



RESEARCH ARTICLE

In vitro and *ex vivo* evaluation of triamcinolone acetate-loaded transferosome gel-based novel carrier for the treatment of osteoarthritis

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ABSTRACT

Aim: Osteoarthritis is one of the prevalent disorders of the bone and is the leading cause of death across the globe. The poor survival rate and limited opportunities for treatment require the use of novel therapies to tackle the disease. **Objectives:** In the present invention, we reported significant *in vitro* and *ex vivo* improvement of BCS Class IV drug, triamcinolone acetate (TMA) in transferosome-based gel. **Materials and Methods:** The optimized transferosome-based suspension (TMA-TS) reported nano-size range with negative zeta potential. Conversion of suspension to gel was initiated using Carbopol 934P as gel base. The **spreadability**, viscosity, and entrapment efficiency were found to be more enhanced in TMA-TG formulation, as compared to pure drug. **Results and Discussion:** The spherical morphology of TMA-TS was confirmed in transmission electron microscopy. *In vitro* dissolution of TMA-TS augments multi-fold release behavior in TMA in phosphate buffer saline 7.4 as dissolution media. Remarkable permeability was observed in goat skin, as confirmed from *ex vivo* permeability experiments, as compared to suspension and pure drug. Stability studies indicated robustness of formulation during 3-month storage period. **Conclusion:** In a nutshell, this microparticulate system is well suited and significantly enhanced the biopharmaceutical attributes of TMA.

KEY WORDS: *In vitro* dissolution, Osteoarthritis, Permeability, Triamcinolone acetate

INTRODUCTION

Transfersomes (TS) are an uncommon kind of liposomes, comprising bilayer previous as phospholipid and edge activator (EA). These vesicles are more versatile than the customary liposomes.^[1] Versatility in these vesicles is certifying to the presence of an EA, which is a solitary chain surfactant with a high range of curve, capable of debilitating the lipid bilayers of the vesicles, and expanding their deformability and adaptability. Sodium cholate, sodium deoxycholate, Spans, Tweens, and potassium glycyrrhizinate were utilized as EAs. In light of these deformable properties, TSs beat the skin entrance trouble by getting themselves through channels of the layer corneum that is short of what one-10th the breadth

of the transfersome. Ultradeformable vesicles containing phospholipids and an EA are new methodology in vesicular medication conveyance and have been alluded to as TS. Versatility in these vesicles is delivered by the presence of an EA, which is generally a solitary chain surfactant with a high sweep of ebb and flow and fit for debilitating the lipid bilayers of the vesicles and expanding their deformability.^[2] Triamcinolone is an engineered corticosteroid (glucocorticoid) it is having a place with calming (steroidal) class. Triamcinolone acetate (TMA)

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DOI:

10.56933/Pharmaspire.2022.14102

Date of Submission: 11 July 2022

Date of Revision: 25 July 2022

Date of Acceptance: 25 July 2022

is a manufactured glucocorticosteroid ties in the objective cell to explicit cytosolic glucocorticoid receptors and accordingly cooperates with glucocorticoid receptor reaction components on DNA, in this way adjusting quality articulation.^[3] Furthermore, this drug has a place with BCS Class IV because of its low dissolvability and low penetrability. TMA is oftentimes recommended by IA, IM, and oral course for the administration of osteoarthritis. Constant oral TMA organization prompts troublesome unfavorable impacts, particularly on the gastric mucosa because of prostaglandin restraint. These results can be encouraged as straightforward sicknesses like dyspepsia, moderate issues like peptic ulcers, and serious concerns like gastrointestinal (GI) drain.^[4] AF can possibly cause nearby aggravation and GI mucosal sores because of its acidic character. Aside from helpful test because of results, physicochemical difficulties of low fluid dissolvability, substance challenge of unsteadiness in soluble, and impartial media just as higher Log p esteem designate it to be figured into TS detailing as a novel transdermal medication conveyance framework. Steroids for osteoarthritis are accessible just in oral or infusion frames that have a few restrictions.^[5,6] Liposomal and niosomal frameworks are not appropriate because of helpless skin porousness, breaking of vesicles, spillage of medication, total, and combination of vesicles. There is a requirement for advancement of a skin conveyance framework to beat the impediments of accessible treatment such as painful infusions, allergic conditions, poor ingestion, poor compliance, and GI issues related with oral corticosteroid. Transporter framework called “transferosomes” are fit for transdermal conveyance of low just as high sub-atomic weight drugs.^[7] Hence, the current examination endeavors to improve the permeability of triamcinolone acetonide-containing transferosome gel (TG) for effective treatment of osteoarthritis.

MATERIALS

TMA was a kind gifted sample from Sun Labs, Ahmedabad, India. Soya phosphatidylcholine was from laboratory ISF College of Pharmacy. Span 80, Tween 80, and Carbopol 934 were purchased from HiMedia, Mumbai, India. Other chemicals and glassware required for this project such as scintillation vials, and centrifuge tubes were obtained from Merck, Mumbai, India.

METHODS

Solubility studies of TMA

Solubility studies of TMA were carried out in distilled water, 0.1 N HCl, and pH 7.4 phosphate buffer (PB), using the shake flask method. An excess amount of TMA was added to 10 mL of distilled water, PB pH 7.4 and 0.1 N HCl, vortexed for 5 min, and kept aside for settlement in

a scintillation vial. The mixtures were shaken for 24 h at 37°C at 100 rpm in a water shaker (PLT-110, High Precision water bath, Hyderabad, India) until equilibrium attained. The supernatant was collected after filtration through 0.22 µm membrane filter and the filtrate was diluted with the respective dissolution medium and assayed by UV-visible spectrophotometer (Lambda 25, Perkin Elmer, Waltham, MA) at $k_{\max}$ of 239 nm.^[8]

Formulation development

Transferosome suspension (TS) of TMA

TMA-TS formulations were prepared by the film hydration method as per earlier published protocol [Figure 1]. Formulations composition is depicted in Table 1. Initially, weighed amount of soya lecithin and drug was dissolved in chloroform: methanol (2:1) mixture. EA solution in the solvent mixture, that is, chloroform methanol was added to the first solution. Dry lipid film was prepared by evaporating the solvent in the rotary flash evaporator (Laborota 4000 WB, Heidolph, Germany) at 68°C. The obtained lipid layer was placed in the vacuum oven to remove any organic solvents. The lipid layer was hydrated with PB saline (PBS) pH 7.4/5.8 buffer (10 mL). It was then allowed to rotate for 1 h at 90°C at 60 rpm to swell at room temperature for 2 h. After swelling, the formulation was sonicated using probe sonicator for 10 min with a 5 min time interval. It was then extruded through membrane filters for size reduction. After the preparation, the formulation was stored in a cool, dry place. The formulation was optimized by changing the formulation and process variables. Optimization was done by changing the EA concentration (%w/v) and lipid concentration (%w/v). The formulation that showed the optimum vesicle size, maximum % entrapment, and flexibility was chosen for further studies. The effect of various concentrations of sodium deoxycholate (f1-f3), Tween 80 (f4-f6), Span 20 (f7-f9) have been thoroughly discussed. Simultaneously, the effect of soy lecithin concentration on physicochemical characteristics of TMA-TS formulation was measured (F1–F9).^[9,10]

Table 1: Characterization of TMS-TS suspension formulation

Formulation code	TAC (mg)	X1 (Lipid)	X2 (EA)	Lipid: EA
F1	10	SPC	SDC	75:25
F2	10	SPC	SDC	85:15
F3	10	SPC	SDC	75:25
F4	10	SPC	Tween 80	75:25
F5	10	SPC	Tween 80	85:15
F6	10	SPC	Tween 80	75:25
F7	10	SPC	Span 20	75:25
F8	10	SPC	Span 20	85:15
F9	10	SPC	Span 20	75:25

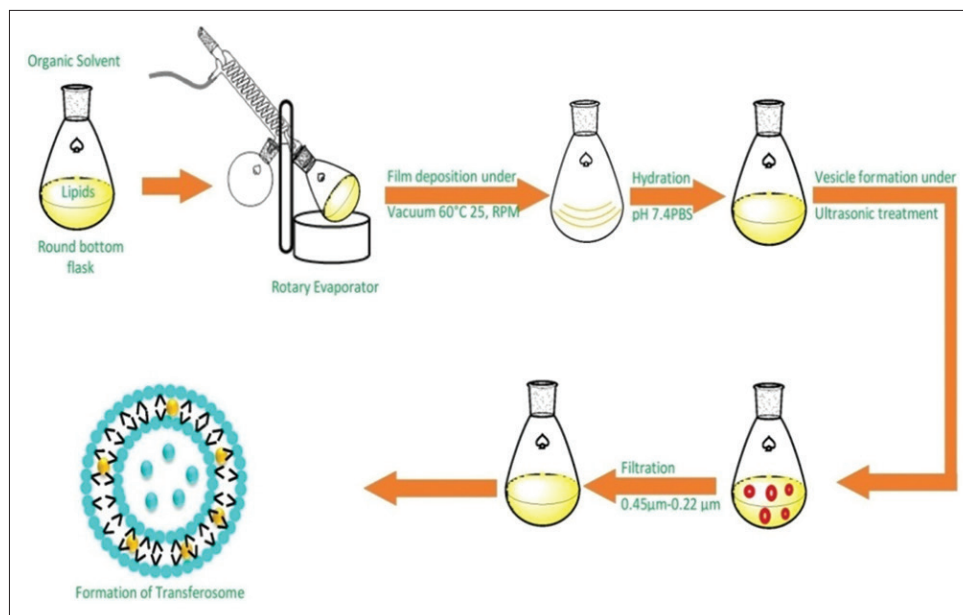


Figure 1: Schematic representation of Transfersome preparation by thin film hydration method

Measurement of vesicle size, polydispersity index (PDI), and zeta potential (ZP)

The vesicle size, PDI, and ZP of TMA-TS formulations were measured using a Malvern Zetasizer (Nano ZS90, Malvern, UK). Formulations were diluted 10 times with double-distilled water to get optimum Kilo Counts Per Second (kCPS) for measurements. ZP of formulations also measured using the same diluted sample using a palladium electrode cell in triplicate. All measurements were carried out at 25°C.^[11]

Transmission electron microscopy (TEM)

Distribution of triamcinolone acetonide TSs was observed under TEM, Hitachi (H-7500). One drop of diluted triamcinolone acetonide TS was deposited on a film-coated copper grid and it was stained with one drop of 2% (w/v) aqueous solution of phosphotungstic acid. Excess of solution was drained off with a filter paper and then grid was allowed to dry for contrast enhancement. The sample was then examined by TEM.^[12]

Entrapment efficiency (EE)

For the determination of EE, the untrapped drug from ultra-adaptable vesicular system was separated by the centrifugation method. Briefly, a Centriscart tube containing 2.5 mL of the formulation was centrifuged at 18,000 rpm for 30 min. After appropriate dilution of the collected supernatant, the drug content was estimated at 239 nm by UV-visible spectroscopy.^[13]

Drug content

The TMA content was measured by placing 100 mL of TMA-TS formulation in a clean volumetric flask (10 mL)

and make up the volume with methanol. This was then stirred for 30 min and allowed to stand for 15 min. After the solution was filtered through 0.22 µm membrane, the filtrate was analyzed spectrophotometrically at 239 nm.^[14]

In vitro drug release studies

The *in vitro* drug release study of TMA-TS formulation was performed using Franz diffusion cells, using PBS pH 7.4 as release media. Dialysis membrane (HiMedia, CSIR, Lucknow, India) with a molecular weight cutoff of 10 kDa and the diameter of 70 mm was used for this study.^[15] One milliliter (2 mg/mL) of formulation was placed in the donor compartment and the receiver compartment was containing 30 mL of PBS pH 7.4. The dissolution medium in the receptor compartment was stirred at 100 rpm and kept at $37 \pm 0.5^\circ\text{C}$ for 24 h. Aliquots (1 mL) were collected at different time intervals (0.5, 1, 2, 4, 6, 8, 10, 12, and 24 h) and replaced with an equal volume of fresh dissolution medium to maintain sink conditions. The aliquots were filtered through 0.45 µm membrane filter. After appropriate dilution, the samples were analyzed using UV-visible spectrophotometer at k_{max} 239 nm against PBS pH 7.4 as a blank. The measurements were done in triplicate. The cumulative amount of TMA released was plotted against time. Data also fitted with release kinetic modeling to determine mechanism of drug release.^[16]

Stability studies

The stability study of optimized TMA-TS formulation was performed at refrigerated and room temperature conditions over 3 months. Samples were periodically withdrawn and evaluated for vesicle size, PDI, ZP, EE, drug content, and elasticity and subjected to statistical treatment.^[17]

Preparation of TMA-TG

TMA-TG (0.5% w/v of TMA) formulation was prepared by adding 0.5% w/v of Carbopol 934 solution to 10 mL of TMA-TFS formulation under continuous stirring. Before the addition, hydrated Carbopol solution was prepared by adding accurately weighed amount (2 g) of Carbopol 934 in 50 mL of distilled water with heating at 50°C in a 100 mL glass beaker and kept for soaking overnight. The pH of the gel was adjusted by the addition 0.2 mL of triethanolamine dropwise and the total weight of the gel was equivalent to 10 g using distilled water.^[18]

Characterization of AF-TG

Prepared AF-TG was evaluated for pH, viscosity, spreadability, stiffness, drug content, and skin permeation studies. Marketed triamcinolone acetonide ointment (0.1% w/w) was used as a control.

pH of TMA-TG

The pH of the TMA-TG formulation was determined using digital pH meter (SD Fine Chemicals Limited, India). Briefly, 0.1 g formulation was dipped in standard pH electrode which was calibrated in various buffers. Measurements were done in triplicate.^[19]

Viscosity

The viscosity of TMA-TG and marketed gel formulation was measured using a Brookfield programmable DV-III Remoter (cup and bob). The sample holder was filled with the sample. The spindle S62 was used to measure the viscosity of preparations. The sample was allowed to settle for 5 min before taking the readings and the results were noted in triplicate.^[20]

Drug content

About 500 mg of gel was weighed and dissolved in 100 mL of methanol, stirred for 30 min, and kept aside for 1 h. The solution was then filtered and analyzed for drug content at 239 nm spectrophotometrically.^[21]

Stiffness

About 100 mg of TMA-TG and marketed formulation were spread with point finger on the skin. The resistance to spread was observed (qualitative test). Three different grades were given based on feeling as +, ++, and +++ (+ highest stiffness, ++ medium stiffness, and +++ lowest stiffness).

Spreadability

In general, the time in seconds taken by both glass slides to slip off from TMA-TG formulation placed in between the slides under the direction of a certain load is considered as spreadability of the formulation. Accurately weighed 1 g of prepared gel formulation was placed between two

glass slides and known weight was placed over an upper glass slide and left for about 5 min. The time required by the upper slide to move on the application of weight to it through the pulley was noted, and the spreadability was calculated using the following formula, in triplicate:

$$S \propto M \times L = T$$

Where, *S* is the spreadability; *M* is the weight applied to upper slide; *L* is the length of the glass slide; and *T* is the time taken to separate the slides from each other.

In vitro drug release studies of TMA-TS and TMA-TG

In vitro release studies of TMA-TG formulation were performed using Franz diffusion cells, as per the procedure described in TMA-TS release studies. Five hundred micrograms of gel formulation used for the release studies.

Ex vivo permeability studies of TMA-TS and TMA-TG

Preparation of skin

Goat skin were used for the skin collection. The goat abdominal surface hair was removed by a depilatory. The skin was surgically removed. The subcutaneous tissue was removed manually, and the dermis side was wiped with isopropyl alcohol for removing residual adhering fat. Full thickness of the skin was washed with PBS, wrapped in aluminum foil, and stored at -20°C till further use (used within 2 weeks of preparation).

Experimental procedure

The *ex vivo* permeation studies of TMA-TG formulation, in comparison with marketed formulations, triamcinolone acetonide were studied using Franz diffusion cells as per the earlier described *in vitro* release study protocol. The goat skin was fixed between the donor and receptor chambers of the diffusion cell. The goat skin was adjusted with the stratum corneum layer facing the permeant (drug), while the dermis faced the receptor compartment. The receiver compartment contained 30 mL of PBS pH 7.4.^[22] The results of TMA permeation were kinetically treated to determine the order of drug permeation. The results of TMA permeated were kinetically treated to determine the best order of drug release from TG formulations. The steady-state flux (*J*) was calculated from the slope of the linear part of the cumulative amount of TMA permeated per unit area (mg/cm²) against a time (h) plot. The statistical significance of the formulations was compared with Student's unpaired *t*-test at *P* < 0.05 level. The permeability coefficient (*P*) of AF through the goat skin was calculated according to the following equations:

$$P \propto J = C_0 \quad (1)$$

*C*₀ is the initial AF concentration.

Stability studies

Stability studies of TMA-TG formulation were conducted by store the formulation at refrigerated and room temperature conditions. Gel formulation was packed in collapsible aluminum tubes and analyzed for pH, drug content, viscosity, and spreadability over 3 months at predetermined time intervals.

RESULTS AND DISCUSSION

Solubility of AF

The saturation solubility of TMA in different media was performed using the shake flask method. The results revealed that the solubility of the TMA was increased from pH 1.2 to 7.4. The solubility of the TMA in PB pH 7.4, methanol, and 0.1 N HCL was 0.742 ± 3.2 mg/mL, 0.847 ± 4.1 mg/mL, and 0.753 ± 1.2 mg/mL, respectively. TMA showed the highest solubility in methanol and was selected as the suitable media for the further studies.

Preparation of TMA-TS

TMA-TS formulations were prepared by the film hydration method. Initial trials were performed with soy lecithin and egg lecithin as the lipids; Tween 80, Span 80, sodium deoxycholate, and as EA. From the physical observations, TS formulations with soy lecithin and Span 80 combinations showed promising results than egg lecithin, Tween 80, and other combinations [Figure 1]. The highest deformability was achieved with Tween 80 and highest % EE was achieved using Span 80. TMA is practically insoluble in water, and up to little extent in Tween 80 affected the EE of TMA, because of its solubilization nature and also HLB value.^[22] Sodium deoxycholate and sodium cholate had lower deformability than Tween 80 due to their steroid-like structures, which are bulkier than the hydrocarbon chains of Tween 80 and generated vesicles of smaller size. The particle size with 100–200 nm size is ideal for transdermal drug delivery. Therefore, sodium deoxycholate was excluded from the study. Span 80 not only showed significantly lowers the deformability than Tween 80 but also it had a significant effect of EE. This could be a result of its high lipophilicity. Hence, TMA-TS formulations were further optimized with soy lecithin and Span 80 as lipid and EA, respectively. The effect of sonication time on vesicle size reduction was studied. Sonication at 15 min with 30% amplitude with 5 min intervals off resulted in the vesicle size range of 200 nm with acceptable PDI. Further, the effect of drug loading, lipid and EA concentration, and pH of hydration medium on TS formulations were studied.

Optimization of EA and lipid concentration

Elasticity of vesicles also known as flexibility index is a crucial response that influences the effectiveness of TS formulations, as it is necessary to transfer through skin.

Hence, Span 80 was selected as EA for the preparation of TMA-TS formulations, based on preliminary studies. Further, the effect of EA concentration on % EE and elasticity of the formulations was studied using 0.05–0.3% w/v (F5–F10). Formulations were prepared by keeping lipid and drug at 1.0% w/v and 0.2% w/v, respectively. The effect of EA concentration on relative deformability and % EE is shown in Figure 1. As the concentration of EA increased from 0.05% to 0.15%, % EE and elasticity of TMA-TS formulation were increased. There were no significant changes in % EE and elasticity observed above 0.15% w/v EA concentration. This could be due to the linear structure of Span 80 tends to compact the bilayer, thus, reducing its flexibility. The effect of lipid concentration on TMA-TS formulations was studied by varying concentration from 0.5 to 2% w/v (F7, F11–F14). The effect of lipid concentration on physical characteristics, assay, % EE, and elasticity of the TMA-TS formulations was studied. As the concentration of lipid increased, the vesicle size of the TS formulation was decreased. Non-significant difference in vesicle size was observed with 1.0–2.0% of lipid concentration.^[23] A similar trend was also observed with PDI and ZP of the formulations. Drug content of the formulation also increased with increasing concentration of the lipids. It is concluded that as the concentration of lipid increases, the % EE of formulations were increased significantly up to 1.0% w/v lipid, beyond this concentration marked decrease or no change was observed. Non-significant changes in the elasticity of vesicles were observed. Hence, 1.0% w/v lipid was used for the formulation of TMA-TS.

Vesicle size, PDI, and ZP

TMA-TS formulation with 0.1% drug load, 1.0% w/v lipid, and 0.15% w/v EA was considered as the optimized formulation (F2). F2 formulation showed vesicle size, PDI, and ZP of 172.1 ± 3.2 nm, 0.153 ± 0.02 , and -4.55 ± 1.3 mV, respectively, with $97.9 \pm 2.4\%$ drug content. Dispersed systems with ± 30 mV considered to be stable. The negative charge of the optimized formulation might influence TMA permeation through goat skin due to electrostatic repulsion between the similar charges on the skin surface and the optimized gel. According to earlier studies, the negatively charged dispersions have improved skin permeation of drugs in transdermal delivery.

TEM

TEM was used to confirm the formation of vesicles in the TMA-TS formulation. The TEM images of F2 formulation are shown in Figure 4. From the lyophilized reconstituted formulation, 10% increase in the particle size was shown, 8% increase in PDI, but no changes were observed in assay, EE, and elasticity of the formulation. The changes in the size and PDI of the formulation might be due to the aggregation of vesicles by lyophilization process. No changes in the chemical characteristics, ZP,

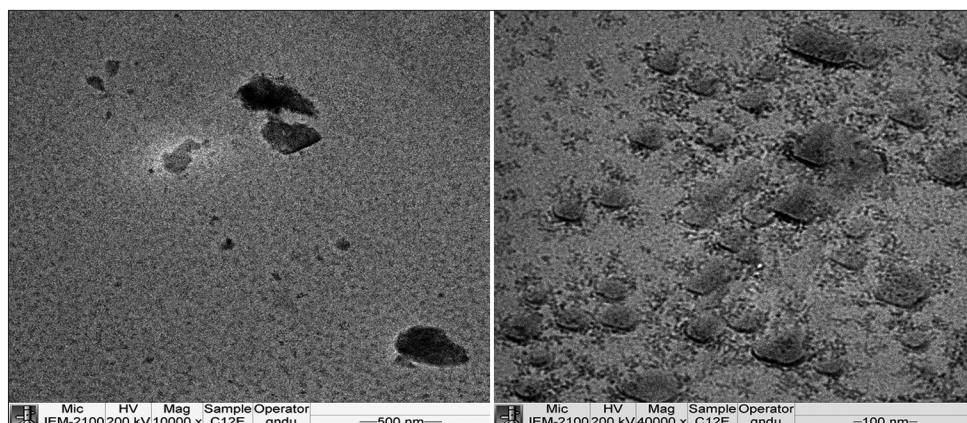


Figure 2: TEM captured images of transferosome suspension of TMA.

and elasticity of the reconstituted lyophilized powder indicate no structural change in the TMA-TS formulation after lyophilization process. This was also confirmed with our earlier reported studies. TMA-TS formulation sample was mounted on aluminum stubs using double adhesive tape and was gold coated in a Hitachi HUS-5 GB vacuum evaporator. TMA-TS formulation showed the formation of round spherical-shaped vesicles.

EE

The EE of optimized TMA-TS formulation was $97.9 \pm 1.8\%$. TS formulations prepared with Span 80 have a higher EE than those prepared with Tween 80. These results may be attributed to the hydrophilic lipophilic balance (HLB) values of these surfactants. The HLB values of Span 80 and Tween 80 were 4.3 and 15, respectively. Hence, according to HLB values, the affinity of surfactant to phospholipid was expected to be higher in the case of Span 80 than in the case of Tween 80 due to the higher lipophilicity of Span 80.^[24]

PREPARATION OF TMA-TG

The optimized F2 formulation was converted into TMA-TG using Carbopol 934 (0.5% w/v) as gelling agent. Triethanolamine (0.2% w/v) was used to neutralize pH of the formed TMA-TG formulation for topical application range. Triethanolamine is a non-ionic neutralizer and it does not disrupt the ionic balance, and no aggregation of the vesicles was observed. Therefore, ionic neutralizers are avoided in the present investigation. The formed TMA-TG formulation was left overnight to remove any entrapped air. The gel was evaluated for physical and chemical characteristics and compared with marketed triamcinolone marketed ointment (0.1% w/w).^[25]

Characterization of TMA-TG

TMA-TG and marketed gel formulations were evaluated for pH, viscosity, spreadability, stiffness, and drug content.



Figure 3: Image of TMA-TG optimized gel formulation.

Results of these parameters are shown in. The pH of the TMA-FG and marketed formulation was 6.75 ± 0.4 and 5.63 ± 0.3 , respectively. These pH values are within the normal range for topical application. Both formulations showed nearly similar viscosity of 13.4 ± 3.02 cP and 22.71 ± 0.4 cP for TMA-TG and marketed gel, respectively.^[26] The spreadability values for F2 and the marketed gel were 6.54 ± 0.5 and 4.23 ± 0.4 g.cm/s, respectively, which indicate that they can be spread easily on skin surface with little stress. TMA content from the TMA-TG and triamcinolone ointment 0.1% was $77.6 \pm 1.2\%$ and $94.5 \pm 1.3\%$, respectively.

In vitro drug release studies of TMS-TS and TMA-TG

In vitro drug release studies of F2 formulation was carried out using the diffusion method. From the release studies, $99.1 \pm 2.7\%$ drug releases within 24 h of study has indicated the sustained release behavior of TMA from TMA-TS formulation. The results of the release studies are shown in Figure 5. The prolonged or sustained release of TMA from TS formulation probably was due to the presence of phospholipid, which when interacted with release medium self-assemble into a bilayer of lipid and which eventually closes into a lipid vesicle. The presence of surfactant acts as a bilayer softening agent, improves the bilayer flexibility and permeability, and then results in slow release of the

drug from the vesicular system.^[27] The release profile of formulation was subjected to kinetics mathematical modeling to determining the release mechanism. The data fit into zero-order, first-order, Higuchi, and Korsmeyer–Peppas model. The regression coefficient values are shown in Table 3. The high r^2 values were observed with zero-order above 0.99. This indicated that the release of drug from TMA-TS formulation followed a zero-order release mechanism. *In vitro* release of TMA-TG formulation showed $83.5 \pm 3.6\%$ drug release within 24 h [Figure 3]. The release of the AF from the TG formulation was significantly ($P < 0.05$) less compared to TMA-TS formulation. This could be due to the presence of gelling agent, and drug slowly diffuses from the surrounding coated nano formulations. Further, drug release followed zero-order release mechanism based r^2 value (0.998) calculated with mathematical model fitting.

Ex vivo skin permeation study of TMS-TS and TMA-TG

The permeation studies for TMA-TG and marketed ointment formulation were performed through goat skin using Franz diffusion cells. The amount permeated versus time profiles of formulations are depicted in Figure 6. From the results, $71 \pm 2.8\%$ and $47.4 \pm 1.9\%$ of % drug permeated from TMA-TG and marketed ointment formulation, respectively. It was found that the cumulative amount of AF permeated from TMA-TG was significantly higher than the amount permeated by marketed gel ($P < 0.05$). The improved permeation of AF from AF-TG may be attributed to the high flexibility of TS, so they can penetrate the skin easily and overcome the barrier function by squeezing through the intracellular lipid of the stratum corneum. Furthermore, after application of TS on the skin, they move from the dry stratum corneum to a deep hydrated layer under the effect of the osmotic gradient, and the presence of surfactant in the structure of TS helps in solubilizing the lipid in stratum corneum, permitting a high penetration of the vesicles. Further, occlusive and bioadhesive properties of vesicles were studied, where occlusion decreases the transepidermal water loss, which further increases skin hydration and permeability. The drug permeation from TMA-TG and marketed ointment formulations was subjected to mathematical release modeling by similar rheological behavior, enhanced permeability, and without cause any skin irritation compared with marketed gel formulation.^[28] Overall, TMA-TG formulation could be considered as an alternative delivery approach for enhanced skin delivery. Pharmacokinetic studies in suitable animal model might be the further confirmatory evaluation.

Data analysis of the permeation study

Steady-state flux and permeability of TMA-TG and marketed formulation were calculated and presented in. From the results, the steady-state flux of TMA was higher

from TMA-TG compared with TMA ointment formulation. The steady-state flux of TMA-TG and marketed gel was 28.8 ± 0.1 and 15.8 ± 0.3 mg/cm²h, respectively. It was found that there was a direct relationship between steady-state flux and permeability coefficients, as represented in. The permeability coefficient of TMA-TG also was 14-fold higher than marketed formulation ($P < 0.05$). These results could be attributed to the high deformability and flexibility of TS, which allowed them to overcome skin barrier properties.

CONCLUSION

The present investigation significantly reported enhanced *in vitro* and *ex vivo* characteristics of TMA following loading into transferosome vesicles. The positive observations were seen during experimentation in the characterization and evaluation of TMA solubility and permeability in TMA-TG based optimized formulation. Hence, the designed delivery system significantly enhances the permeation of drug and well suited for future direction for the treatment of osteoarthritis.

CONFLICTS OF INTEREST

None.

ACKNOWLEDGMENTS

Nil.

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