RESEARCH ARTICLE

Methocarbamol Transferosomal Gel For Topical Delivery: *In-vitro*, *Ex-vivo* and *In-vivo* Evaluation

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ABSTRACT

Because of their capacity to permeate across the stratum corneum's barriers to penetration, transferosomes are one of the vesicular transporters that have recently attracted a lot of research and attention. The current study aimed to analyze and improve methocarbamol transferosomal transdermal gels. Reverse phase evaporation was used to create thirteen different formulations of methocarbamol-loaded transfersomes. Using face-centered central composite designs, the influence of independent process variables, like soy lecithin content and surfactant concentration, on dependent variables, such as entrapment effectiveness and the vesicle size of methocarbamol transfersomes, was assessed. The *ex-vivo* permeation flux of methocarbamol and the lipid to surfactant ratio/skin absorption efficiency of the drug and transferosomal gels were optimized. The optimized formulation showed an entrapment efficiency of 95%, a vesicle diameter of 428 nm, a drug content of 96.6% and a zeta potential of −28.9 mV. *In-vitro* and *ex-vivo* drug release rate in optimised gels was found to be 86.43 and 84.12% for 24 hours, respectively. This tranferosomal delivery method for transdermal methocarbamol may be preferable to the standard of care.

Keywords: Vesicular carriers, Methocarbamol transferosomal gels, Reverse phase evaporation, Central composite design, Entrapment efficiency, Vesicle size, Soy lecithin content, Surfactant concentration.

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INTRODUCTION

Transdermal drug delivery techniques in smaller doses have been investigated as an important and effective way to easily and safely administer drugs to achieve local and systemic effects for prolonged periods of time while avoiding the first step of hepatic metabolism.[1] Transdermal drug delivery must extend beyond the stratum corneum, the top layer of the skin's protective layer, the attainment of therapeutic medication concentrations in specific skin layers.[2] The physicochemical characteristics of the delivered drug and the vehicle used have an impact on the transdermal drug release mechanism as well. Over the past decades, various strategies are explored to overcome the difficulties involved with transdermal drug delivery.[3] Drug distribution through the skin has recently been explored using submicron-sized vesicular drug-carrier systems such as liposomes, transferosomes, ethosomes, microemulsions, etc.[4] Transferosomes are among these vesicular carriers that have received extensive research and are now becoming more significant for topical use because of their capacity to circumvent stratum corneum penetration challenges.[5,6]

Transferosomes are primarily synthetic vesicles that are far more deformable than typical liposomes.[7] 'Ultra-deformable liposomes' is another name for these. By combining surfactants in the right amounts, it is possible to distort liposomes for improved drug molecule penetration through the skin. They are stretchy by nature, which makes it easier for them to fit as an unbroken vesicle through the skin's tiny pores. The transfersomes' deformable characteristics are caused by the existence of edge activators.[8] The transepidermal osmotic gradient between the skin surface and the inside of the skin drives elastic transport. They can pass through pores that are smaller than themselves thanks to their flexibility.[9,10]

The response surface methodology (RSM) examines how various explanatory variables affect one or more response variables. An experimental design often comprises choosing the ideal arrangement of independent elements as well as the level of each aspect to be researched. Nevertheless, it is crucial to limit the number of runs while still attaining the desired findings because experimental runs are time- and money-consuming. Many methods, including Box-Behnken (BB), full factorial, and central composite designs,[11] are used to accomplish this. Response surface analysis and optimization with factorial designs are efficient methods for reducing the amount of time needed to generate pharmaceutical dosage

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forms and enhancing research output.[12]

A skeletal muscle relaxant called methocarbamol is used in conjunction with rest, physical therapy, and other treatments to ease the pain and discomfort of acute musculoskeletal problems. It shares a structural similarity with guaifenesin and is used to alleviate spasm.[13] The current study was targetted to prepare transferosmal gel containing methocarbamol for enhanced transdermal penetration and understand the impact of experimental variables such as soy lecithin and surfactant concentration on %entrapment efficiency and vesicle size using a central composite design (CCD)-based response surface methodology.

As we explore this new area of nanotechnology, it becomes imperative to recognize that this new area provides a key component factor their application in a range of compositions to optimize the permeability of many pharmaceutical compounds. This is evidenced by the fact that various transfersome products are currently being used in cutting-edge clinical trials. Interestingly, several innovative solutions in the area of dermal and transdermal delivery may soon find success in the global market.

MATERIALS AND METHODS

Materials

Methocarbamol a gift sample from Khandelwal Laboratories Pvt Ltd, Mumbai, India. Soy lecithin, Tween 80 and Carbopol-934 were acquired from Sigma Aldrich Ltd, India.

Methods

Preparation of methocarbamol-loaded transfersomes

Reverse phase evaporation was used to create transferosomes, albeit with various alterations as stated in the literature. Cholesterol and soy lecithin were first taken as lipids into container. Surfactant tween 80 was added to the same beaker and mixed with diethyl ether and chloroform to dissolve it. Setup was left at room temperature for 24 hours until a thin layer formed. A probe sonicator (FS-600, Frontline Electronics, and Machinery Pvt. Ltd., India) was used to sonicate the drug solution (1000 mg/mL in water) onto the thin film for two minutes at a frequency of 20 kHz. To create transferosomal suspensions, the membrane was hydrated with sodium deoxycholate, an edge deactivator, in phosphate-buffered saline (pH 7.4). As a chemical permeation enhancer, 2% volume/volume dimethyl sulfoxide (DMSO) was added to each suspension. Then, different transferosomal suspensions that had been created were passed through filter paper (No. 40). These transferosome suspensions were then added at 1% w/v. Carbopol and stored in a cool, dark environment to avoid oxidation.[14,15]

Experimental design

In the current study, version 12 of the advanced design software was used to optimize two main properties of transferosomes, mainly vesicle size and percent entrapment efficiency. The independent variables selected were component A is Cholesterol: Soya lecithin and component B is the surfactant. Vesicle size and %entrapment efficiency are dependant variable chosen. The lower values of components A and B were selected as 10 mg and the higher value is 90 mg. Based on the above independent and dependent variables response surface plots were designed using the CCD. The experimental ranges for each element were chosen based on early experiment findings and the viability of producing transfersomes at extreme values. Table 1 lists the batch codes and factors. The software's solution was used to determine which optimized formulation to use.[16,17]

Characterization of Transfersomes

Fourier transform infrared spectroscopy studies

Dry potassium bromide was combined with 1 to 3 mg of the material before it was tested in transmission mode over a 4000 to 400 cm^1 wave number range[18,19] for FTIR. Figures 1 and 2 depicts the distinctive drug absorption of various functional groups and illustrates the peaks in Table 2.

Drug loading, entrapment efficiency and vesicles size

Vesicles size assessed by utilizing dynamic light scattering (DLS) along Malvern Zetasizer 3000 HSA. The polydispersity index (PDI), is a quantity for size distribution width, and the

Table 1: Methocarbamol transferosomal gel formulation

STD	*Run*	*Factor 1 A: Soya lecithin :Cholesterol*	*Factor 2 B:Surfactant*	*Drug (Methocarbamol in mg)*	*Carbopol 934 (%w/v) (%)*
8	1	90	90	50 mg/10 mL	1
9	2	106.569	50	50 mg/10 mL	1
13	3	50	50	50 mg/10 mL	1
6	4	50	-6.56854	50 mg/10 mL	1
3	5	50	50	50 mg/10 mL	1
4	6	50	50	50 mg/10 mL	1
7	7	50	106.569	50 mg/10 mL	1
2	8	50	50	50 mg/10 mL	1
11	9	90	10	50 mg/10 mL	1
1	10	-6.56854	50	50 mg/10 mL	1
5	11	10	10	50 mg/10 mL	1
12	12	10	90	50 mg/10 mL	1
10	13	50	50	50 mg/10 mL	1

Table 2: FTIR Interpretation of Methocarbamol and Excipients

S.No.	*Functional Group*	*Wavenumber (cm^1)*	*Pure Methocarbamol*	*Methocarbamol+Soyalecithin+Tween 20+ Tween 80*
1	NH_2	3300-3000	3437.84	3501.98
2	COOH	1760-1690	1733.67	2921.13
3	CH_3	2000-1500	1506.82	1734.75
4	C=O	1660-1690	1677.79	1646.90
5	$(C_6H_6)_2$	1500-400	1506.82 – 524.27	1457.05 – 506.77

Table 3: Table showing the results for entrapment efficiency percentage, drug content and vesicle size of methocarbamol

S.No.	*Formulation number*	*Vesicle size (nm)*	*Entrapment efficiency (%)*	*Drug content (%)*
1	MTG1	297	71	92.1
2	MTG2	428	95	96.6
3	MTG3	405	87	92.4
4	MTG4	252	85	92.2
5	MTG5	405	87	92.4
6	MTG6	405	87	92.4
7	MTG7	369	68	90.32
8	MTG8	405	87	92.4
9	MTG9	287	94	95.4
10	MTG10	601	89	93.9
11	MTG11	473	91	94.2
12	MTG12	557	71	91.1
13	MTG13	405	87	92.4

Table 4: Results showing stability studies of methocarbamol transferosomal gel

S.No.	*No. of days*	*Evaluation parameter*	*Initial*	*MTG2*
1	30	Appearance	Light yellow color	No change
2	60	pH	5.6	No change
3	90	%drug content	96.7	No change

Table 5: The comparison of PK parameters for marketed formulation and MTG2

PK parameters	*Marketed formulation*	*MTG2*
C_{max} (ng/mL)	1069.98 ± 17.43	1124.43 ± 13.54
AUC_{0-t} (ng.h/mL)	7321.22 ± 36.35	8432.84 ± 47.54
AUC_{0-inf} (ng.h/mL)	8392.22 ± 38.43	10517.32 ± 46.24
T_{max}(h)	2.5 ± 0.08	4.0 ± 0.4
$t_{1/2}$ (h)	8.4 ± 0.28	11.02 ± 0.14

average diameter are obtained via DLS.[20] Before the experiment, the material was diluted with ultra-purified water. The complete amount of the produced transferosomal suspensions underwent a 30 minutes ultracentrifugation at 10°C at an rpm of 20,000.

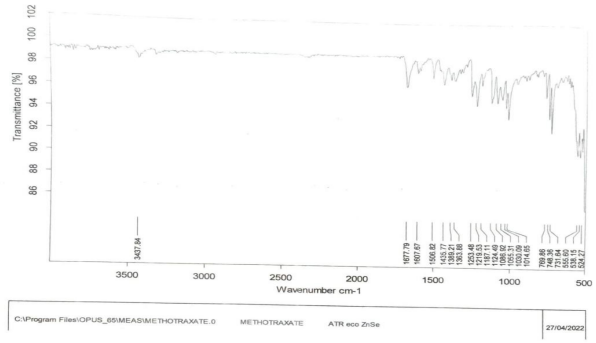


Figure 1: FTIR spectrum of methocarbamol

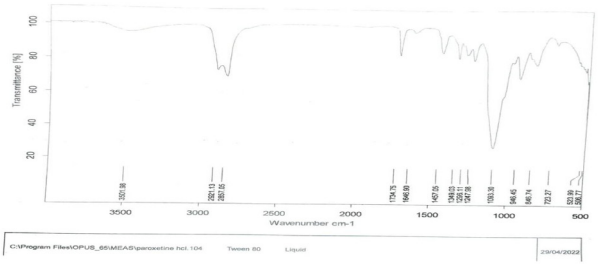


Figure 2: FTIR spectrum of Methocarbamol+ Soyalecithin + Tween 20+ Tween 80

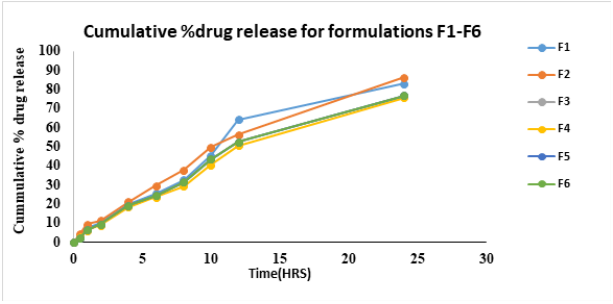


Figure 3: Graph showing %cumulative drug release for formulations F1-F6

Table 6: Fit Summary

Source	*p-value Sequential*	*R^2 Adjusted*	*R^2 Predicted*	
Linear	< 0.0001	0.9327	0.8763	Suggested
2FI	0.1578	0.9408	0.8399	
Quadratic	0.5516	0.9358	0.7336	
Cubic	0.0003	0.9967	0.9113	Aliased

After centrifugation, 9 mL of phosphate saline buffer (pH 7.4) was added to dilute 1-mL of the supernatant, absorbance taken at 267 nm for methocarbamol. Below formula was utilized to compute the drug entrapment efficiency and drug loading.[21] The results are shown in Table 3.

$$\% EE = \frac{Qt - Qs}{Qt} * 100$$

$$\% DL = \frac{Qt - Qs}{Qu} * 100$$

Table 7: Sequential model sum of squares [Type I]

Sources	*Sum of squares*	*df*	*Mean of squares*	*F-value*	*p-value*	
Mean *vs* Total	2.152 E+06	1	2.152 E + 06			
Linear *vs* Mean	1.104 E+05	2	55180.35	84.10	< 0.0001	Suggested
2FI *vs* Linear	1369.00	1	1369.00	2.37	0.1578	
Quadratic *vs* 2FI	811.69	2	405.85	0.6486	0.5516	
Cubic *vs* Quadratic	4218.31	2	2109.15	65.10	0.0003	Aliased
Residual	162.00	5	32.40			
Total	2.269 E+06	13	1.745 E + 05			

Table 8: Statistics summary

Source	*SD*	*R²*	*R² adjusted*	*R² predicted*	*PRESS*	
Linear	25.61	0.9439	0.9327	0.8763	14464.79	Suggested
2FI	24.02	0.9556	0.9408	0.8399	18722.02	
Quadratic	25.02	0.9625	0.9358	0.7336	31148.85	
Cubic	5.69	0.9986	0.9967	0.9113	10368.00	Aliased

Table 9: Response 1: Vesicle size

Source	*Sum of squares*	*df*	*Mean square*	*F-value*	*p-value*	
Model	1.104E+05	2	55180.35	84.10	< 0.0001	significant
A-Cholesterol	1.103E+05	1	1.103E+05	168.19	< 0.0001	
B-Surfactant	13.94	1	13.94	0.0213	0.8870	
Residual	6561.00	10	656.10			
Lack of fit	6561.00	6	1093.50			
(Pure) Error	0.0000	4	0.0000			
(Cor) Total	1.169E+05	12				

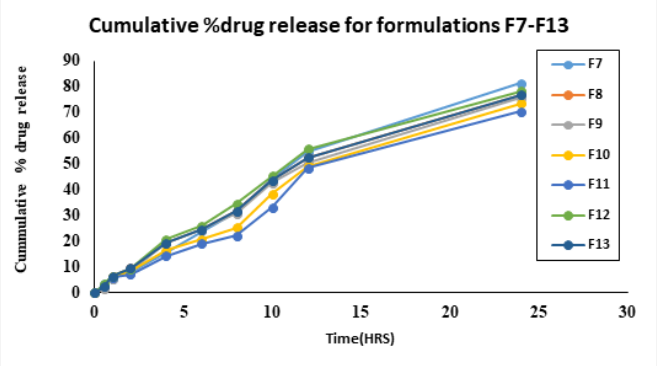


Figure 4: Graph showing %cumulative drug release for formulations F7-F13

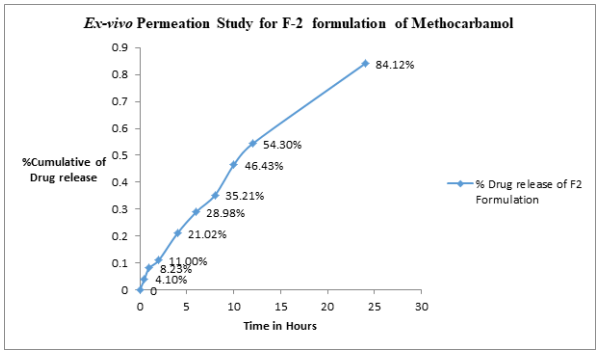


Figure 5: Graph indexing %cumulative drug release for formulations F2 formulation of methocarbamol

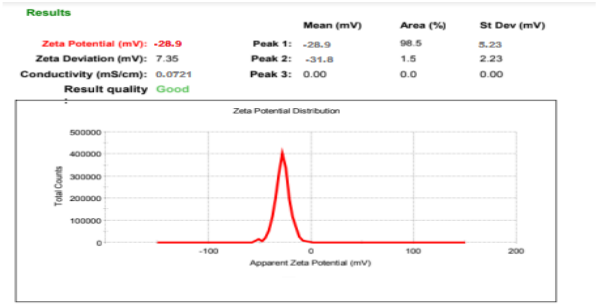


Figure 6: Zeta potential of MTG2 formulation

In-vitro drug release study

In-vitro release experiments of various transferosome preparations were performed using a modified Franz diffusion cuvette. A diffusion chamber assembly with a 2.5 cm diffusion area held the cellophane membrane in place. The liquid agitated at 100 rpm while maintaining a temperature of 37 ± 0.5°C consistently. Phosphate buffer of 22.5 mL, pH 5.5. At appropriate intervals, aliquots 2 mL were removed and immediately an equivalent amount of fresh medium. Calculation and a plot of drug release rate *versus* time was constructed[22,23] and was shown in Figures 3 and 4.

Ex-vivo study

Swiss-albino mice were employed for an *ex-vivo* study with modified artificial Franz diffusion chamber. With an effective

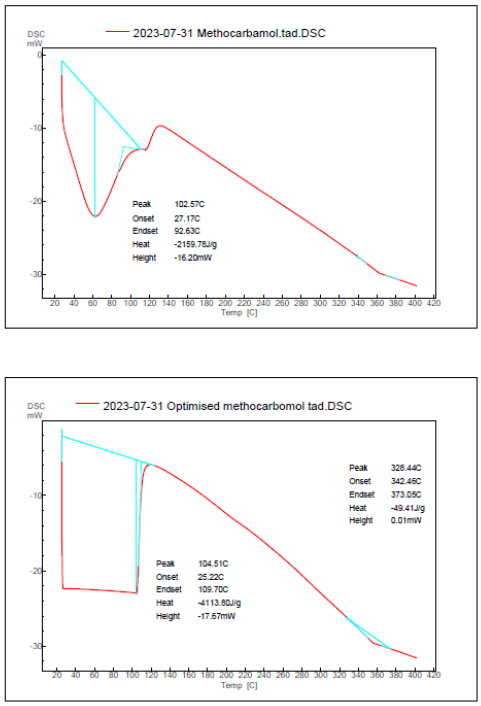


Figure 7: DSC images of MTG2

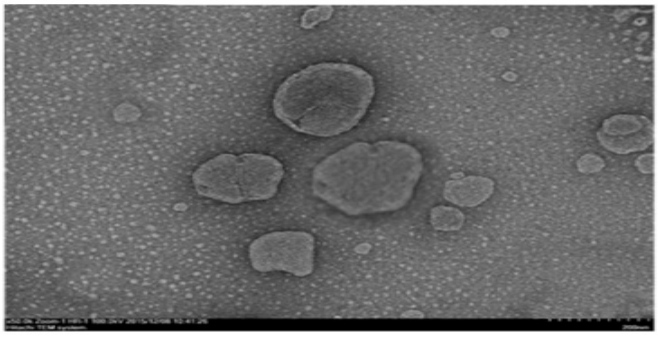
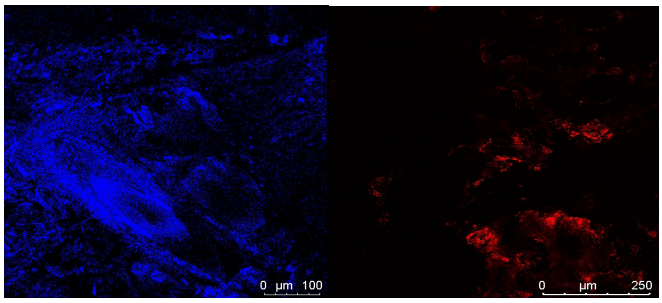


Figure 8: TEM images of MTG2



A) B)

Figure 9: CLSM image of methocarbamol and transferosomal methocarbamol gel

Table 10: Fit statistics

Std. Dev.	*25.61*	*R²*	*0.9439*
Mean	406.85	R²Adjusted	0.9327
%C.V.	6.30	R² Predicted	0.8763
		Precision	26.9964

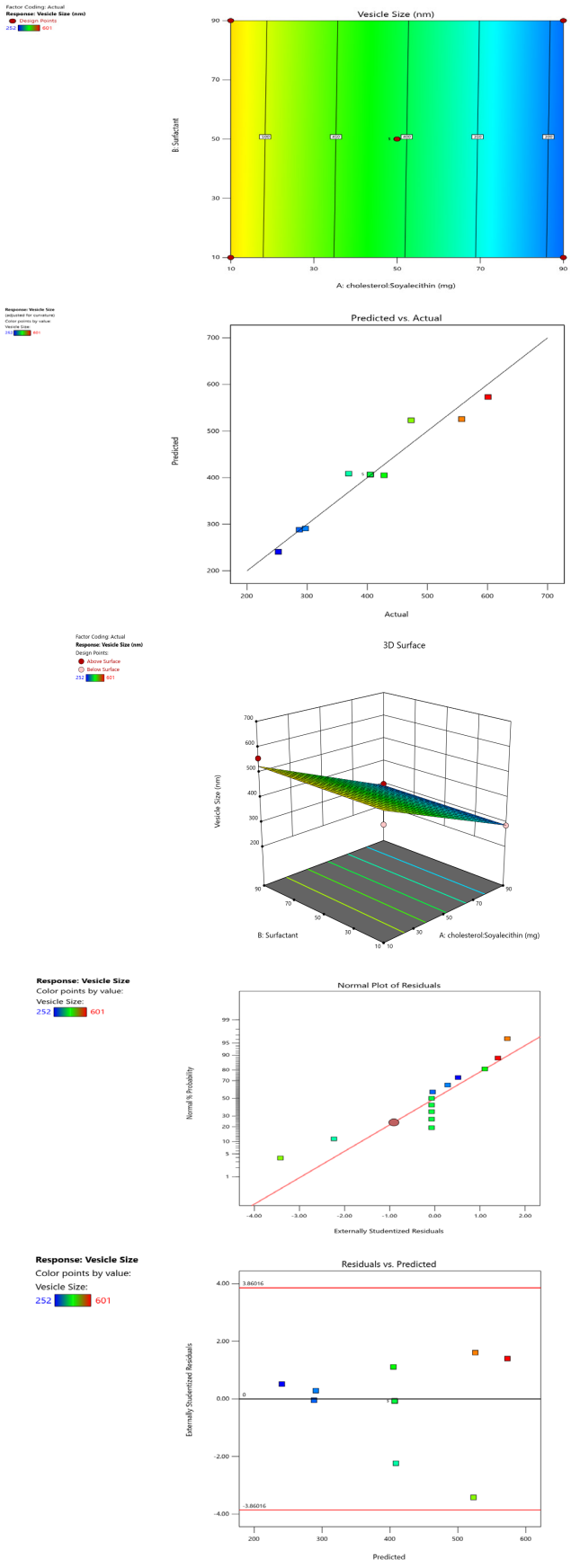


Figure 10: Effect of variables on vesicle size-3D surface plots

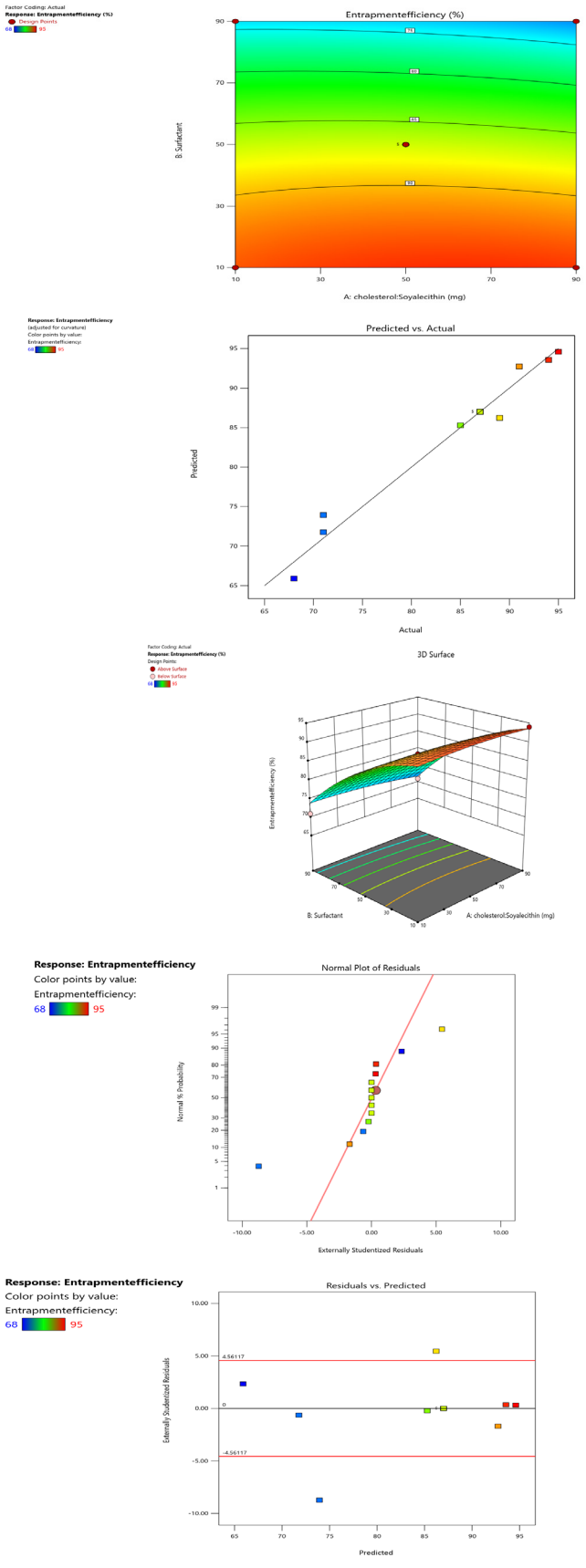


Figure 11: Plots in 3D illustrating how different variables affect the effectiveness of entrapment

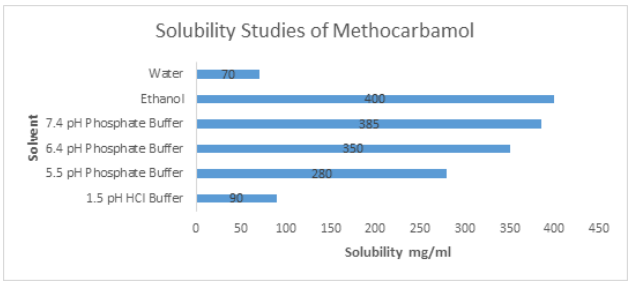


Figure 12: Solubility chart of methocarbamol

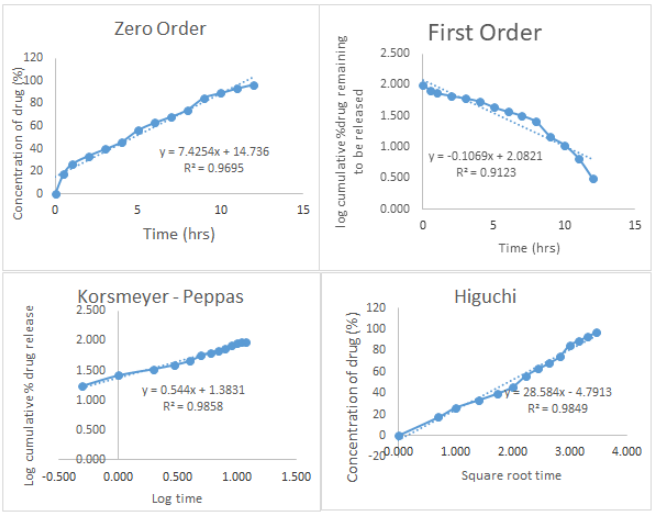


Figure 13: Drug release kinetics for F2 formulation of methocarbamol transferosomes

diffusion surface area of 2.5 cm^2, the skin was fixed between the donor and recipient diffusion cell chambers. Freshly made phosphate buffer with a pH of 5.5 was placed inside the receptor chamber. The chamber was regulated at 37°C and a heated plate magnetic stirrer was used to continuously stir the solution in the receiving chamber at 300 rpm. Methocarbamol (2 mL) was carefully introduced, solution of 5 mL was removed from the receiving partition absorbance measured, and then quickly replaced with same volume of fresh PBS at pH 5.5.[24] The results are shown in Figure 5.

Measurement of pH

A digital pH meter was used to find out what the gel's pH was. A precise 0.25-gram transferosome-based gel was mixed with 25 mL of clean water. With a buffer solution of pH 4.0, 7.0, and 9.0, the pH meter was set up each time it was used.

Spreadability index

For a good therapeutic response, the formulation's spreadability must give enough dose availability for the skin to absorb. To measure spreadability, 2 to 5 g of gel was placed between two slides, the weight was incrementally added to the weight pan, and the time it took for the top plate to face a distance of 10 cm after adding 80 g of weight was recorded. Less spread time is indicated by good spreadability. The formula that follows determines it.

Table 11: Fit summary

Source	*Sequential p-value*	*Lack of fit p-value*	*Adjusted R²*	*Predicted R²*	
Linear	< 0.0001		0.8628	0.7812	
2FI	0.6698		0.8507	0.6514	
Quadratic	0.0065		0.9544	0.8109	Suggested
Cubic	0.1810		0.9678	0.1409	Aliased

Table 12: Sequential Model Sum of Squares [Type I]

Source	*Sum of Squares*	*DF*	*Mean Square*	*F-value*	*p-value*	
Mean vs Total	92907.77	1	92907.77			
Linear vs Mean	824.73	2	412.37	38.72	< 0.0001	
2FI vs Linear	2.25	1	2.25	0.1942	0.6698	
Quadratic vs 2FI	79.48	2	39.74	11.23	0.0065	Suggested
Cubic vs Quadratic	12.27	2	6.13	2.45	0.1810	Aliased
Residual	12.50	5	2.50			
Total	93839.00	13	7218.38			

Table 13: ANOVA for Quadratic model

Source	*Sum of Squares*	*DF*	*Mean Square*	*F-value*	*p-value*	
Model	906.46	5	181.29	51.24	< 0.0001	significant
A-Cholesterol	0.8824	1	0.8824	0.2494	0.6328	
B-Surfactant	823.85	1	823.85	232.85	< 0.0001	
AB	2.25	1	2.25	0.6359	0.4514	
A^2	2.72	1	2.72	0.7680	0.4099	
B^2	79.24	1	79.24	22.40	0.0021	
Residual	24.77	7	3.54			
Lack of Fit	24.77	3	8.26			
Pure Error	0.0000	4	0.0000			
Cor Total	931.23	12				

Table 14: Fit statistics

Std. Dev.	*1.88*	*R²*	*0.9734*
Mean	84.54	Adjusted R²	0.9544
CV. %	2.23	Predicted R²	0.8109
		Adeq Precision	22.4611

$$S = m * l/t$$

Viscosity

The Brookfield viscometer was used to measure the viscosity of the produced transdermal transferosomal gels of methocarbamol at an optimal speed of 10 rpm.

Zeta potential & polydispersity index

Zetasizer (Malvern Instruments Ltd, Worcestershire, UK) utilised to calculate the zeta potential. Using a transparent zeta disposable cuvette, the device was operated at a constant temperature of 25°C.[25] The cumulant analysis was used to determine the particles' mean diameter and polydispersity index (PI) and the results are shown in Figure 6.

Stability studies

Tests were conducted on parameters including pH, preparation appearance, and active ingredient content in optimized preparations of transferosomal gels produced during stability testing for approximately 3 months at room temperature[26, 27] and the results are tabulated in Table 4.

Differential scanning calorimetry

DSC was used to conduct thermal testing of individual drug-polymer combinations to determine their physical compatibility. Amounts of the sample ranging from 1 to 2 mg were placed in sealed aluminum sample pans during each scan at a temperature of 10°C/min in a nitrogen environment between 25 and 350°C. As a reference, an empty aluminum pan was used[28] and the results are shown in Figure 7.

Transmission electron microscopy

Transmission electron microscopy was used to examine the morphology of the improved preparation. On a copper grid covered in foil, a drop of the diluted transferosome suspension was applied, stained with a drop of 2% (w/v)

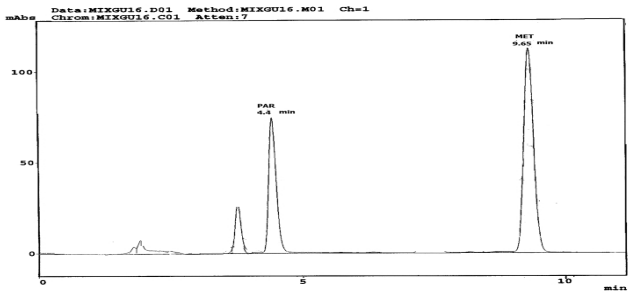


Figure 14: Standard HPLC chromatogram of methocarbamol in rabbit plasma

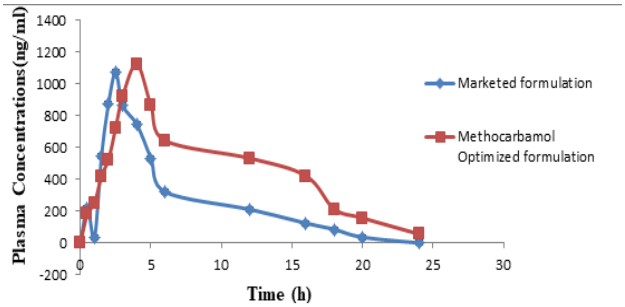


Figure 15: Comparison of plasma drug concentrations for marketed formulation and MTG2

aqueous phosphotungstic acid solution, and then let to dry to improve contrast. Figure 8 displays the outcomes of the analysis performed on the materials using transmission electron microscopy at a magnification of 72,000 x.[29]

Confocal-laser scanning microscopy

Wistar rat abdomen skin was removed, and transfersomes made with rhodamine B dye were placed evenly and non-occlusive for 24 hours. The skin was cleansed with rubbing alcohol and distilled water before being placed on the slide with SC facing up. The slide was made with a 5 to 10 m thickness. A 540 nm stimulating argon laser beam and a 625 nm emission Confocal-laser scanning microscopy (CLSM) were then used to examine the slide's z-axis.[30] Rhodamine B dye applied using the same technique as above was compared to a simple solution and the results are shown in Figure 9.

Methocarbamol in-vivo study

Overnight fasted rabbits were given access to water the day before administration. By using a randomized cross-over model, 12 male white rabbits of weight 2 to 2.5 kg were chosen for the *in-vivo* investigation and examined for the presence of any disorders. Before the commencement of each trial, the animal weight was measured and noted. Following that, six rabbits received oral doses of commercially available methocarbamol. Prior to the study the hair of the other six rabbits at the abdomen site of the gel application was cut. The skin was carefully cleaned with warm water, then with an alcohol swab, and dried dry before the gel was applied. After shaving the skin, a 20 cm^2 area of gel containing methocarbamol was applied. After oral administration, blood samples of around 0.5 mL collected from jugular vein into heparinized tubes in regular periods. As soon as possible, 0.1-mL of the whole blood was accurately transferred to another tube, which was then frozen at 20°C and kept until analysis. To prevent clot formation, a 1-ml blood sample was taken at regular intervals and 1-mL heparin solution was previously added to the sample container. Samples are centrifuged at 2500 rpm for 30 minutes. After dilution, 1-mL of the clear filtrate was obtained and 20 mL solution was subjected to HPLC examination after the separation of supernatant, corrected with 500 mL of mobile phase, vortexed strained using 0.45 mm syringe filters.[31] It was determined that a validated analytical method was satisfactory and demonstrated to be sufficient for determining the presence of methocarbamol in plasma. Table 5 reveals the summary of the connected pharmacokinetic variables for rabbit plasma.

Pharmacokinetic analysis

The pharmacokinetic parameters analyzed were C_{max}, T_{max}, Auc0-t, and $Auc_{0-\infty}$. AUC0-t and $AUC_{0-\infty}$ values were calculated using the linear trapezoidal rule.[32]

RESULTS AND DISCUSSION

Using the statistical method of formulation optimization based on the design of experiments, several pharmaceutical dosage forms have been created by running a number of tests. A response surface design, which provides details on the individual effects, pair-wise interactions, and curvilinear variable influences of the many design aspects under investigation, is a crucial component of the composite design. Present inquiry, Design-Expert Software Version 12 proposed a central composite design with a total of 13 experimental formulations of transferosomal gels containing methocarbamol for two factors: Soya lecithin content and surfactant concentration. As a reaction, the impacts of these variables on percent entrapment efficiency and vesicle size of designed transferosomal gels containing methocarbamol were investigated. Table 1 gives a summary of the design matrix, together with the experimental strategy and observed response values. The central composite design was used to fit the observed response values obtained from all trial formulations, resulting in model equations with distinct primary components and interaction variables for each response under consideration. One-way ANOVA was used to analyze these models statistically ($p < 0.05$). $p < 0.05$ for either of the calculated results, indicating the significance of the models.

ANOVA for Linear Model

This method is significant since F-value is 84.10. 0.01% of the time may noise be the cause of an high F-value. The $p<0.05$, model terms are deemed significant. In this context, the model term A is significant. In case value is more than 0.1000, model terms have no significance. If our model has a lot of meaningless terms (apart from those required to maintain hierarchy), model reduction may improve it.

The difference between the predicted R2 of 0.8763 and the adjusted R2 of 0.9327 is less than 0.2, indicating that they

are reasonably in agreement. Adeq precision measures the signal-to-noise ratio. Ideal proportion is at least 4. Based on the aforementioned values, our signal is sufficiently strong at a ratio value of 26996. Using this paradigm, the design space can be explored (Table 6).

Response 1 (vesicle size)

Effect of variables on Vesicle size-3D surface plots is shown in Figure 10 and Tables 7-11.

Response 2 (Entrapment Efficiency percent)

The model F-value of 51.24 indicates the model ought to be regarded significant. This level of F-value may result in noise 0.1% of the time. Important model terms in this condition include B and B2. If the value is more than 0.1000, model terms are not significant. If our model has a large number of meaningless terms, model reduction might improve it (Figure 11 and Tables 12-14).

It is thought that there is a reasonable agreement when the difference between the predicted R^2 of 0.8109 and the adjusted R^2 of 0.9544 is less than 0.2. The signal-to-noise ratio is accurately measured. The ideal ratio is at least 4. Based on the aforementioned values, our signal is sufficiently strong at a ratio value of 22.461. Using this paradigm, the design space can be explored.

Solubility studies for Methocarbamol

To provide a better film-formation ability and better stability followed by hydration, all of the excipients, including the drug dissolved completely in the solvent and get a clear transparent solution. The formulation's solvents may also act as penetration boosters to increase medication flux through the membrane. In this study methocarbamol has higher solubility in ethanol and less solubility in water hence ethanol enhances the permeation of the formulation (Figure 12).

Chemical Compatibility Studies (FTIR)

All of the typical peaks of methocarbamol were present in the combination FTIR spectral data of excipients along with drug, demonstrating drug compatilibility with the polymers (Figures 1 and 2).

Vesicle Size, Entrapment Efficiency & Drug content

Using response surface methods, Box-Behnkan design, and characterizing the transferosomes beforehand to create homogeneous batches, 13 distinct transferosomes were synthesized in this study. In Table 3, the vesicle size & entrapment efficiency of transferosomes were displayed.

As transferosomal properties are one among the nano formulations the results of the prepared transferosomes of methocarbamol were found to exhibit vesicle size between252 to 601 nm which is said to be an optimum nano size for increasing the permeability and bioavailability of the drug. The surfactant concentration on any vesicle or carrier preparation plays a very vital role. It was told in the literature that surfactant concentration and entrapment efficiency increases vice-versa. As the surfactant concentration in the current research was run through design expert software the concentration of surfactants was kept optimum. Consequently, the entrapment efficiency ranged from 68 to 95% for all formulations. This seems that all the formulations have good entrapment efficiency and no leakage. MTG2 formulation showed better %entrapment efficiency when compared to other formulations. All of the formulations had a drug concentration that varied between 90.32 to 96.6%. MTG2 formulation showed as high as 96.6% when compared to other formulations.

***In-vitro* diffusion Studies for Methocarbamol and Release Kinetics**

The dissolution profiles of 13 different methocarbamol-loaded transferosomal formulations are shown in Figures 3 and 4. The *in-vitro* release tests revealed that methocarbamol from nanosponges was released quickly and completely. All the formulations showed a drug release of above 75% after 24 hours. F2 showed as high as 86.43% drug release at 24 hours. The *in-vitro* drug release data were fitted to the Korsmeyer and Peppas release model in order to understand the mechanism of drug release from the transferosomes, and the interpretation of release exponent values sheds light on the mechanism of drug release from the dosage form. The optimized formulation revealed zero order kinetics with an anomalous diffusion mechanism, according to these values (super case II transport).

***Ex-vivo* Permeation Study for F-2 Formulation of Methocarbamol**

Based on the results of 2 major responses (Dependent variables) selected for design expert software for optimization, formulation MTG2 of methocarbamol was noticed to produce more entrapment efficiency (95%) and optimum Vesicle Size. Hence these two formulations were considered for further evaluation. The *ex-vivo* %drug release of optimized formulation (F2) and the release was found to be 84.12% for 24 hours (Figures 5 and 13).

pH results

As the transferosomal gel needs to be applied topically the pH values reported between 5.5 to 6.This seems to be optimum for transdermal application. MTG2 formulation's pH was discovered to be 5.7.

Spreadability index

As the transferosomal gels exhibit pseudo-plastic flow they are supposed to be spread efficiently and uniformly. The result of MTG2 which is 11.34 gcm/sechas proved that the spreadability of the formulation is optimum.

Viscosity

MTG2 was found to have a viscosity less than 3,500 Centi poise i.e., 2890 cps which seems to be in gel consistency and not in cream or oil consistency.

Zeta potential and polydispersity index

According to Figure 6, appropriately high MTG2 formulation zeta potential (-28.9 mv), the complexes would be stable and its ability to aggregate would be negligible. The colloidal suspensions are homogeneous if the polydispersity index is narrow.

Stability studies

The formulations underwent three months stability test at room temperature in the dark, and were evaluated for appearance, pH, and percentage entrapment efficiency. There were no significant changes in the initial values of appearance, pH, and percentage entrapment efficiency with MTG2. These findings demonstrate the methocarbamol transferosomes were identified to be stable at room temperature and they have the capacity for protection of drug molecules.

Differential scanning calorimetry

The formulation's thermal stability is demonstrated by a DSC thermogram. According to DSC research, in the powder form drug was present and was dispersed uniformly among the transfersomes.

Transmission electron microscopy

The TEM shows the shape of vesicles, and the TEM images for MTG2were found to be spherical with a uniform surface.The uniform surface and shape of vesicles have proved that there won't be any drug leakage happening from the formulations.

CLSM studies

The drug's vesicle penetration depth was investigated using CLSM. The CLSM's strong fluorescence showed that the developed transfersomal formulation was distributed uniformly throughout the strata of the skin. Rhodamine B dye was put into an improved transfersomal formulation, and a confocal laser scanning microscopic image of the formulation revealed that it penetrated much deeper and fluoresced much more intensely than the rhodamine B dye solution. This effective transfersome transport of rhodamine B shows that it has improved penetration and fusion with lipids membrane in the deeper layers of skin, supporting the findings of previous researchers.[33] As a result, the plain dye solution was not able to penetrate to the same degree as the transfersomal formulation.

Pharmacokinetic analysis

The ability of methocarbamol was investigated *in-vivo* using tests on rabbits. The profiles of plasma concentrations of the gel-optimized methocarbamol in the rabbits are depicted in Figure 14. The C_{max} of the marketed formulation and methocarbamol gel was observed as 1069.98 ± 17.43 ng/mL and 1124.43 ± 13.54 ng/mL respectively. Likewise, the T_{max} of the marketed formulation and methocarbamol gel was observed as 2.5 ± 0.08 hours and 4.0 ± 0.4 hours respectively was considerably altered in comparision with the oral suspension. T_{max} results were more after application of gel rather than oral treatment in all rabbits. This variation in T_{max} was caused by the barrier function of stratum corneum as well as the continuous drug release from the gel. Lower T_{max} values were seen with oral suspension administration because it is a typical instant-release dose type. $AUC_{0-\infty}$ for the optimized formulation was higher, which is 10517.32 ± 46.24 ng.h/ml when compared to the marketed formulation, which is 8392.22 ± 38.43 ng.h /mL. The AUC_{0-t} of the gel was significantly more when in comparision with marketed formulation. Gel formulation of methocarbamol has significant drug concentration in the blood compared to commercial formulation and suggested better systemic absorption (Figure 15).

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