RESEARCH PAPER

Effect of Penetration Enhancers on Permeability of Atorvastatin Gel

Ahmed Abbas Abdullah Al-Ramel[1], Swati Bharama Jadhav[2], Nagesh Chandrasekhara Setty[2*], Suma Naduvinamani[2], Yousif M. Elmosaad[3*], Pavan Kumar Sreenivasalu[4], Sreeharsha Nagaraja[5]

[1] Department of Public Health, College of Applied Medical Sciences, King Faisal University, Al Ahsa - 37912, Saudi Arabia. Email: 224003059@student.kfu.edu.sa

[2] Department of Pharmaceutics, Maratha Mandal's College of Pharmacy, Belgaum, Karnataka, India

[3*] Department of Public Health, College of Applied Medical Sciences, King Faisal University, Al Ahsa - 37912, Saudi Arabia (Corresponding Author). Email: yelmosaad@kfu.edu.sa

[4] Department of Restorative Dentistry and Endodontics, College of Dentistry, King Faisal University, Al-Ahsa - 31982, Saudi Arabia. Email: psreenivasalu@kfu.edu.sa

[5] Department of Pharmaceutical Sciences, College of Clinical Pharmacy, King Faisal University, Al-Ahsa - 31982, Saudi Arabia. Email: sharsha@kfu.edu.sa

[*] **Corresponding Author:** Nagesh Chandrasekhara Setty, nagesh1273@gmail.com

Yousif M. Elmosaad, yelmosaad@kfu.edu.sa

Received: 15th Jan, 2026; **Revised:** 27th Feb, 2026; **Accepted:** 20th Mar, 2026; **Available Online:** 5th Apr, 2026

ABSTRACT

This study focused on the design and development of atorvastatin (ATR) hydrogel using natural polymers like xanthan gum, guar gum and synthetic polymer used was carbopol-940. Twelve formulations were prepared and evaluated for spreadability, consistency, drug content and drug release study. By comparing all the evaluation parameters, F9 was chosen as best formulation which has good homogeneity, spreadability, drug content and drug release profile i.e., 84.15%. Then in F9 formulation different penetration enhancers (PE) were added such as camphor, menthol and thymol with varying concentration from 1-5%. Total 9 formulations were prepared. Then evaluation parameters of hydrogel were carried out repeatedly. Based on this study TF1 formulation was chosen as best which has 93.04% drug release with good homogeneity and spreadability. Skin penetration efficiency study, ex-vivo study and deposition study were carried out for both F9 and TF1 formulations. And this study showed the superiority of ATR-loaded with enhancer over ATR hydrogel and this concludes that ATR with PE is capable of permeating drug in deeper layers of skin.

Keywords: Penetration enhancer, Atorvastatin, Rheumatoid arthritis, Skin penetration efficiency study, Topical gel.

How to cite this article: Al-Ramel AAA, Jadhav SB, Setty NC, Naduvinamani S, Elmosaad YM, Sreenivasalu PK, Nagaraja S. Effect of Penetration Enhancers on Permeability of Atorvastatin Gel. Int J Drug Deliv Technol. 2026;16(25s): 667-676. DOI: 10.25258/ijddt.16.25s.81

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The principle function of skin is to defend it from surrounding environment additionally it behave as barrier for absorption of drugs. Skin structure mainly contains three layers i.e epidermis, dermis and hypodermis. Stratum corneum (SC) is the outermost layer of epidermis and it act as barrier for drug penetration There are different penetration enhancers used in topical and transdermal delivery are physical enhancer, chemical enhancer, natural penetration enhancer and miscellaneous enhancer used to increase the drug penetration by modifying the stratum corneum properties.[1,2]

Penetration enhancers are also called as accelerants or sorption promoters. Penetration enhancers such as terpenes, obtained from natural sources have gaining much importance in pharmaceutical and cosmetic formulation.[3] Because it has many advantages such as safe to use, high enhancement effect and at 1-5% concentration it has minimal irritation.[4]

Permeability enhancement is required to treat chronic disease like arthritis, as desired amount of drug may not reach to the desired site at desired rate with presently available therapy.

In the world around 18 million people are affected by rheumatic arthritis. The prevalence of rheumatic arthritis (RA) in India is ranging from 0.28-0.7%. It is an autoimmune disease results in destruction of joints and synovial hyperplasia. The most affected site in RA is wrist. RA is more common in women as compared to men. The different inflammatory mediators play principal function in development of the disease includes tumor necrosis factor-alpha (TNF-α), C-reactive protein (CRP), IL-18 and IL-12(interleukins), monocyte chemoatrractant protein-1(MCP-1), RANKL (receptor activator of nuclear factor-kB ligand), fractalkine, MMP-9(matrix metalloproteinase-9) along with molecules of adhesion.

The currently available treatment for RA includes NSAID'S (non-steroidal anti-inflammatory drugs), corticosteroids, DMARDs (disease modifying anti-rheumatic drugs), and biologics. The continued use of these agents has many adverse effects like osteoporosis, ischemic necrosis, muscle disease, impaired wound healing, gastric ulcers, candidosis, pancreatitis and infections like bacterial or fungal. These drugs are administered as oral formulation such as tablet, capsule and suspension by oral route which has many disadvantages like ineffective drug delivery at synovial joint, GI disturbances, toxicity and expensive treatment. Hence now a day's topical gels are used frequently because it has many qualities such as easy to remove, systemic drug absorption, diminished dose, inexpensive treatment, reduced side effects and it has controllable action.[5, 6,7]

Statins along with their antilipidaemic effect also has immunomodulatory and anti-inflammatory effect. Atorvastatin is classified under statin group and it will inhibit the production of cytokines, interkeukin-2(IL-2), interkeukin-12, TNF-α.Treatment using atorvastatin decreases the production of pro-inflammatory cytokines and chemokines. Atorvastatin partially prevent the release of TNF-α which is a central cytokine in mediating inflammatory pain. Atorvastatin also regulates the MHC-class II expression in endothelial cells and show immunomodulatory effect.[8, 9]

The purpose of the study was to formulate atorvastatin hydrogel loaded with penetration enhancers to improve the permeation of atorvastatin in deeper layers of skin.

MATERIAL AND METHOD

Material

Atorvastatin (drug), xanthan gum (XG) and guar gum (GG) were purchased from Yarrochem, Mumbai, Carbopol-940 and camphor (Loba chemie, Mumbai), thymol and menthol (Flora laboratory Mumbai), sodium benzoate (Milton chemicals, Mumbai), triethanolamine and rhodamine 6G (SD fine-chem Ltd, Mumbai), methanol (Fischer scientic,Mumbai).

Instruments

Brookfield viscometer (CAP 2000+ Brookfield Engineering Laboratories, MA, USA), UV-Vis spectrophotometer (Model UV 1800 Shimadzu Corporation, Japan), Franz Diffusion Cell (Local manufacturer, India), Magnetic stirrer (Servewell instruments pvt.ltd.), Digital pH meter (Servewell instruments pvt.ltd.).

Preformulation studies

Preformulation testing is the first step in the analytical development of dosage forms. Preformulation research looks into the physical and chemical properties of a drug-both alone and when combined with excipients.[10]

Identification of pure drug

Determination of melting point

Thiel's tube was used to determine the melting point of a drug sample. Take capillary tube which is sealed at one end in which small quantity of sample was placed and kept in thiel's tube and the melting temperature of drug sample was noted.[11]

FTIR spectroscopy

The IR spectrum is used for the analysis of pure drug samples.

Method- A few milligrams of solid sample was mixed with dry KBr (10-200mg). The mixture is pulverized using mortar and pestle in an evacuated dye and the mixture was subjected to a pressure. The combination sinters, resulting in a clear, translucent disc. In the mid-infrared band the potassium bromide employed was entirely transferent.[12, 13]

Determination of λmax

The *λmax* of atorvastatin was determined using phosphate buffer solution (pH-6.8) which was scanned in UV spectrophotometer at 200-400 nm.[14]

Compatibility studies of pure drug and excipients

FTIR spectroscopy

FT-IR graph aid in the identification of drug and detection of drug-excipient interactions. Shimadzu fourier transform infrared spectrometer was used to access the compatibility of drug sample along with polymers.

Differential scanning calorimetry

Differential scanning calorimetry was used to investigate the drug-excipient interaction. The compounds to be tested were placed in a standard aluminium pan, and a scan in the range of 100 degrees per minute. Each sample weighed between 10 and 12 mg. Each scan includes a substraction of a scan taken with the reference sample as a baseline (water/buffer).[12, 13]

FORMULATION DEVELOPMENT

Preparation of atorvastatin hydrogels

Total 12 formulations of atorvastatin hydrogels were prepared by varying concentration of XG (xanthan gum), GG (Guar gum) and carbopol-940 as per table no. 1. The polymers were soaked in distilled water overnight. The polymer was distributed in the water based drug solution using magnetic stirrer at medium speed. Then add sodium benzoate in it. In the carbopol-940 gel triethanolamine was added to adjust the pH.[15]

Table no.1 Preparation of atorvastatin gel

Formulation code	ATR(mg)	XG (mg)	GG (mg)	C-940 (mg)	Sodium benzoate(mg	D/W (ml)
F1	250	300	-	-	0.025	10
F2	250	400	-	-	0.025	10
F3	250	500	-	-	0.025	10
F4	250	600	-	-	0.025	10
F5	250	-	150	-	0.025	10
F6	250	-	200	-	0.025	10
F7	250	-	250	-	0.025	10
F8	250	-	300	-	0.025	10
F9	250	-	-	100	0.025	10
F10	250	-	-	150	0.025	10
F11	250	-	-	200	0.025	10
F12	250	-	-	250	0.025	10

All formulations were made for 10gm.

Preparation of atorvastatin gel using terpenes

From F1-F12 formulations, F9 formulation was selected as best formulation. In this formulation various terpenes such as camphor, thymol and menthol with different concentrations were added by dissolving in small amount of ethanol. Add sodium benzoate in it as shown in table no.2. The formulations were stirred using magnetic stirrer at medium speed. Finally add triethanolamne (TEA) to adjust the pH of formulation.[15]

Table no. 2 Preparation of atorvastatin gel with terpenes

Formulation code	ATR (mg)	C-940 (mg)	Camphor (mg)	Menthol (mg)	Thymol (mg)	Sodium benzoate (mg)	D/W (ml)
TF1	250	100	0.1	-	-	0.025	10
TF2	250	100	0.3	-	-	0.025	10
TF3	250	100	0.5	-	-	0.025	10
TF4	250	100	-	0.1	-	0.025	10
TF5	250	100	-	0.3	-	0.025	10
TF6	250	100	-	0.5	-	0.025	10
TF7	250	100	-	-	0.1	0.025	10
TF8	250	100	-	-	0.3	0.025	10
TF9	250	100		-	0.5	0.025	10

The formulations were made for 10gm.

Evaluation parameters of atorvastatin gel without terpenes

1. Appearance and colour

The prepared hydrogels were placed in the container and visually inspected for their colour.[16, 17]

2. Homogeneity

Homogeneity of the formulations was tested.[16, 17]

3. Grittiness

Prepared formulations were tested using optical microscope for the appearance of any grits.[16, 17]

4. pH determination

A digital pH meter was used to determine the formulation's pH. 1gm of gel was distributed in 100ml of D/W (distilled water). Then electrode was inserted into this solution and note down the reading. Before the pH was measured, the pH meter was calibrated using buffer solutions 4, 7, and 10. [16, 17]

5. Consistency

A Brookfield viscometer with spindle number 6 was used to measure the gel's consistency.

On the center of the viscometer plate, the necessary quantity of gel was applied. At rotation speed 10 rpm and temperature 25˚c was maintained, the viscosity of the preparation was tested.[18]

6. Determination of spredability

On a pre-marked glass plate required amount of hydrogel was putted inside the circle (diameter 2cm), onto which the alternative glass plate was placed over this 500gm weight was placed for 5mins. An increase in spreading area of hydrogel was recorded. The percentage spread by area was determined using the equation [1].

$$\text{Percentage spread by area} = \frac{Af}{Ai} \times 10 \qquad \text{Eq.1}$$

Where Ai = initial diameter (2cm), Af = final area after spreading.[19, 20]

7. Drug content determination

Specified quantity of gel was added in 10ml of volumetric flask containing methanol (stock solution-

A). From stock solution-A 1ml of solution was pipetted off, and make up the volume 10ml using phosphate buffer solution and sonicated for few minutes (stock solution-B). From stock solution-B, 1ml solution was pipetted out and diluted with phosphate buffer solution. A UV-visible spectrophotometer was used to measure absorbance in order to estimate the drug content.[15]

8. *In vitro* diffusion study

The hydrogel's *in vitro* diffusion study was conducted using a Franz diffusion cell. A dialysis membrane was clamped between the diffusion cell's donor and receptor compartments. The receptor chamber was filled with 20ml of Phosphate buffer solution (PBS). The diffusion cell was kept on hot plate at temperature 37˚c, and the PBS existing in receptor chamber was agitated constantly at 100rpm with the help of magnetic stirrer. The donor compartment contained the prepared hydrogel. At regular intervals, 1ml of the sample was removed from the receptor compartment and replaced with an equivalent volume of PBS. The absorbance of the sample was then measured using a UV spectrophotometer.[21]

Evaluation parameters of atorvastatin gel with terpenes

Appearance, colour, homogeneity, grittiness, pH, consistency, spredability, drug content and *in vitro* diffusion study of the gel with terpenes were performed as previously described for the hydrogel formulation without terpenes.

***Ex vivo* permeation studies**

Rat skin preparation -

Male Sprague rat was purchased from the Maratha Mandal's Nathajirao G. Halgekar Institute of Dental Sciences and Research Centre Animal House. Rat hair removed using electric cliper and skin fat, debris were removed. Fresh rat skin was used for experiment. It has been approved by the IAEC committee.

Rat skin and Franz diffusion cell were used in the *ex vivo* permeation investigations. The skin was cut into proper size and mounted in such a way that SC of the skin is faced the donor cell and dermis towards the receiver compartment. In receptor compartment 25ml of PBS was kept and in the donor chamber 250mg of hydrogel was placed. The *ex vivo* studies were carried out for both formulations without terpenes (control) and with terpenes. The Franz diffusion cell was kept on magnetic stirrer and agitated with the help of magnetic bead at a temperature 37±2˚c for 24hrs. Then, at regular intervals, 1ml of the sample was removed from the receptor compartment and replaced with an equivalent volume of PBS. The withdrawan solutions were estimated spectrophotometrically at 238nm.[21, 22] Flux, permeability coefficient (Kp) and enhancement ratio(ER) were calculated using the following equations.

$$\text{Flux, J} = \frac{\text{Amount of drug permeated}}{\text{Time}} \times \text{Diffusion area of skin} \quad \text{Eq. (2)}$$

$$\text{Kp}\left(\frac{\text{cm}}{\text{hr}}\right) = \frac{\text{Flux of the formulation}}{\text{Initial amount of drug in the donor compartment}} \quad \text{Eq. (3)}$$

$$\text{ER, Enhancement ratio} = \frac{\text{Flux of the formulation}}{\text{Control formulation}} \quad \text{Eq. (4)}$$

Skin deposition study

Skin deposition experiments are conducted to determine drug deposition on skin after permeation studies are finished. To get rid of extra formulation, the treated skin was gently cleaned with methanol after a 24-hour treatment. The skin was divided into smaller pieces, homogenized with methanol, and sonicated for 60 minutes in an ultrasonic bath. The solution was centrifuged for 10min at 100rpm. The obtained solution was filtered using nylon membrane filter and diluted with phosphate buffer solution. The amount of atorvastatin deposited into skin was determined using UV visible spectrophotometer.[23]

Skin penetration efficiency study

Fluorescence microscopy was utilized to analyze the permeation ability of terpenes through skin layers. The rat skin mounted on the Franz diffusion cell was treated for eight hours with the TF1 formulation loaded with rhodamine 6G (Rh 6G). Following treatment, the skin was separated from the diffusion cell and thoroughly cleaned with water to remove any remaining formulation After being treated, the skin sample was divided into smaller pieces and preserved in 10% buffered formalin. Following fixation, the skin samples were put in paraffin wax and sliced with a microtome into sections that were 4.5 µm thick. The obtained thin tissue section was kept on glass slide and that were inspected under fluorescence microscope for the appearance of fluorescence in skin layers. The same procedure was followed for F9 formulation.[23, 24]

Stability study

Stability test was conducted for the gel formulation at 40 ±2˚C and 75 ±5% relative humidity for 90 days in accordance with ICH recommendations. The drug

content, drug release study, and physical appearance are monitored on a regular basis.[18]

RESULT AND DISCUSSION

Total 21 formulations were prepared, where F1-F12 were prepared as plain gel without terpenes and TF1-TF9 were prepared using different terpenes.

Preformulation studies

Melting point of atorvastatin was found to be 163.77˚c, which is nearer to reported melting point that confirms the purity of sample. Atorvastatin *λmax* was observed at 238nm shown in fig.1. The physical mixture's FTIR spectra showed no peaks or the elimination of existing peaks, indicating that atorvastatin and excipients are not interacting; this showed that the atorvastatin is compatible with each of the polymer used in the formulation shown in fig.2. The DSC curve of ATR shows exothermic peak at 163.77˚c, near to its melting point at 160.64˚c, thus indicates crystalline nature shown in fig.3.

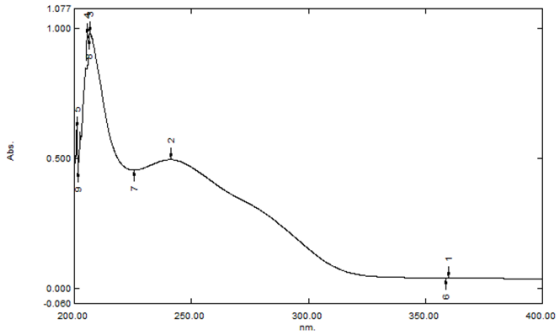
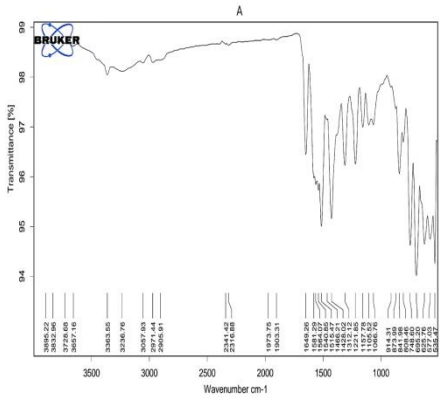
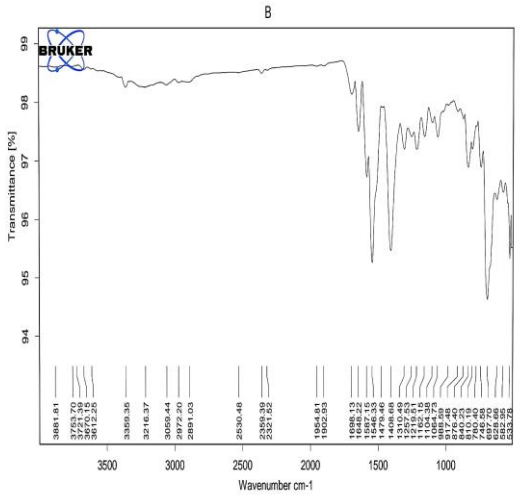
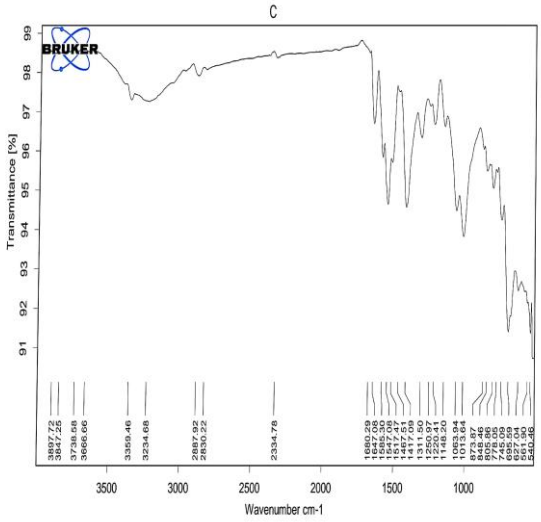


Fig. 1: UV spectrum of atorvastatin in PBS (pH 6.8).







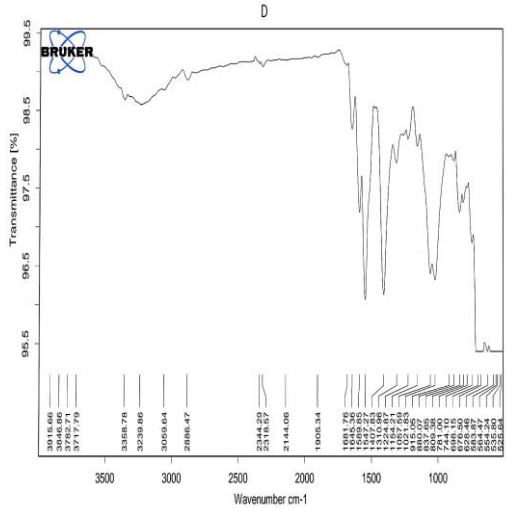


Fig.2: FTIR Spectra of A. Atorvastatin, B. Physical mixture (ATR, C-940, Sodium benzoate), C. ATR, GG, Sodium benzoate, D. ATR, XG, Sodium benzoate.

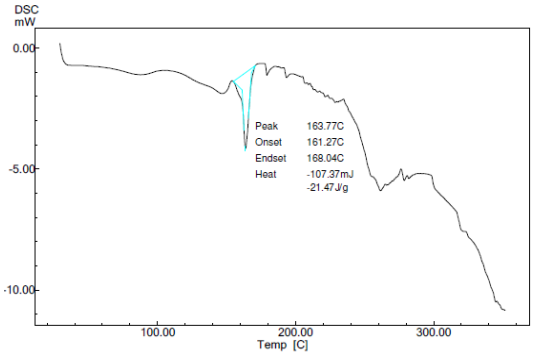


Fig. DSC of ATR

Evaluation of hydrogel

Findings from the visual examination of 21 hydrogels showed that all the formulation are white in colour, except F5-F8 which contains guar gum are opaque in colour. The prepared hydrogel has good homogeneity, smooth texture with no clumps. The physicochemical characteristics of all formulation without and with terpenes are given in Table no.3 and 4respectively. pH of all formulations is similar to the skin pH, this shows that formations are compatible with skin pH. All formulations showed good content uniformity. As the polymer concentration increases viscosity increases and spredability decreases and vice versa.

Table no. 3 Evaluation of atorvastatin hydrogel

Formulation code	pH	Viscosity (cP±SD)	Spredability (%)±SD	Drug content (%)±SD
F1	5.78±0.017	966±0.014	323.33±2.88	96.45±0.046
F2	5.61±0.015	1275±0.022	311.66±2.88	92.52±0.04
F3	5.52±0.005	2381±0.018	270±5	93.48±0.10
F4	5.53±0.015	2766±0.016	253.33±2.88	89.02±0.02
F5	5.67±0.015	834±0.02	323.33±2.88	91.56±0.069
F6	5.66±0.005	863±0.032	303.33±2.88	93.42±0.046
F7	5.87±0.005	1209±0.019	286.66±2.88	88.65±0.046
F8	5.62±0.005	2287±0.020	260±2.88	87.02±0.046
F9	5.21±0.015	797±0.011	315±5	99.12±0.069
F10	5.19±0.005	1453±0.025	278.33±2.88	81.42±0.046
F11	5.14±0.011	1725±0.013	263.33±2.88	86.38±0.100
F12	5.03±0.01	2353±0.023	240±5	87.52±0.069

n=3

Table no.4 Evaluation of hydrogel with terpenes

Formulation code	pH	Viscosity (cP±SD)	Spredability (%)±SD	Drug content(%)
TF1	6.09±0.017	1025±0.022	293.33±2.88	97.36±0.069
TF2	6.03±0.015	1214±0.014	310.66±2.88	96.68±0.200
TF3	6.01±0.005	1532±0.0133	353.33±2.88	97.49±0.046
TF4	6.30±0.015	2135±0.032	278.33±2.88	95.06±0.023
TF5	6.07±0.015	2149±0.023	286.66±2.88	94.36±0.21
TF6	6.13±0.005	2263±0.07	286.66±2.88	97.24±0.042
TF7	6.48±0.005	2019±0.024	258.33±2.88	97.69±0.04
TF8	6.50±0.005	2307±0.022	263.33±2.88	96.32±0.06
TF9	6.40±0.015	2357±0.019	243.33±2.88	95.50±0.04

n=3

In vitro diffusion study

Drug release of atorvastatin from preparation containing xanthan gum are given in following order F1>F2>F3>F4 and for guar gum based hydrogel, the release profile was given in following order F5>F6>F7>F8. Similarly formulation containing carbopol-940, the order of release was ranked as F9>F10>F11>F12 as shown in figures: 4 and 5. Drug release profiles of all 12 formulations were studied. F9 formulation was chosen as best preparation which has highest drug release i.e 84.69% for 8hrs. The drug release of the F9 formulation with carbopol-940 was high because there was less interparticular cross-linking between the drug and C-940. Additionally, the low polymer concentration caused low viscosity and reduced flow resistance, which increased the rate of drug release.[25]

In selected F9 formulation different penetration enhancer such as camphor, thymol, menthol with various concentration like 1%, 3% and 5% were added for preparation of hydrogel. Total nine formulations (TF1-TF9) were prepared and evaluated. TF1 formulation contains thymol-1% was selected as best formulation because it has highest drug release i.e 93.04% as shown in the figure: 6.

Previous research demonstrated that lipophilic terpenes increase the penetration of hydrophobic drugs while hydrophilic terpenes, such menthol, promote the penetration of hydrophilic pharmaceuticals. Therefore, high lipophilicity of terpene is necessary to boost the penetration of lipophilic drugs. So, the TF1 formulation has highest drug permeation contains thymol, has log P

value-3.28 is greater as compared to menthol and camphor has log P value 3.2, 2.13 respectively. In earlier research, it was stated that the menthol and camphor has no or slight effect on penetration of lipophilic drugs. Hence ATR is lipophilic drug, menthol and camphor decreases the drug release. Structure of enhancer also influences the drug permeation. Bulkier PE is less active than smaller PE. Hence thymol is a monocyclic phenol monoterpene and camphor is a bicyclic ketone monoterpene, so terpenes containing ketone group decreases the permeation of ATR [26, 27, 28]. By considering all this aspects, TF1 formulation was selected as best. Further *ex vivo* permeability study, skin deposition study and skin penetration study were performed for both TF1 and F9 formulation.

***Ex vivo* permeation study**

Using Franz diffusion cell, the *ex vivo* skin penetration of the TF1 formulation across the rat skin was assessed and contrasted with that of the F9 formulation as a control. The cumulative amount of ATR permeated for 24hrs from hydrogel containing PE (17.94 mg/cm²) was higher compared to that of hydrogel without PE (16.036mg/cm²). The permeation flux of ATR from TF1 0.238mg/cm²/h was greater than F9 formulation (0.212mg/cm²/h). The permeability coefficients of TF1 and F9 formulations were found to be 3.80×10^{-2} and 3.40×10^{-2}cm/h respectively. The enhancement ratio of TF1 was found to be 1.118. This represents that PE successfully increased the permeation of ATR.

By studying all the aspects the study found that better penetration of hydrogel which containing penetration enhancer as compared to gel without enhancer. The study found that the drug release using rat skin was less as compared to cellulose membrane, because of the complex composition of skin which provides greater hindrance for drug molecules for penetrating at the time of diffusion process.

Skin deposition study

For better understanding of the drug retention behavior inside the skin layers, the skin deposition study is conducted. The total drug retained into the skin via control formulation (0.122mg/cm²) was slightly higher as compared to that of formulation containing PE (0.0781mg/cm²). The possible reason could be that, terpene used as penetration enhancer, increase the percutaneous absorption of compounds by various mechanisms such as altering the lipid composition of SC or by making the drug more diffusible in SC or by increasing drug partitioning into SC [29]. Due to this the skin deposition of control formulation is more which do not contain PE.

Skin penetration efficiency study

The penetrating capacity of terpene is demonstrated using fluorescence microscopy in contrast to the control, Rh-6G F9 hydrogel. Rh 6G hydrogel exhibited fluorescence restricted only to the superficial SC layer. But in Rh 6G-TF1 hydrogel with terpene, consistent strong fluorescence was seen across the sc, epidermis and dermis. The significant increase in fluorescence intensity suggests that Rh 6G TF1 hydrogel with terpene is effectively delivered to deeper skin layers and has the potential to improve permeability of skin and transfer the drug molecule deeper into the skin layers shown in figure;7.

Stability study

Stability study was performed for best preparation TF1. The preparation was reserved in packed container for 3 months at 40˚c and 75% relative humidity. The formulation was evaluated at different time intervals for 90 days. The colour, pH, drug content and drug release of the formulation remain unchanged. This shows that the formulation is physically and chemically stable even after 3 months.

The diffusion exponents N for F9 and TF1 formulation were 0.715 and 0.7319, less than 1 represents non-fickian mechanism.

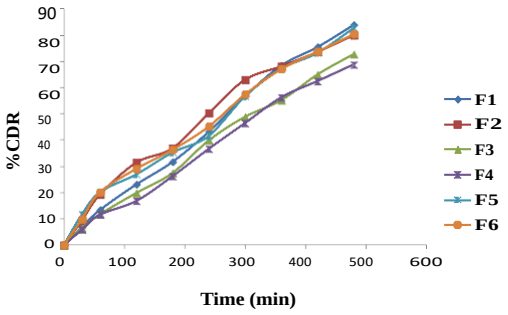


Fig.4 *In vitro* drug release profiles of hydrogels from F1-F6

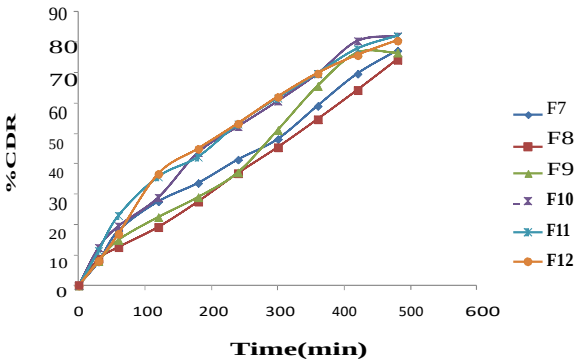


Fig .5: *In vitro* drug release profiles of hydrogels from F7-F12

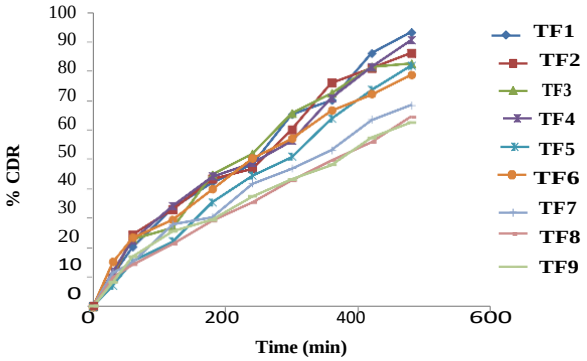


Fig.6: *In vitro* drug release profiles of hydrogels with terpenes

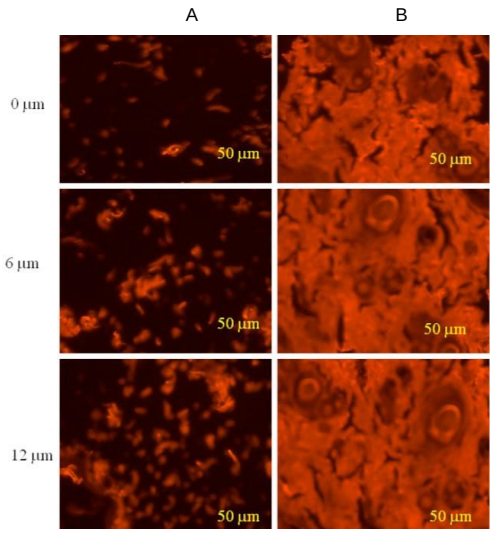


Fig.7: Fluorescence photomicrographs of rat skin after 8h application of (A) Rhodamine-6G F9 hydrogel without terpene, and (B) Rhodamine-6G-TF1 loaded terpene.

CONCLUSION

In the present study, atorvastatin hydrogels were formulated with and without penetration enhancers with an intension to increase the permeability of the drug across the skin where it can be achieved better treatment for rheumatic arthritis. The ATR hydrogel loaded with penetration enhancer showed an improved *in vitro* drug release compared to hydrogel without PE. The *ex vivo permeation* study of ATR gel loaded with enhancer was significantly greater as compared to plain hydrogel. Results of fluorescence microscopy showed the ability of penetration enhancer to improve skin penetration and effectively transport the drug molecules to the deeper skin layers.

ABBREVIATIONS

ATR- Atorvastatin, PE- Penetration enhancer, RA- Rheumatic arthritis, FTIR- Fourier-transform infrared spectroscopy, DSC- Differential scanning calorimetry, SC- Stratum corneum, TNF-α- Tumor necrosis factor-alpha, CRP- C-reactive protein, IL-18- Interleukin-18, IL-12- Interleukin-12, MCP-1- Monocyte chemoattractant protein-1, RANKL- Receptor activator of nuclear factor-kB ligand, MMP-9- Matrix metalloproteinase-9, NSAID'S- Non-steroidal anti inflammatory drugs, DMARDs-Disease modifying anti rheumatic drugs, XG-Xanthan gum, GG- Guar gum, C-940-Carbopol-940, KBr- Potassium bromide, TEA-Triethanolamine, PBS- Phosphate buffer solution, cp - Centipoise, IAEC- Institutional Animal Ethics Committee, Rh 6G- Rhodamine 6G, µm- Micrometer, ICH- International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use.

ACKNOWLEDGMENT

The author's express sincere gratitude to the principal of Maratha mandal's college of pharmacy, Belgavi and Management, MM group of institutions, Belgavi for providing required facilities.

FUNDING

This work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia [Grant No. KFU261902].

CONFLICT OF INTERESTS

The authors declared no conflict of interest.

REFERANCES

1. Ruela AL, Perissinato AG, Lino ME, Mudrik PS, Pereira GR. Evaluation of skin absorption of drugs from topical and transdermal formulations. Brazilian Journal of Pharmaceutical Sciences. 2016 Jul;52:527-44.
2. Roy N, Agrawal M, Chaudhary S, Tirkey V, Dhwaj A, Mishra N. Review article on permeation enhancers: a major breakthrough in drug delivery technology. International Journal of Pharmaceutical Sciences and Research. 2017 Mar 1;8(3):1001
3. Patil UK, Saraogi R. Natural products as potential drug permeation enhancer in transdermal drug delivery system. Archives of dermatological research. 2014 Jul;306(5):419-26.
4. Lim PF, Liu XY, Chan SY. A review on terpenes as skin penetration enhancers in transdermal drug delivery. Journal of Essential Oil Research. 2009 Sep 1;21(5):423-8.
5. Stoilova S. Integrative Treatment for Arthritis.
6. Thakur S, Riyaz B, Patil A, Kaur A, Kapoor B, Mishra V. Novel drug delivery systems for NSAIDs in management of rheumatoid arthritis: An overview. Biomedicine & Pharmacotherapy. 2018 Oct 1;106:1011-23.
7. Patil PB, Datir SK, Saudagar RB. A review on topical gels as drug delivery system. Journal of Drug Delivery & Therapeutics. 2019 May 2;9.
8. Barsante MM, Roffê E, Yokoro CM, Tafuri WL, Souza DG, Pinho V, Castro MS, Teixeira MM. Anti-inflammatory and analgesic effects of atorvastatin in a rat model of adjuvant-induced arthritis. European journal of pharmacology. 2005 Jun 15;516(3):282-9.
9. Jury EC, Ehrenstein MR. Statins: immunomodulators for autoimmune rheumatic disease?. Lupus. 2005 Mar;14(3):192-6.
10. Chaurasia G. A review on pharmaceutical preformulation studies in formulation and development of new drug molecules. International journal of Pharmaceutical sciences and research. 2016 Jun 1;7(6):2313.
11. Gangurde AB, Awais M, Bairagi VA, Abdurrahman, Sanaurrehman, Karishma, Rajashri. Formulation Development and in Vitro Evaluation of Fast Dissolving Tablets of Ramipril Solid Dispersion. Indo American Journal of Pharmaceutical Sciences. 2018 Aug 1;5(8):8409-16.
12. Manikandan M, Kannan K, Manavalan R. Compatibility studies of camptothecin with various pharmaceutical excipients used in the development of nanoparticle formulation. Int J Pharm Pharm Sci. 2013;5(4):315-21.
13. Trivedi MK, Panda P, Sethi KK, Gangwar M, Mondal SC, Jana S. Solid and liquid state characterization of tetrahydrocurcumin using XRPD, FT-IR, DSC, TGA, LC-MS, GC-MS, and NMR and its biological activities. Journal of pharmaceutical analysis. 2020 Aug 1;10(4):334-45.
14. Indian Pharmacopoeia Commission**.** Indian Pharmacopoeia**.** Vol. 1. Ghaziabad (India): Indian Pharmacopoeia Commission; 2010. p. 559.
15. Zakaria AS, Afifi SA, Elkhodairy KA. Newly developed topical cefotaxime sodium hydrogels: antibacterial activity and in vivo evaluation. BioMed research international. 2016;2016(1):6525163.
16. Laxmi RJ, Karthikeyan R, Babu PS, Babu RN. Formulation and evaluation of antipsoriatic gel using natural excipients. Journal of Acute Disease. 2013 Jan 1;2(2):115-21.
17. Chen MX, Alexander KS, Baki G. Formulation and evaluation of antibacterial creams and gels containing metal ions for topical application. Journal of pharmaceutics. 2016;2016(1):5754349.
18. Singh V, Chaubey N. Design and evaluation of topical hydrogel formulation of aceclofenac for improved therapy. J. Drug Deliv. Ther. 2019 Sep 1;9:118-22.
19. Ahad A, Aqil M, Ali A. Investigation of antihypertensive activity of carbopol valsartan transdermal gel containing 1, 8-cineole. International journal of biological macromolecules. 2014 Mar 1;64:144-9.
20. Al-Suwayeh SA, Taha EI, Al-Qahtani FM, Ahmed MO, Badran MM. Evaluation of skin permeation and analgesic activity effects of carbopol lornoxicam topical gels containing penetration enhancer. The Scientific World Journal. 2014;2014(1):127495.
21. Chen M, Shamim MA, Shahid A, Yeung S, Andresen BT, Wang J, Nekkanti V, Meyskens Jr FL, Kelly KM, Huang Y. Topical delivery of carvedilol loaded nano-transfersomes for skin cancer chemoprevention. Pharmaceutics. 2020 Nov 27;12(12):1151.
22. Vasanth S, Dubey A, GS R, Lewis SA, Ghate VM, El-Zahaby SA, Hebbar S. Development and

investigation of vitamin C-enriched adapalene-loaded transfersome gel: a collegial approach for the treatment of acne vulgaris. AAPS PharmSciTech. 2020 Jan 8;21(2):61.

23. Peram MR, Jalalpure S, Kumbar V, Patil S, Joshi S, Bhat K, Diwan P. Factorial design based curcumin ethosomal nanocarriers for the skin cancer delivery: in vitro evaluation. Journal of liposome research. 2019 Jul 3;29(3):291-311.

24. Sarwa KK, Mazumder B, Rudrapal M, Verma VK. Potential of capsaicin-loaded transfersomes in arthritic rats. Drug delivery. 2015 Jul 4;22(5):638-46.

25. Auda SH, El-Rasoul SA, Ahmed MM, Osman SK, El-Badry M. In-vitro release and in-vivo performance of tolmetin from different topical gel formulations. Journal of Pharmaceutical Investigation. 2015 Jun;45(3):311-7.

26. Furuishi T, Kato Y, Fukami T, Suzuki T, Endo T, Nagase H, Ueda H, Tomono K. Effect of terpenes on the skin permeation of lomerizine dihydrochloride. Journal of Pharmacy & Pharmaceutical Sciences. 2013 Sep 20;16(4):551-63.

27. El-Kattan AF, Asbill CS, Michniak BB. The effect of terpene enhancer lipophilicity on the percutaneous permeation of hydrocortisone formulated in HPMC gel systems. International journal of pharmaceutics. 2000 Apr 5;198(2):179-89.

28. Sapra B, Jain S, Tiwary AK. Percutaneous permeation enhancement by terpenes: mechanistic view. The AAPS journal. 2008 Mar;10(1):120-32.

29. Prasanthi D, Lakshmi PK. Terpenes: Effect of lipophilicity in enhancing transdermal delivery of alfuzosin hydrochloride. Journal of Advanced Pharmaceutical Technology & Research. 2012 Oct 1;3(4):216-23.