RESEARCH ARTICLE

Tofacitinib Citrate-Loaded Topical Gel for the Treatment of Rheumatoid Arthritis: Formulation and *In-vitro-In-vivo* Characterization

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ABSTRACT

This study aims to create and assess the anti-arthritic activity of tofacitinib citrate (TC) as a topical gel formulation. Using the gelling agent Carbopol 980 P, seven gel formulations were formed. The gel formulations were assessed for pH, drug content, spreadability, homogeneity, and *in-vitro* diffusion profile. Out of all the formulations, G7 exhibited superior release characteristics (99.1%) in comparison to the other formulations. The DSC research revealed a strong endothermal peak at 214.17°C and demonstrated an interaction with the excipients taken into consideration in this investigation. While carrying out the *in-vivo* study, formulation G7 successfully reduced inflammation induced by CFA. Ultimately, the optimal formulation for the "G7" can be determined & successfully treats inflammation associated with arthritis. As per stability studies, it was specified that there are no such major changes in the long-term and accelerated stability testing study.

Keywords: Tofacitinib citrate, Topical gel, Carbopol 980 P, Arthritis, Inflammation, Stability.

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INTRODUCTION

The US Food and Drug Administration (USFDA) granted a license for tofacitinib, the first inhibitor of Janus kinase (JAK) to treat moderate to severe RA.[1-2] A specific small-molecule inhibitor of JAK3 and JAK1, as well as, to a lesser extent, tyrosine kinase (TYK2), is tofacitinib.[3-4] Crucially, tofacitinib can also regulate synovitis, structural joint degeneration, and T-cell activation [5]. Oral tofacitinib citrate (TC) dosages of 5 mg are administered twice a day.[6-7] Tofacitinib lowers the immune system and increases susceptibility to infections when taken orally at large doses (5 mg and 10 mg daily). Clinical trials have recently demonstrated good therapeutic effects for the treatment of psoriasis symptoms when 2% tofacitinib ointment is applied topically.[8-9] Tofacitinib applied topically can circumvent the drawbacks of oral therapy, including pre-systemic metabolism, gastrointestinal issues, dose escalation, non-tissue distribution, and systemic adverse effects (down in neutrophil count).[10-13] Tofacitinib is also required at a lower dose for topical and transdermal administration than for oral administration, which lessens the likelihood of systemic side effects [14]. For topical and transdermal distribution, the stratum corneum serves as a barrier.[15] Merely a barrier preventing drugs from passing through the skin is formed by corneocytes in the intercellular matrix that make up the stratum corneum.[16] Solid lipid nanoparticles (SLNs), are developed to augment drug permeation through the skin. The present study primarily involved developing TC-loaded topical gel; *in-vitro* evaluation and *in-vivo* efficacy in rat models.

MATERIALS AND METHODS

Materials

Tofacitinib citrate was attained as a gift sample from Unison Pharmaceuticals, India. Carbomer 980P was provided by Guapha Pharmaceuticals, India. The dialysis membrane was acquired from Hi Media Laboratories Pvt. Ltd. Analytical reagent (AR) grade reagents and solvents were all used.

Methods

Preparation of Gel loaded with SLN

Carbomer 980P was placed in a 100 ml beaker, and lukewarm water was added. To prevent lump formation, a magnetic stirrer (Remi, Mumbai) rotating at 70 RPM was used for mixing. Triethanolamine was placed in the resultant liquid to correct the pH and produce a gel. Propylene glycol, glycerol, and benzalkonium chloride were combined in another beaker. To this mixture, TC nanoparticles are added and thoroughly mixed. Lastly, a magnetic stirrer spinning at 100 RPM was used to mix the drug dispersion into the Carbomer 980P gel.

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Table 1: Formulation composition of gel loaded with optimized nanoparticles

Ingredients	*G1*	*G2*	*G3*	*G4*	*G5*	*G6*	*G7*
Lipid Nanoparticle (Equivalent quantity of 10 mg TC)	17	17	17	17	17	17	17
Carbomer 980P (g)	0.5	1.0	1.5	2.0	0.5	1.0	1.5
Propylene glycol (mL)	25	20	10	5	-	-	-
Glycerol (mL)	-	-	-	-	25	20	10
Benzalkonium chloride (%)	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Triethanolamine (mL)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Purified water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

According to Table 1, seven formulations were created, stored in a refrigerator at 15°C, and characterized.

Drug content

A measured quantity of prepared gel was added in 100 ml of pH 6.8 ethanolic phosphate buffer. For an hour, the gel solution was shaken constantly using a mechanical shaker. A UV-visible spectrophotometer (Shimadzu UV-1800, Japan) was used to study the solution at 289 nm.

pH determination

The gel was brought into direct contact with the pH meter (Systronic Digital, Model: 335, Mumbai). After allowing the pH meter to equilibrate, the pH is then recorded.

Viscosity

The produced gels' viscosity was evaluated by a Brookfield viscometer (model DV-II+Pro, USA). By whirling the spindle No. 68 at 30 and 50 rpm, viscosity was measured**.**

Spreadability

Wooden blocks and glass slides were utilized for measurement. Approximately 500 mg of gel that had been created was placed in the pan. The amount of time looked for the upper slide to completely detach from the secure slides was noted.

$$S = ML/T$$

Where S = spreadability, M = weight secured to the top slide, L = length of a glass slide, and T = duration of the slide's separation.

Homogeneity

For uniformity testing, every generated gel formulation was characterized. Visual inspection of the gel was conducted following its settling in appropriate containers. The gels were examined to see how they looked and whether there were any obstructions.

***In-vitro* Diffusion Study**

The drug TC was released *in-vitro* from the gel using the diffusion technique and a Franz cell. Prior to usage, equal-sized pieces of 6 cm by 2.5 cm cellophane membrane were cut, and distilled water was used to soak them for a whole day. The tests are done in 10 milliliters of phosphate buffer pH 6.8 saline maintained at 37 ± 0.5°C by a magnetic stirrer and an IKA Auto Temp Regulator, Germany continuous heating device. A single gram of gel was added to the donor compartment. One milliliter aliquot sample was taken out and substituted with a similar volume of fresh buffer at regular intervals. Through a UV spectrophotometer with a wavelength of 289 nm; the amount of drug diffusing across the membrane was determined.

***In-vivo* study of Paw edema volume induced by *complete Freund's Adjuvant* (CFA)**

The study was carried out by CPCSEA guidelines. Before carrying out the in-vivo study, the study project was permitted by the Institutional Animal Ethical Committee with approval number**.** A total of 48 rats per six-month-old was considered. (Wister rats, 200–250 gm of both genders) were used. Four groups of twelve animals were created. All rats anesthetized with 3% isoflurane in oxygen developed arthritis after receiving a three-week intradermal injection of CFA (0.01 mL/kg) straight into the hind metatarsal foot pad.

Tofacitinib citrate gel was prepared by dispersing Tofacitinib citrate in 2% agar gel.

Group A: Received saline water (1.80Ml/kg)+CFA;

Group B: Treated with CFA + Tofacitinib citrate gel (Reference drug) applied topically.

Group C: Treated with CFA+G7 (equivalent drug amount of 10 mg) applied topically*;*

Group D: treated with CFA+G7 (equivalent drug amount of 20 mg) applied topically.

Hind paw volume

It was measured by plethysmograph (PLM 01 Plus, Germany) by the below-given formula.

Increase in paw volume

It was measured for a time of 21 days and paw volume was measured for 0, 5, 10, 15, and 21 days.

Stability study of best gel loaded with optimized nanoparticle

Stability experiments for the optimized formulation were performed under accelerated conditions by the ICH using a well-closed container or a sealed vial was utilized. The vial contains "G7," which was stored in a stability chamber (Stability models 3907, Thermofisher, Mumbai). Gel formulations were stored under the conditions stated in Table 4 for the duration given. The gel formulation's physical qualities, such as appearance, color, presence of clogs, consistency, and phase separation, were assessed. Gel was also assessed for

Table 2: Characterization of gels

Formulations	*Characterization of gels*				
	Drug content (%)	*pH*	*Viscosity(cp)*	*Spreadability(mm)*	*Homogeneity*
G1	87.7 ± 2.8	6.7	398 ± 3	77 ± 7	+++
G2	86.9 ± 4.3	6.2	473 ± 2	57 ± 8	++
G3	92.3 ± 4.9	6.8	504 ± 6	64 ± 2	++
G4	90.8 ± 5.2	6.6	584 ± 7	50 ± 8	++
G5	88.3 ± 3.7	6.7	406 ± 2	70 ± 6	+++
G6	83.4 ± 5.3	7.2	488 ± 9	59 ± 3	++
G7	93.6 ± 4.5	6.4	530 ± 3	55 ± 4	++

All data are stated as Mean ± Standard error of the mean (n = 3)

chemical factors such as pH change, drug concentration, and viscosity.

RESULTS AND DISCUSSION

According to Table 2, the drug content of all formulations ranged from 83.4 to 93.4%. No such variations were seen in pH readings, which fall within the typical pH range for skin. It was determined that viscosity varied between 398 and 584 cp. Formulations containing the greatest quantity of Carbomer 980P (2.0 g) exhibited the highest viscosity value (584 cp), whilst formulations containing the least amount of Carbomer 980P (0.5 g) exhibited the lowest viscosity (398 cp). Similar patterns may be noticed in the investigation of spreadability; a high concentration of Carbomer 980P demonstrated the lowest value, whilst a low concentration of gelling agent exhibited the highest spreadability. In the "Homogeneity" investigation, all formulations exhibited the absence of aggregated mass and the appearance of clog mass.

In-vitro Dissolution Study

As per the study Carbomer 980P significantly contributed to drug release as a gelling agent. It was determined that an increase in Carbomer 980P significantly slowed the drug's release. The "G1" formulation accelerated drug release by 99.7% at 10 hours. The "G4" formulation had a prolonged drug release of 75.1% at 14 hours. However, "G7" containing 1g of Carbomer 980P released 99.1% after 14 hours. Similar comparisons were done between formulations based on Propylene glycol and glycerol. Gels containing glycerol released the drug more slowly than gels containing the same amount of propylene glycol. As evidenced by G2 and G6, etc. G2 released 99.6% of the drug while G6 only released 91.7%.

ATR Study of Best Gel Formulation

TC comprises three major structural units, which are pyrrole, pyrimidine, and piperidine. The IR peaks were identified and correctly interpreted. A maximum of 1731.26 cm^{-1} owed to C=C stretching and a maximum of 841.45 cm^{-1} owing to C-H stretching. Due to C-N stretching in the pyrrole ring, a strong peak emerged at 1207.00 cm^{-1}. Similarly, a wean peak contributed by N-H stretching in pyrrole emerged at 3497.76 cm^{-1}. At 3367.48 cm^{-1}, a peak emerged in the piperidine ring, which was attributed to N-H stretching. The carbonyl (C=O)

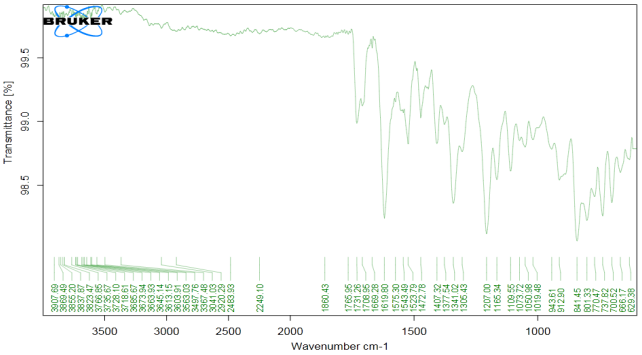

Figure 1: ATR spectra of tofacitinib citrate

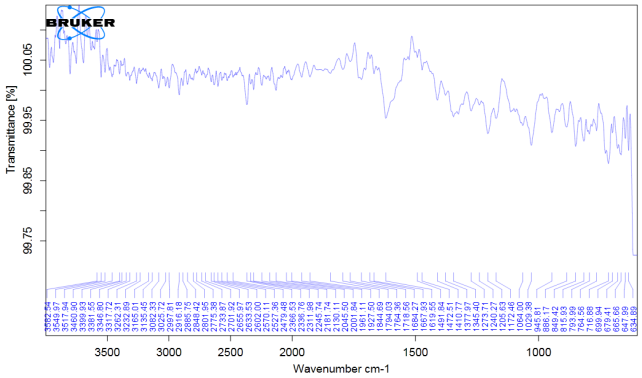

Figure 2: ATR spectra of best gel (G7) loaded with optimized nanoparticles.

group is responsible for the peak at 1619.80 cm^{-1}. In addition, a prominent distinctive peak at 1341.02 cm^{-1} was noticed, as instigated through C-N stretching in the pyrimidine ring is shown in Figure 1.

Characteristic sharp peaks were detected at 1667.93 cm^{-1}, few more characteristic peaks were detected at 2366.53, 1345.40, and 1205.63 cm^{-1} is shown in Figure 2.

DSC Study of Best Gel Formulation

DSC of tofacitinib citrate was shown in Figure 3.

Figure 4 depicts the sharp endothermal peak at 214.17°C was identified by DSC analysis; this value is significantly different from the pure TC as previously reported at 218.05°C. This determined a notable alteration in the endothermal peak.

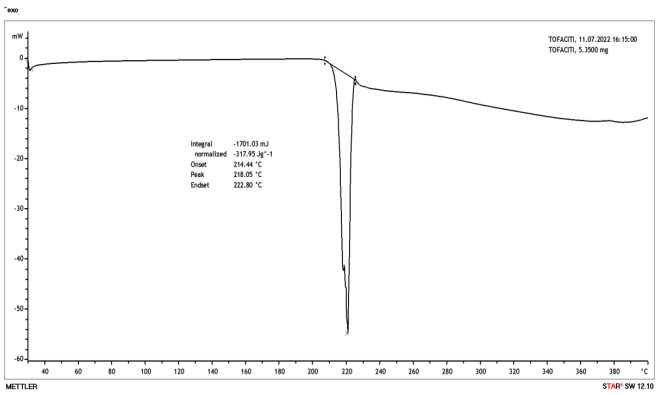

Figure 3: DSC of tofacitinib citrate

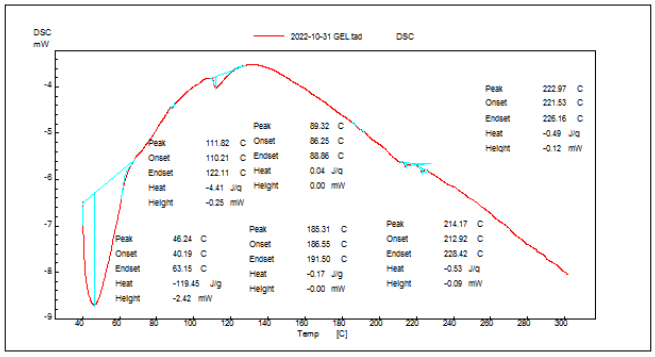

Figure 4: DSC thermogram of best gel (G7) loaded with optimized nanoparticle

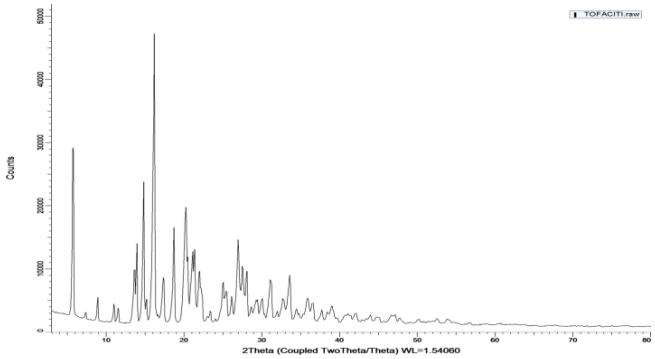

Figure 5: XRD of tofacitinib citrate

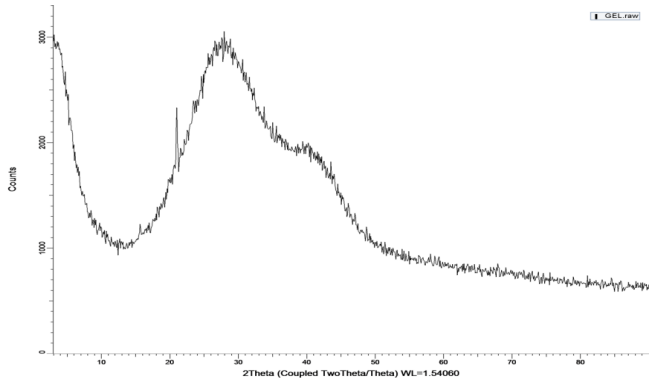

Figure 6: XRD spectra of best gel (G7) loaded with optimized nanoparticle

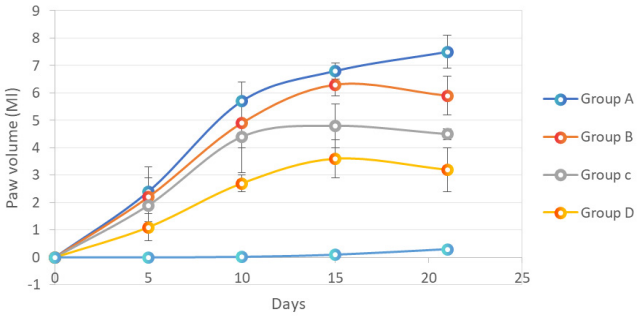

Figure 7: Paw volume in CFA-induced inflammation in all group

Figure 8: Control group of hind paw

XRD Study of Best Gel Formulation

In comparison to pure TC, G7 displayed a larger peak at 30.12 (2Theta) with a 3000 intensity. In addition, a second broad peak of 2000 intensity occurred at 40.28 (2Theta). In the presence of excipients and solvents, the peak's broadness may be due to the finite deposition of the drug crystal as shown in Figure 5.

Figure 6 shows the XRD spectra of best gel (G7) loaded with optimized nanoparticle

In-vivo Study

While performing the study, formulation "G7" successfully reduced inflammation which is estimated as paw volume that CFA induces. The result was further signified from the $p < 0.05$ by one-way ANOVA. Finally, it can be concluded that the best formulation, "G7," successfully treats inflammation associated with arthritis, as highlighted in Figure 7

The control group of hind paw was picturized in Figure 8 and inflammation in hind paw with CFA-treated rats is shown in Figure 9. The CFA-treated paw of rat applied topically with optimized lipid nanoparticles loaded in the gel was shown in Figure 10.

Stability Study of best Gel loaded with Optimized Nanoparticle (G7)

It observed in Table 4, there are no such major changes in the long-term and accelerated stability testing study [63-65]. This ascertained the stability of the best formulation "G7".

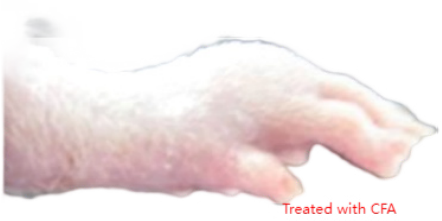

Figure 9: Inflammation in hind paw with CFA-treated rats

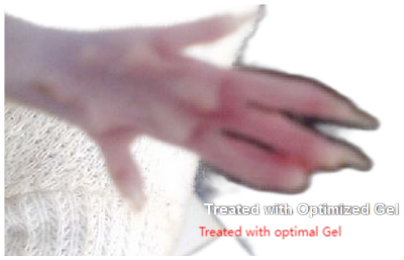

Figure 10: CFA-treated rats applied topically with optimized lipid nanoparticles loaded in the gel

Table 3: One-way analysis of data

Summary of Data						
	Treatments					
	Group'A'	*Group'B'*	*Group'C'*	*Group'D'*	*Control*	*Overall*
N	5	5	5	5	5	25
∑X	22.4	19.3	15.6	10.6	0.42	68.32
Mean	4.48	3.86	3.12	2.12	0.084	2.733
$\sum X^2$	140.74	103.35	66.26	31.7	0.1004	342.1504
Std.Dev.	3.1776	2.6857	2.0969	1.5189	0.1276	2.545

Outcome Details				
Source	*SS*	*df*	*MS*	
Between-treatments	59.3244	4	14.8311	*F* = 3.08592
Within-treatments	96.1211	20	4.8061	
Total	155.4455	24		

The value of the f-ratio is 3.08592. .039367 is the *p-value*. At $p < 0.05$., the finding is significant.

Table 4: Stability study of G7 as per ICH guideline

Long term: 5 ± 2°C/60% RH ± 5%					
Evaluation parameter	*Duration of study (months)*				
	0	*3*	*6*	*9*	*12*
pH	6.7	6.7	6.6	6.5	6.7
Drug content	93.6	92.9	91.6	90.7	90.9
Viscosity	530	528	527	525	520

Accelerated: 0 ± 2°C/75% RH ± 5%			
Evaluation parameter	*0*	*3*	*6*
pH	6.7	6.5	6.5
Drug content	93.6	91.5	88.6
Viscosity	530	519	514

CONCLUSION

Seven topical gel formulations of tofacitinib citrate (TC) were prepared and evaluated for their anti-arthritic activity. The gel was evaluated for pH, drug content, spreadability, homogeneity, *in-vitro* diffusion profile, and *in-vivo* study. It was discovered that the formulation G7, which contains 1.5% Carbopol 980 P, is a potentially effective topical gel for the treatment of arthritis. While performing the *in-vivo* study, formulation G7 successfully reduced inflammation induced by CFA. Further clinical research may enhance the usefulness of this formulation for patients with inflammatory joint disorders.

REFERENCES

1. Taylor P.C., Moore A., Vasilescu R., Alvir J., Tarallo M. A structured literature review of the burden of illness and unmet needs in patients with rheumatoid arthritis: a current perspective. *Rheumatol. Int.* 2016;36(5):685–695.
2. Dorner T., Strand V., Cornes P., Goncalves J., Gulacsi L., Kay J., Kvien T.K., Smolen J., Tanaka Y., Burmester G.R. The changing landscape of biosimilars in rheumatology. *Ann. Rheum Dis.* 2016;75(6):974–982.
3. Ramaiah Maddi, Balaji Maddiboyina, Ramya Krishna Nakkala, Harekrishna Roy, M Akiful Haque, Azmath Farhana, & Swapna S. Exploring the Therapeutic Potential of Probiotics in Fighting Respiratory Viral Infections. Journal of Population Therapeutics and Clinical Pharmacology, 2022; 29(04), 886–900.
4. Fearon U., Canavan M., Biniecka M., Veale D.J. Hypoxia, mitochondrial dysfunction and synovial invasiveness in rheumatoid arthritis. *Nat. Rev. Rheumatol.* 2016;12:385–397.
5. Svendsen A.J., Junker P., Houen G., Kyvik K.O., Nielsen C., Skytthe A., Holst R. Incidence of chronic persistent rheumatoid arthritis and the impact of smoking: a historical twin cohort study. *Arthritis Care Res.* 2016;69(5):616–624.
6. Yang M., Feng X., Ding J., Chang F., Chen X. Nanotherapeutics relieve rheumatoid arthritis. *J. Control Release.* 2017;252:108–124.
7. McInnes I.B., Schett G. Pathogenetic insights from the treatment of rheumatoid arthritis. *Lancet.* 2017;389:2328–2337.
8. Ursini F., Leporini C., Bene F., D'Angelo S., Mauro D., Russo E., De Sarro G., Olivieri I., Pitzalis C., Lewis M., Grembiale R.D. Anti-TNF-alpha agents and endothelial function in rheumatoid arthritis: a systematic review and meta-analysis. *Sci. Rep.* 2017;7:5346.

9. Tanaka Y. Current concepts in the management of rheumatoid arthritis. *Korean J. Intern. Med.* 2016;31(2):210–218.
10. McInnes I.B., Schett G. Pathogenetic insights from the treatment of rheumatoid arthritis. *Lancet* 2017;389:2328–2337.
11. Jones G., Nash P., Hall S. Advances in rheumatoid arthritis. *Med. J Aust.* 2017;206(5):221–224.
12. Bhalekar M.R., Madgulkar A.R., Desale P.S., Marium G. Formulation of piperine solid lipid nanoparticles (SLN) for treatment of rheumatoid arthritis. *Drug. Dev. Ind Pharm.* 2017;43(6):1003–1010.
13. Raj R., Mongia P., Ram A., Jain N.K. Enhanced skin delivery of aceclofenac via hydrogel-based solid lipid nanoparticles. *Artif. Cells Nanomed. Biotechnol.* 2016;44(6):1434–1439.
14. Garg N.K., Singh B., Tyagi R.K., Sharma G., Katare O.P. Effective transdermal delivery of methotrexate through nanostructured lipid carriers in an experimentally induced arthritis model. *Colloids Surf. B Biointerfaces.* 2016;147:17–24.
15. Kumar LA, Pattnaik G, Satapathy BS, Swapna S, Mohanty D. Targeting to Brain Tumor: Nanocarrier-Based Drug Delivery Platforms, Opportunities, and Challenges. J Pharm Bioallied Sci. 2021;13(2):172-177.
16. Singh C, Yashwant, Gupta AK. Formulation and the Study of Finished Products Used for Anginal Disease. International Journal of Pharmaceutical Quality Assurance. 2022;13(4):385-388. DOI: 10.25258/ijpqa.13.4.07