneration, fatty change, congestion and edema in dermis.

Changes in the skin treated with aqueous gel when compared with control showed that carbopol permeates or enhances permeation. With BT9 formulation major changes in the dermis were observed which can be attributed to the action of ethanol and propylene glycol.

CONCLUSION

Transdermal TT gels were formulated successfully using Carbopol polymer. The formulations were optimized by the experimental design,Taguchi robust design.SN Ratio plots indicated significant effect of ethanol and propylene glycol on permeation of TT. The optimized ratio of cosolvents were found to be ethanol (60%) and propylene glycol (10%). Therefore, gels with cosolvents can be used as penetration enhancers in transdermal delivery TT.

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