

Formulation and optimization of gentamicin hydrogel infused with *Tetracarpidium conophorum* extract via Central composite design for topical delivery

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ABSTRACT

INTRODUCTION: Response surface methodology coupled with statistically designed experiment has been found to be very useful in optimising multivariable processes. The aim of this study is to evaluate the influence of two independent variables, a ratio of permeation enhancers/antioxidants (Transcutol and ethanolic extract of *Tetracarpidium conophorum* EETC) and stirring rate, on the flux and permeation of gentamicin hydrogel.

METHODS: A modification of free radical initial polymerization was utilized to formulate the gentamicin hydrogel. A 3² factorial central composite design (CCD) was then utilized to investigate the effect of independent variables of permeation enhancer Transcutol: EETC (X1), Stirring speed (X2) via 14 formulation batches which were evaluated for dependent variables Flux (Y1) and amount of drug permeated after 12 hours (Y2) ex-vivo.

RESULTS: The results of analysis of variance (ANOVA) carried out to determine the fit of the models revealed that the models were statistically significant ($p < 0.05$) and did not show lack of fit ($R^2 > 0.80$). The regression equation generated for flux was $Y1 = 19.35 - 25.82X1$

$- 0.044X_2 + 0.0097X_1X_2 + 11.86X_2^2$ and for cumulative permeation of gentamicin in 12 hours $Y_2 = 315.50 - 189.67X_1 + 0.28X_2 - 1.29X_1X_2 + 123.55X_2^2$. The validity of the statistical models used for predicting flux and drug permeation was confirmed by carrying out three confirmation experimental runs at the identified optimum conditions. The results showed that there was no significant difference between the experimental results and those predicted by the statistical models.

DISCUSSION AND CONCLUSION: The excellent correlation between the predicted and measured values shows the validity of statistical models ($R^2 = 0.95$). Utilization of antioxidant and permeation enhancer has been used for the first time to investigate the influence on dependent variables. Optimization of gentamicin hydrogel using central composite statistical design is valid for prediction of drug permeation and flux utilizing variables in formulation.

Keywords: Hydrogels, polymer, *Tetracarpidium conophorum*, Response surface methodology, gentamicin

INTRODUCTION

Gentamicin is a water soluble aminoglycoside antibiotic derived from *Micromonospora purpurea*, and actinomycete. It is used for treatment of infections caused by susceptible strains of *Pseudomonas aeruginosa*, *Proteus* species (indole-positive and indole-negative), *Escherichia coli*, *Klebsiella-Enterobacter-Serratia* species, *Citrobacter* species and *Staphylococcus* species (coagulase-positive and coagulase-negative). When required for topical administration, it is usually formulated as creams as well as ointments which possess various disadvantages in terms of reduced stability, erratic drug release and decreased skin permeability when compared to hydrogels.¹

The water holding capacity and permeability are the most important characteristic features of a hydrogel.² Biocompatibility is the third most important characteristic property required by the hydrogel as it calls for compatibility of the gel with human natural tissue without causing

any toxicity upon its degradation.² In addition to the above characteristics, the soft and rubbery nature of hydrogels minimises irritation to surrounding tissue. Their highly porous structure which can easily be tuned by controlling the density of cross-links in the gel matrix and the affinity of the hydrogels for the aqueous environment in which they are swollen³ is also an advantage. The porosity of hydrogels also permits loading of drugs into the gel matrix and subsequent drug release at a rate which is dependent on the diffusion coefficient of the small molecule or macromolecule through the gel network.³

Tetracarpidium conophorum, commonly called the African walnut plant, whose ethanolic extract would make up the second component of the formulation of study, is a perennial climbing shrub 10 to 20 feet long, found growing wild in forest zones of sub-Saharan Africa, including Nigeria. Studies have shown that the African walnut possess some beneficial properties like antibacterial,⁴ antioxidant^{4,5} and immune-stimulating activities. It is commonly used in Nigerian folkloric medicine for the treatment of bacterial infections and ailments caused by oxidative stress.⁶ Photochemical screening of ethanolic extracts of *tetracarpidium conophorum* showed presence of alkaloids, saponins, glycosides, flavonoids and tannins in studies carried out by Ezealisiji *et al.* The antibacterial properties of this plant extract can be attributed to the presence of these secondary metabolites.⁶ Incorporating these extracts into a three-dimensional polymer network formed by hydrophilic polymer chains via either physical or chemical bonds, hydrogels will be utilized to form a novel drug delivery system comprising of components that will work synergistically to facilitate wound healing.⁷

In the development of topical dosage form, an important issue was to design an optimized pharmaceutical formulation with appropriate penetration rate within a short time period with minimum trials. Traditional experiments require more effort, time, and materials when a complex formulation needs to be developed. Recently, response surface methodology (RSM) via central composite design (CCD) coupled with statistically designed experiment has been found to be very useful in optimising multivariable processes and it has been successfully applied to the optimisation of many bioprocesses.^{8, 9, 10, 11} Based on the principle of design of experiments, the methodology encompasses the use of various types of experimental designs, generation of polynomial equations, and mapping of the response over the experimental domain.

In this investigation, we explored the utility of RSM via CCD for the optimization of topical Gentamicin hydrogel production using a two variable central composite design via free

radical initial polymerization of the alkyl acrylate polymer. The developed optimized formulation was evaluated for performance related *in vitro* drug release and *ex vivo* permeation study. Physicochemical characterization of the Gel was carried out via rheological studies, drug content evaluation, Fourier transform infrared spectroscopy (FTIR) and the mechanism of release was evaluated via varying kinetic models.

EXPERIMENTAL

Chemical and Reagents

Gentamicin sulphate (BP grade) was obtained as a gift from Drugfield Pharmaceuticals Limited (Ogun State, Nigeria), Carbopol Ultrez 21[®] was obtained as a gift from Metchem Limited (Mumbai India/ Lubrizol corporation USA), Carbopol 940[®] (Lubrizol corporation USA), Propylene glycol, Triethanolamine (Merck German), Transcutol[®] was obtained as a gift from Gattefosse (Cedex, France), O-Phthalaldehyde OPA from Fluka (Steinheim Germany). N-acetyl cysteine (NaC) Sodium Hydroxide from Sigma Aldrich (St. Louis, USA). All other chemical and reagents were of analytical grade.

Extraction of ethanolic extract of *Tetracarpidium conophorum* (EETC)

The plant was collected from farms in Nkwere Local Government Area, Imo state, Nigeria, and identified by Mr Oyebanji O.O of the Department of Botany, University of Lagos, Lagos, Nigeria. A voucher specimen assigned reference number LUH6972 was deposited in the institutional herbarium for reference.

The methanolic extract of the leaves was obtained using a method by Amaeze *et al.*, 2011. The plants were air dried for 14 days and the leaves were separated and grounded using a Retsch rotor mill ZM 200. 200g of the finely grounded leaves of *T.conophorum* was weighed and placed in a container with 2 liters of ethanol poured into it and allowed to macerate for 24hrs and then filtered. The extraction was done thrice and the combined filtrate centrifuged at 3000 rpm using a Sorvall ST 8 centrifuge. The extract of *T.conophorum* (EETC) obtained was freeze dried, transferred into a glass vial and kept in a desiccator at 20°C until analysis.

Preparation of gentamicin loaded acrylate copolymer based hydrogels

0.1%w/w of gentamicin sulphate was dissolved in aliquots of purified water and propylene glycol was titrated in drops into the mixture. The permeation enhancer Transcutol:EETC (antioxidant extract) in varying ratios 2%v/v and 10%v/v propylene glycol were incorporated into the aqueous phase of the formulation. At 25°C the gel phase was prepared by dispersing the alkyl acrylate cross-polymers, Carbopol® Ultrez 21 (1.5% w/v) in purified water using a mechanical stirrer at a predetermined stirring rate. The pH was adjusted with the cross linking agent triethanolamine (TEA) to pH of 5.5. Both the aqueous fraction and the gel fraction were then mixed at a varying stirring rates to form the polymeric hydrogel. The hydrogels were stored in sealed glass containers for further analysis.

Fourier transform infrared spectroscopy (FTIR)

Gentamicin, EETC, and Carbopol Ultrez 21® compatibility was evaluated by Fourier transform infrared (FT-IR). Physical mixtures of gentamicin, the polymers (Carbopol Ultrez 21) and excipients (1:1) were separately mixed with three parts of potassium bromide and they were compressed to form pellets with a hydraulic press at 10 tons pressure. The FT-IR absorption spectra of all samples were recorded in the range of 400-4000/cm by potassium bromide disc method using FT-IR spectroscopy (Bruker, South Africa). The optimized hydrogel formulation was also analysed via FTIR. Physical appearance of the samples and the appearance (or disappearance) of peaks in the spectra were observed to access any possible physical or chemical interactions.

Experimental Design

A two variable, central composite design was used for the formulation of the gentamicin hydrogels. The independent variables tested included Transcutol:EETC ratio and the stirring speed. These variables were varied over five levels and replicated six times at the centre point to result in a total of fourteen experimental runs. The ranges of the independent variables are shown in Table 1. Two responses namely flux ($\mu\text{g}/\text{cm}^2/\text{hr}$) and the amount of drug permeated after 12 hours ($\mu\text{g}/\text{cm}^2$) were chosen for optimisation using RSM. The experimental observations were analysed using Design Expert® 7.0.0 software (Stat-ease, Inc. Minneapolis,

USA). The coded and actual values of the independent variables were calculated using Equation (1).

$$x_i = \frac{X_i - X_o}{\Delta X_i} \quad (1)$$

Where x_i and X_i are the coded and actual values of the independent variable respectively. X_o is the actual value of the independent variable at the centre point and ΔX_i is the step change in the actual value of the independent variable. The experimental data was fitted according to Equation (2) as a second-order polynomial equation including main effects and interaction effects of each variable. Analysis of variance (ANOVA) and response surface plots were generated using Design Expert and the optimised value of the independent variables for optimum response was determined using numerical optimisation.

$$Y_i = b_o + \sum b_i X_j + \sum b_{ij} X_i X_j + \sum b_{ii} X_i^2 + e_i \quad (2)$$

where Y_i is the dependent variable or predicted response, X_i and X_j are the independent variables, b_o is offset term, b_i and b_{ij} are the single and interaction effect coefficients and e_i is the error term.

Physical evaluation of hydrogel formulation

The hydrogels were physically examined for colour, homogeneity and consistency.

pH Evaluation

The pH of the hydrogels was recorded with pH meter (Ashford, UK), via ensuring electrode was in contact with the formulated hydrogel for 45 seconds to allow for equilibration. Experiments were performed in triplicate.

Rheological studies

The viscosities of the varying formulations were determined at 25°C at varying RPM with the aid of a cone and plate viscometer with spindle- 4, (Brookfield Engineering Laboratories, DV-E Digital viscometer ID:12020N15).

Drug content determination

1g of hydrogel was dissolved in 10mls of water, centrifuged at 500 rpm for 45 minutes and filtered using a 0.5µm millipore filter. Utilizing a 1:50 dilution, the concentration of gentamicin was obtained using a UV/Visible spectrophotometer (UV-Vis 2600 shimadzu Analytical and measuring instruments) after derivatization utilizing O-Phthalaldehyde reagent by Kowalczyk's method (12). Phthalaldehyde reagent was formulated prior to use by dissolving 20mg of O-Phthalaldehyde in 1.0ml of methanol to 1.5mls of a 10% N-acetylcysteine and diluting to 10mls with 0.2mls⁻¹ solution of borate buffer pH 10. Gentamicin an aminoglycoside antibiotic, does not absorb UV light due to its weak chromophore hence the need for derivatization. The phthalaldehyde reagent was stored in amber coloured bottles and kept in a dark cupboard prior to use. The reaction of the amine group in the aminoglycoside with the O-Phthalaldehyde in the presence of N-acetyl cysteine as a nucleophile to yield a fluorescent isoindole which is measured at 332nm absorbance.¹² This method is superior to that used by Nnamani *et al.*,¹ where mercaptoethanol which emits a characteristic unpleasant odour during the derivatization process.

Preparation of Wistar Rat Abdominal Skin

The hair of ether anesthetized wistar rats weighing between 150-200g was carefully removed with electrical clippers, and the full thickness of skin was removed from the abdominal region. Utilizing heat separation techniques, the epidermis was prepared. The epidermis was prepared surgically by heat separation technique¹³ which involved soaking the entire abdominal skin in water at 60°C for 45s, followed by careful removal of the epidermis. The epidermis was washed thrice with water and used for *ex vivo* permeability studies.

Ex vivo permeation studies

Permeation study was carried out using skin obtained from male wistar rats (skin thickness 0.45-08.mm) mounted on modified Franz diffusion cells with diffusion area of 3.71cm². The receptor compartment contained 30 mls phosphate buffer (pH of 7.4 at 37.1°C ± 0.2°C). 1g of each hydrogel formulation was applied on the skin surface in the donor compartment area with the stratum corneum facing downwards in the donor compartment. An aliquot of 1 ml was withdrawn at predetermined time intervals and replaced with equal volume of fresh media. The samples were analysed using a UV/Visible spectrophotometer (UV-Vis 2600 Shimadzu

Analytical and measuring instruments) after derivatization utilizing O-Phthalaldehyde reagent absorbance was measured at 332nm absorbance. All experiments were performed in triplicate. Cumulative amounts of drug permeated ($\mu\text{g}/\text{cm}^2$) was plotted against time in hours and drug flux ($\mu\text{g}/\text{cm}^2/\text{hr}$) at steady state was calculated by dividing the slope of the linear portion of the curve by the area of the exposed skin surface 3.71cm^2 . The permeation coefficient was deduced by dividing the flux by initial drug load as shown in Equation 3 and 4

$$\text{PEc} = \delta Q(A \delta t)^{-1} / C_o \quad (3)$$

$$\text{PEc} = J_{ss} / C_o \text{ (cm/hr)} \quad (4)$$

where PEc is the permeation coefficient (cmhr^{-1}); C_o is the initial drug concentration in the drug compartment; J_{ss} represents the steady state flux ($\mu\text{g}/\text{cm}^2/\text{hr}$), where Q indicates the quantity of substances crossing the rat skin, A is the area of the rat skin exposed and t is the time of exposure in hours.

The optimized gentamicin hydrogel derived from statistical analysis was then compared with marketed gentamicin formulation via *ex vivo* permeation studies and the data obtained was evaluated using one-way analysis of variance followed by Turkeys test at $P < 0.05$

Ex vivo permeation kinetics of drug release

The mechanism of drug release from the hydrogels was analysed by fitting the release data to various release kinetic models. The zero-order (K_o), first order (K_f), and Korsemeyer Peppas (K_p) model was used to find out the model with the best fit.^{1,11}

Accelerated stability testing

ICH guidelines (40 °C/75 %RH) were followed in the accelerated stability testing of the optimized hydrogel formulation. The hydrogels were packed in amber colored jars and kept in a stability chamber with set temperature and relative humidity. The formulations were subjected to accelerated stability testing at both room temperature and at 40°C and parameters were recorded on day 0, 10, 15, 30, 90. The formulations were evaluated for pH, assay, gel index, percentage of drug released at 12 hours.

Statistical analysis

The data were expressed mean comparison with the standard was evaluated using one-way analysis of variance (ANOVA) (\pm SD). Significant differences ($p < 0.05$) of mean values were determined by Tukey test.

RESULTS

Fourier transform infrared spectroscopy

The individual spectra and the physical mixture spectra were recorded and analysed. The fingerprint region and absorbance values relating to the relevant bioactive functional groups of the individual spectra analysed and the physical mixtures showed an absence of interaction between gentamicin, carbopol and transcutool and EETC as shown in Figure 1. Absorbance patterns corresponding in position and relative intensity to those in the FTIR spectra of the individual components were observed with no significant change in FTIR spectra after introduction of the polymers and the permeation enhancers thus indicating a lack of physical or chemical interaction as shown in Figure 1.

Fourier transform infrared spectroscopy peaks of A) Gentamicin and the physical mixtures of Carbopol[®] (B) Physical mixtures of Carbopol[®] and EETC, (C) Physical mixtures of Carbopol[®] and EETC:Transcutool (D) Hydrogel formulation containing Carbopol[®], EETC, Transcutool and all other excipients highlighting some major bands/peaks. Some major bands/peaks on the spectra were 3089.75cm^{-1} O-H stretching, 1706.88cm^{-1} carboxyl group which is characteristic of the principal absorption peaks of Carbopol[®]. Gentamicin was characterized by principal peaks at 610.22cm^{-1} and $1100-1400\text{cm}^{-1}$. The spectral data of EETC confirmed the presence of functional groups such as hydroxyl, ester group and aldehyde group among others 2920.21cm^{-1} - C-H stretching depicting alkenes and aryl groups, 1730.32cm^{-1} - aldehyde/ketone - C=C- stretching at 1440cm^{-1} . For compatibility study the FTIR spectra were compared and there was no disappearance or major shift of important peaks in the physical mixtures of Carbopol[®], EETC and Transcutool spectrum.

Gel Characterization.

All the formulations had a pale greenish colour and a good gel like consistency. The formulated hydrogels formulated using the composite design had drug content variation from 94.5% - 102.9% with pH of all the 14 hydrogel formulations ranged of 5.50 - 5.95 after neutralization.

with triethanolamine as shown in Table 2 . This pH range is important for use on the surface of wound to facilitate wound healing at an acidic pH (1,3, 12).

Rheological Measurements

Spindle 4 was used for the viscometric characterization of hydrogels. Characterization was done at 20 - 60rpm which is the working range for this spindle. As the shear rate increased there was a corresponding decrease in the viscosity of the gels. This was evaluated exponentially using the Power Law as shown in Equation 3.

$$T = K D^n \quad (5)$$

where T is shear stress; K is gel index (GI) or consistency index; D is shear rate; and n is flow index. Gel indices computed ranged from 1.02 to 2.11 as shown in Table 2.

Statistical Modelling and Analysis

Analysis of the experimental data using the Design Expert software revealed that the quadratic model was suitable for describing the formulation of the hydrogels. The final statistical models for predicting the flux and the amount of drug permeated after 12 hours are given in Equations 6 and 7.

$$Y = 19.35 - 25.82X_1 - 0.044X_2 + 0.0097X_1X_2 + 11.86X_1^2 \quad (6)$$

$$Y = 315.50 - 189.67X_1 + 0.28X_2 - 1.29X_1X_2 + 123.55X_1^2 \quad (7)$$

The values of the responses as predicted by Equations 4 and 5 are presented in Table 3 alongside the experimental data for comparison. The results of analysis of variance (ANOVA) carried out to determine the fit of the statistical models for flux and drug permeation are presented in Tables 3 to 5.

Tables 3 to 6 show the results of analysis of variance (ANOVA) carried out to determine the fit of the statistical models representing the flux and drug permeation after twelve hours. Tables

3 and 4 respectively shows that the models for flux and drug permeation were statistically significant with very low p values of 0.0001 and 0.0019 respectively. The single effect model terms representing the effect of transcuto:EECTC ratio and stirring speed for both models (Equations 5 and 6) were significant indicating changes in the values of these variables could affect the flux and drug permeation.

Table 5 shows that the models for flux and drug permeation had high R^2 values of 0.90 and 0.82 respectively. The R^2 value indicates the degree to which a model is able to predict a response. The closer the R^2 value is to unity, the better the model can predict the response (15). The high R^2 values obtained for both models show that there was significant fit between the observed and predicted values of flux and drug permeation. Table 5 also shows that the standard deviation of the observations was relatively small compared to the mean values of flux and drug permeation showing that there was very little dispersion about the mean for the data predicted by both models. The experimental runs were carried out with a high reliability and precision as seen from the relatively low values of coefficient of variation (CV) obtained for flux and drug permeation (8.75 and 12.27 respectively) (16). The adequate precision values of both models were greater than four. This shows that the models had adequate signals and thus could be used to navigate the design space (17)

Effect of independent variables on hydrogel formulation

Figures 3 and 4 are response surface plots showing the effect of Transcutol:EECTC ratio and stirring speed on the flux and drug permeation of the hydrogels respectively. Lower levels of stirring speed and transcuto:EECTC ratio enhanced the flux of the formulated hydrogel as shown in Figure 3. This is evidenced by the fact that the flux increased with a reduction in stirring speed. This observation was recorded at all values of Transcutol:EECTC ratio investigated. A similar trend was also observed for the Transcutol:EECTC ratio for all values of stirring speed investigated.

DISCUSSION

Gentamicin is freely soluble in water (hydrophilic) and much of the drug was present in the aqueous phase of the formulations, loosely attached at or near the particle surface, because the more hydrophilic the substance, the weaker the interaction with particle surface, and eventually the compound could be localized in the surfactant layer.¹ When more drug particles at the periphery of the particle surface eventually encounter the polymeric cross-linked gel-matrices, stabilization would occur.^{18,19} Maximization of skin uptake and delivery of such a drug that is hydrophilic such as Gentamicin in a hydrogels would thus be affected by increased stirring speeds above 60-77rpm. Figure 4 shows that the drug permeation after twelve hours reduced with increase in stirring speed. This trend was however more significant at a higher ratio of transcutol to EETC compared to when lower ratio was used. An increase of stirring speed above this point will ensure decreased porosity of the polymeric system increasing entrapment of the transcutol:EETC within the hydrophilic matrix due to excessively intense agitation during formulation. This may consequently result in decreased release rates as seen in Figure 2 and 4 inadvertently negatively influencing permeation of gentamicin through the skin as seen in Figure 4.¹ A burst effect as result of increase stirring speed may also account for the decreased drug permeation with increase stirring speed as the drug is freed from the polymeric matrices and as such cannot be transported through the biological membrane utilizing transcutol:EETC, this effect will account for why there is a reduced flux at higher stirring speeds as shown in Table 3 where the experimental values closely correlated with the predicted responses.

EETC has been studied for toxicity and biocompatibility and has been seen to be non toxic to and biocompatible with mammalian cell lines thus informing its use in this formulation development.^{4,5,6} EETC is very high in antioxidants which lower inflammatory markers and facilitate wound healing by promoting fibroblast migration, this combination of EETC with transcutol (2-(2-Ethoxyethoxy)ethanol) a chemical permeation enhancer synergistically causes diffusional resistance of the stratum corneum increasing migration of gentamicin through the skin via increased solubility in the stratum corneum. Transcutol:EETC at high concentrations facilitate interaction with stratum corneum lipids to increase fluid into the skin producing increased flux, P_{Ec} and ultimately drug permeation. There was an inverse relationship between the drug permeation after twelve hours and the transcutol:EETC ratio. This ensures that increased permeation occurs in the first 12 hours of hydrogel application. The flux obtained ranging from 9.05 to 14.42 $\mu\text{g}/\text{cm}^2/\text{hr}$ accounting for release at the linear portion of the gentamicin permeation curve in Figure 1 which represents the first 4 hours of drug release and permeation showed that GeH4 with flux 14.42 $\mu\text{g}/\text{cm}^2/\text{hr}$ having the highest flux which reflects increased permeation at an optimal stirring speed. This trend was observed both at high as well

as at low values of stirring speed. However, the correlation between the drug permeation and transcutol:EETC ratio was more significant at high values of stirring speed due to increased porosity of the hydrogel matrix. The mechanism of release predominantly observed was the Higuchi model thus relating that initial drug concentration in the hydrogel matrix is much higher than drug solubility with drug diffusion taking place in one dimension with edge effect being negligible, this accounts for increased release through pores in the matrix hydrogel system.

Optimization of hydrogel formulation

Numerical optimization was carried out to maximize the flux and drug permeation using the Design Expert software. The optimum conditions were chosen from the results obtained from the software possessing the highest desirability. These conditions are summarized in Table 6. The implication of these results is that the maximum flux and drug permeation can only be obtained if the independent variables are fixed at the values shown in Table 7. Accelerated stability testing of the optimized formulation showed that no variation in pH, assay, gel index, percentage of Drug released at 12 hours was observed as shown in Table 8.

Validation of Statistical Models

The validity of the statistical models used for predicting flux and drug permeation was confirmed by carrying out three confirmation experimental runs at the identified optimum conditions (Table 6). The results showed that there was no significant difference between the experimental results and those predicted by the statistical models. The excellent correlation between the predicted and measured values shows the validity of statistical models. Figure 5 shows the percentage of drug released from the optimized formulation in comparison with a marketed formulation. *Ex vivo* permeation study showed an improved release rate was obtained compared to the marketed topical formulation with 100% release occurring at 12 hours with a significant effect ($P < 0.05$) compared to the marketed brand which had 90% release at the same. This result is in consonance with the optimum value of drug permeation given in Table 7. Flux was obtained as $16.9 \mu\text{g}/\text{cm}^2/\text{hr}$ compared to $9.98 \mu\text{g}/\text{cm}^2/\text{hr}$ for the marketed formulation and amount of drug permeated after 12 hours was $260 \mu\text{g}/\text{cm}^2$ compared to $176 \mu\text{g}/\text{cm}^2$ for the marketed formulation. The results are in consonant with the optimized values given in Table 7

from CCD with the solubilizing effect of the permeation enhancer / antioxidant ratio ensuring optimal flux and transdermal permeation within 12 hours at 60 rpm. All the gel formulations were stable at room temperature and under stressed conditions as shown in Table 8 after accelerated stability testing.

Conclusion

Optimization of gentamicin hydrogel using central composite statistical design is valid for prediction of drug permeation and flux utilizing variables in formulation preparation i.e stirring speed and permeation enhancer: antioxidant ratio, showing their interaction with each other. The contour plots aided in prediction of the value of transcutol:EETC and stirring speed which would provide an optimized gentamicin hydrogel with optimal and drug permeation after 12 hours. The evaluation of therapeutic efficacy in an animal model is recommended for further studies.

Acknowledgement

The authors would like to thank Drugfield Pharmaceuticals Limited(Ogun State, Nigeria) for the gift of Gentamicin sulphate, Metchem Limited (Mumbai India) for the gift of Carbopol[®] Ultrez 21 and Gattefosse (Cedex, France) for the gift of Transcutol[®].

Table 1: Experimental range of independent variables for a two factor CCD

Independent variables	Symbols	Coded and Actual Levels				
		-1.414	-1	0	1	1.414
Transcutol:EETC (-)	X ₁	1/3	3/7	2/3	0.902	1
Stirring speed (RPM)	X ₂	60	77.57	120	162.43	180

Uncorrected proof

Table 2 Observed responses in the central composite design for gentamicin hydrogels

Hydrogel Formulation	pH	Assay (%)	PEc (cm/hr)	Gel index (G1)	Kinetic modelling		
					Zero order (Ko)	First Order (Kf)	Higuchi model (Kh)
GeH1	5.67 ± 0.05	98.65 ± 0.11	1.77	1.02	0.912	0.567	0.943
GeH 2	5.69 ± 0.04	98.73 ± 0.28	1.91	1.76	0.893	0.329	0.954
GeH 3	5.78 ± 0.32	99.43 ± 0.78	1.72	1.89	0.903	0.510	0.964
GeH 4	5.51 ± 0.09	101.02 ± 0.21	1.85	1.76	0.911	0.622	0.932
GeH 5	5.86 ± 0.03	98.82 ± 0.88	1.70	1.65	0.954	0.476	0.954
GeH 6	5.54 ± 0.48	99.72 ± 0.01	1.69	1.97	0.963	0.619	0.922
GeH 7	5.88 ± 0.11	100.07 ± 0.29	1.73	1.56	0.945	0.409	0.973
GeH 8	5.89 ± 0.77	98.99 ± 0.89	1.71	2.00	0.953	0.743	0.917
GeH 9	5.61 ± 0.37	98.62 ± 0.67	1.77	1.97	0.944	0.599	0.972
GeH 10	5.92 ± 0.11	99.89 ± 0.32	1.78	1.56	0.891	0.421	0.954
GeH 11	5.93 ± 0.07	100.33 ± 0.06	1.93	2.11	0.932	0.511	0.911
GeH 12	5.55 ± 0.32	101.77 ± 0.03	1.72	1.99	0.894	0.613	0.919
GeH 13	5.63 ± 0.04	102.31 ± 0.15	1.73	1.34	0.843	0.412	0.963
GeH 14	5.78 ± 0.12	100.99 ± 0.45	1.72	1.69	0.909	0.592	0.951

Table 3: Experimental design matrix for gentamicin hydrogel formulation

Run	Factors				Responses			
	Coded values		Actual values		Flux ($\mu\text{g}/\text{cm}^2/\text{hr}$)		Drug permeation ($\mu\text{g}/\text{cm}^2$)	
	X ₁	X ₂	X ₁	X ₂	Actual	Predicted	Actual	Predicted
GeH1	1	1	0.902	162.43	6.91	6.37	124.43	102.10
GeH 2	1	-1	0.902	77.57	9.25	9.43	186.93	176.70
GeH 3	-1.414	0	1/3	120.00	12.75	13.64	222.4	249.07
GeH 4	-1	-1	3/7	77.57	14.24	13.83	254.8	235.96
GeH 5	0	0	2/3	120.00	9.8	9.34	178.02	175.02
GeH 6	0	-1.414	2/3	60.00	11.21	11.64	196.26	209.49
GeH 7	0	0	2/3	120.00	8.57	9.34	163.78	175.02
GeH 8	1.414	0	1	120.00	7.63	7.69	114.21	128.71
GeH 9	0	1.414	2/3	180.00	5.59	7.03	110.21	140.55
GeH 10	0	0	2/3	120.00	9.71	9.34	184.98	175.02
GeH 11	-1	1	3/7	162.43	11.51	10.38	243.99	213.06
GeH 12	0	0	2/3	120.00	9.98	9.34	183.99	175.02
GeH 13	0	0	2/3	120.00	9.82	9.34	176.43	175.02
GeH 14	0	0	2/3	120.00	9.05	9.34	165.34	175.02

Table 4: ANOVA results for model representing flux

Sources	Sum of Squares	df	Mean Squares	F value	p value
Model	59.92	4	14.98	20.71	0.0001
X ₁	35.41	1	35.41	48.94	< 0.0001
X ₂	21.18	1	21.18	29.28	0.0004
X ₁ X ₂	0.038	1	0.038	0.053	0.8238
X ₁ ²	3.29	1	3.29	4.55	0.0617

Table 5: ANOVA results for model representing drug permeation

Sources	Sum of Squares	df	Mean Squares	F value	p value
Model	20265.19	4	5066.30	10.50	0.0019
X ₁	14486.89	1	14486.89	30.02	0.0004
X ₂	4753.28	1	4753.28	9.85	0.0120
X ₁ X ₂	667.96	1	667.96	1.38	0.2695
X ₁ ²	357.05	1	357.05	0.74	0.4120

Table 6: Statistical information for ANOVA

Parameter	Response	
	Flux	Drug permeation
R-Squared	0.90	0.82
Mean	9.72	178.98
Standard Deviation	0.85	21.97
C.V %	8.75	12.27
Adeq. Precision	14.68	11.20

Table 7: Summary of optimum conditions for the formulation of hydrogels

Variables	Optimum value
Stirring speed (rpm)	60
Transcutol:EETC ratio (-)	1/3
Flux ($\mu\text{g}/\text{cm}^2/\text{hr}$)	16.1
Drug permeation after 12 hours ($\mu\text{g}/\text{cm}^2$)	258

Table 8 Accelerated stability testing on the optimized (GeH) gentamicin hydrogel at 40 °C/75 %RH ($p \leq 0.05$)

Duration	pH	Assay (%)	Gel index (G1)	Flux	Drug permeation after 12 hours ($\mu\text{g}/\text{cm}^2$)	Appearance and homogeneity
Day 0	5.77 \pm 0.05	99.65 \pm 0.11	1.93 \pm 0.02	15.93 \pm 0.22	254.03 \pm 1.01	Satisfactory
Day 10	5.79 \pm 0.04	99.73 \pm 0.28	1.96 \pm 0.03	15.83 \pm 0.74	251.72 \pm 2.11	Satisfactory
Day 15	5.78 \pm 0.32	99.43 \pm 0.78	1.99 \pm 0.01	16.09 \pm 0.32	253.32 \pm 0.93	Satisfactory
Day 30	5.78 \pm 0.07	100.02 \pm 0.21	1.96 \pm 0.01	15.99 \pm 0.18	250.11 \pm 1.02	Satisfactory
Day 90	5.76 \pm 0.03	99.98 \pm 0.03	1.93 \pm 0.04	15.93 \pm 0.07	254.2 \pm 0.97	Satisfactory

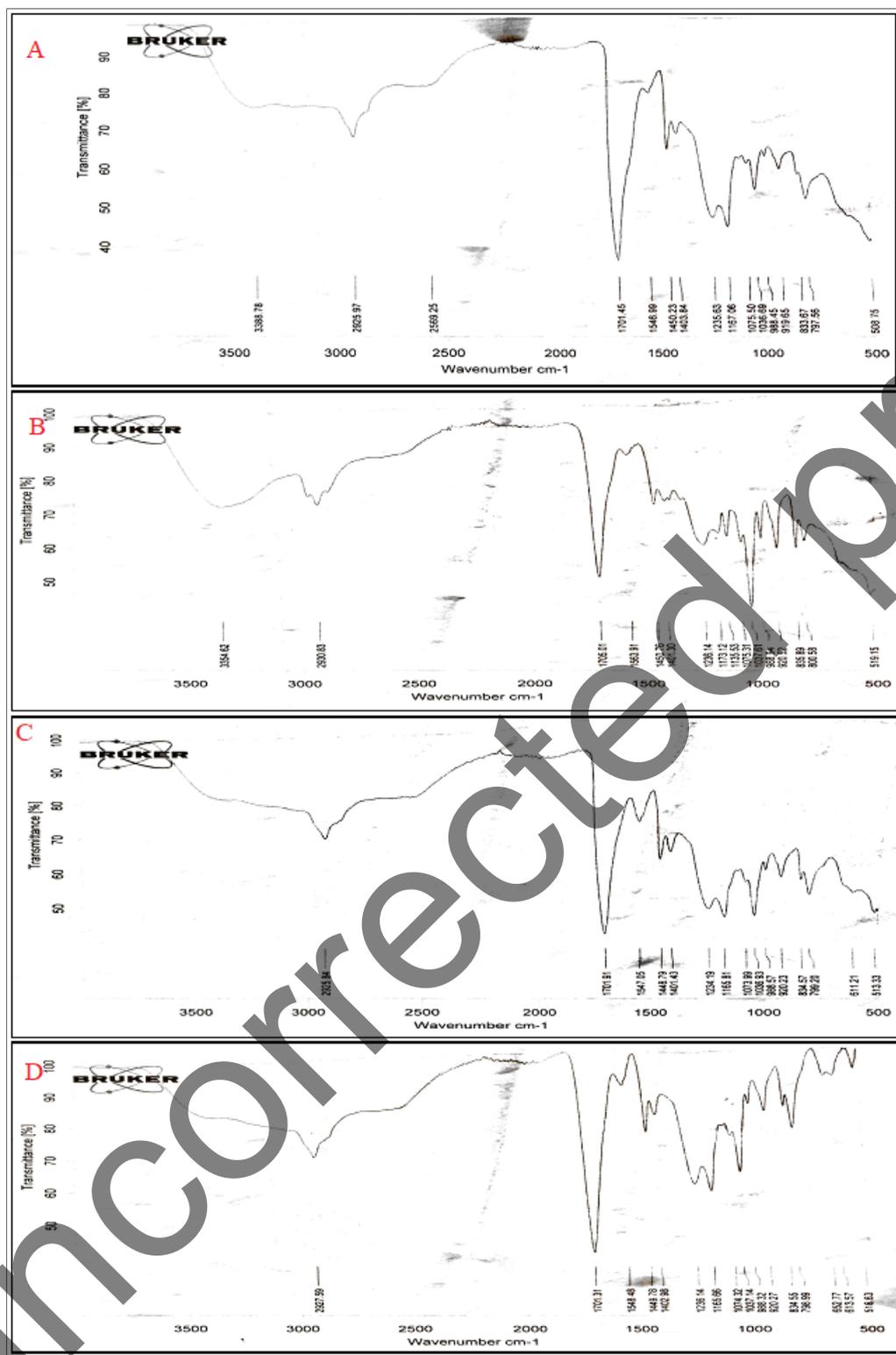


Figure 1: Fourier transform infrared spectroscopy peaks of A) Gentamicin and the physical mixtures of Carbopol[®] (B) Physical mixtures of Carbopol[®] and EETC, (C) Physical mixtures of Carbopol[®] and EETC:Transcutol (D) Hydrogel formulation containing Carbopol[®], EETC Transcutol and all other excipients.

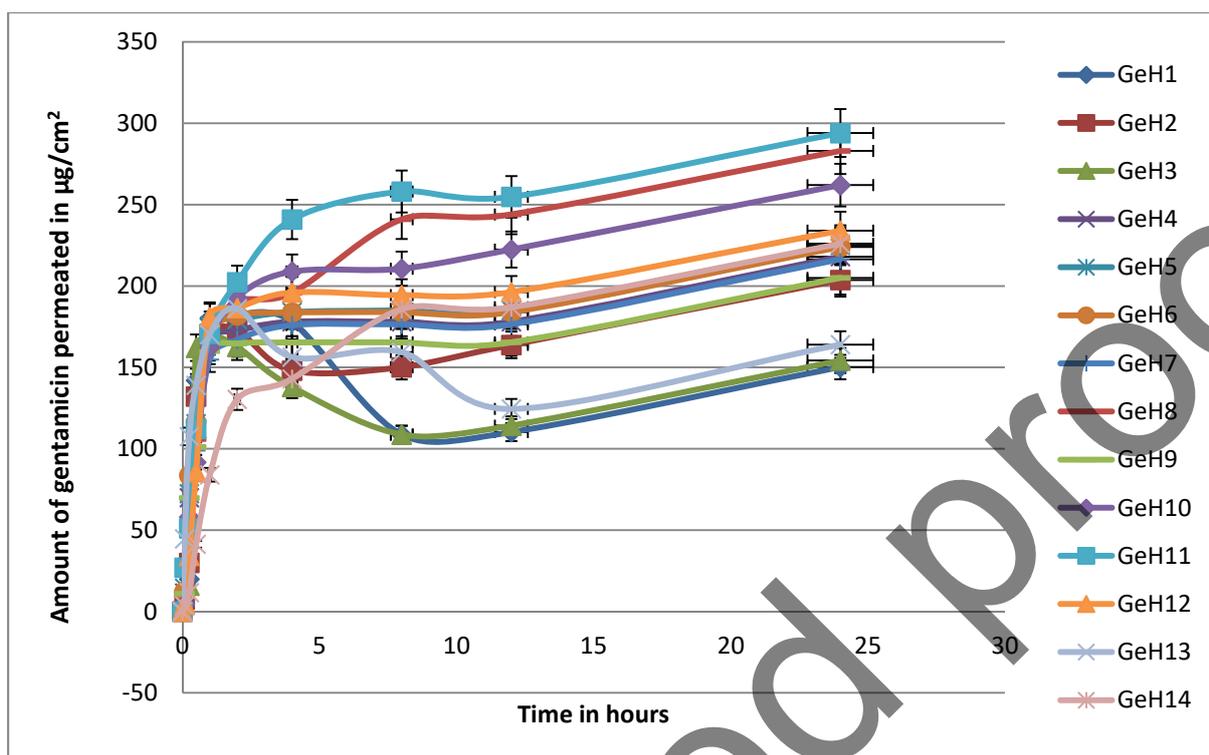


Figure 2 *Ex vivo* permeation profile of the varying gentamicin hydrogel formulations GeH1-GeH14

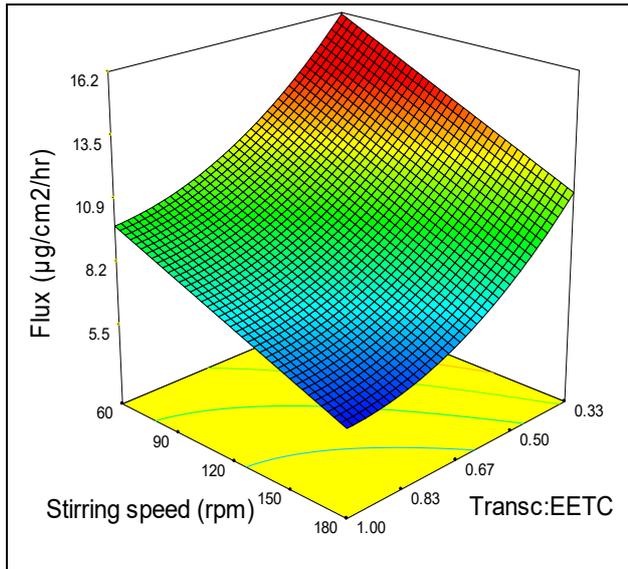


Figure 3: Effect of Transcutol:EETC ratio and stirring speed on flux

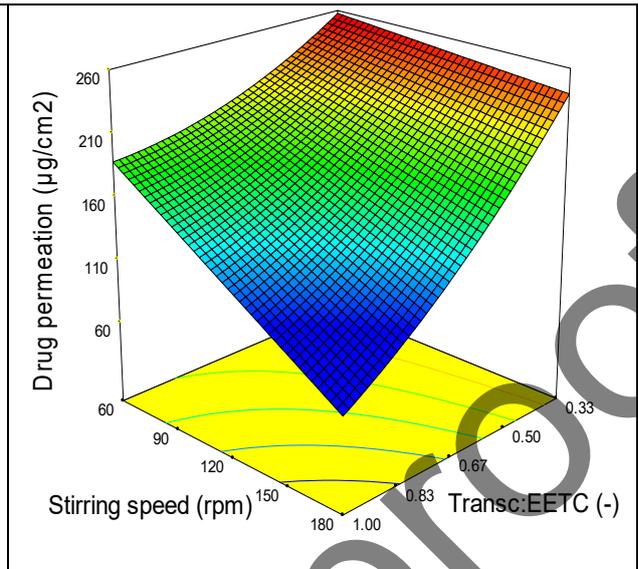


Figure 4: Effect of Transcutol:EETC ratio and stirring speed on drug permeation after 12 hours

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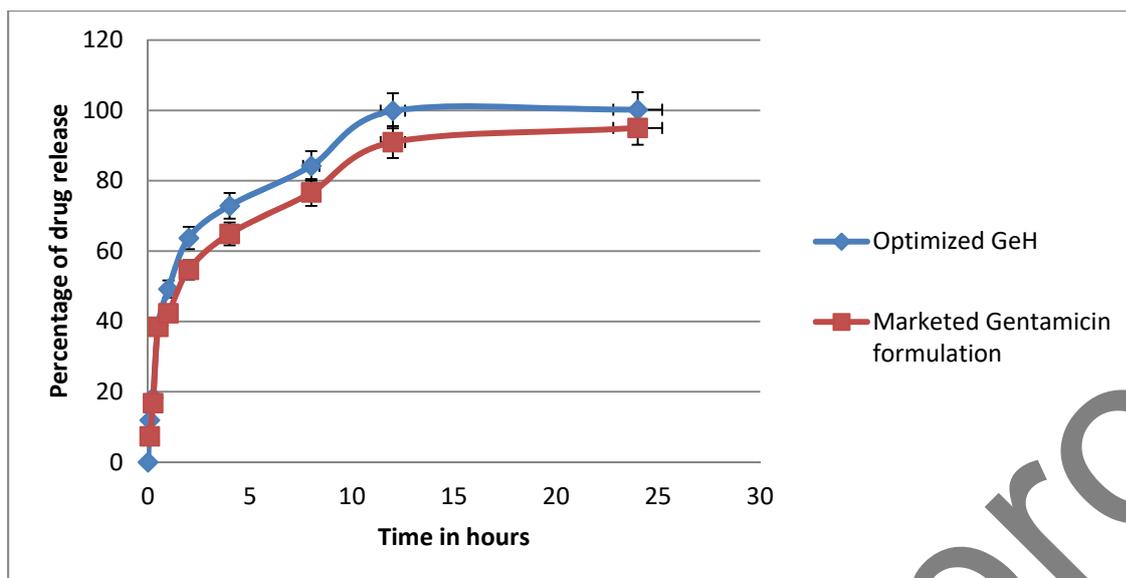


Figure 5 Comparison of Percentage of gentamicin release from the optimized hydrogel formulation and marketed gentamicin topical formulation against time in hours.

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