FORMULATION AND EVALUATION OF TOPICAL NANOGEL CONTAINING GRISEOFULVIN FOR THE TREATMENT OF FUNGAL INFECTION

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ABSTRACT

The term" Nanogel" refers to a hydrogel nanoparticle with a network of cross-linked hydrophilic polymers. The present investigations were aimed at formulating topical gel containing nanogel of griseofulvin and assess its effectiveness on superficial infections. Nanogel pharmaceutical formulations are gaining popularity because of their ability to serve both as hydrophobic and hydrophilic and as a gel. These products are well-known for their ease of use, spreadability, controlled release, and ability to hydrate dry skin. Natural essential oils have been shown to promote the cutaneous permeability of topical formulations, enhancing medication safety and efficacy. Furthermore, the physicochemical properties of the optimized nanogel formulation were characterized by techniques such as Fourier transform infrared (FT-IR) spectroscopy. Herein, we developed NEG for the enhanced permeation of Griseofulvin against candidiasis. Nanogels are made up of cross-linked polymer that expand in a suitable detergent. Several hydrophilic functional groups in hydrophilic polymers enable nanogels to absorb large amounts of water. Incredibly stable, adaptable, and responsive to sensory influences, nanogels have the potential to be a safe and effective medication delivery vehicle for biological products. Peptides, hormones, DNA, vaccines, chemotherapeutic treatments, and a wide range of pharmaceutical goods can all be delivered using these devices.

KEYWORDS: *Nanoparticles, Nanogel, Nanotechnology, polymer*

INTRODUCTION

Nanotechnology, a unique technique, opens up a plethora of opportunities for drug production and delivery (nanomedicine) approaches that include the characterization, synthesis and design of molecules or materials, as well as devices, with effective function at the nanometre scale. The primary goal of this technique is to improve current therapeutic and diagnostic procedures.[1]

As an advanced DDS, various nanotechnological techniques such as protein-based nanoparticles, lipid-based nanoparticles, Nano emulsions, nanocrystals, nanodiamonds, carbon nanotubes, nanosuspensions, and Nanogels have been introduced, with Nanogels being the most advantageous over other DDS techniques.[2]

When Nanogels are dispersed in aqueous media, their swollen networks soften and are able to encapsulate the required volume of water. By allowing the formation of spontaneous interactions between the polymer matrix and the agents, desired biological or drug molecules can be loaded into the Nanogels, resulting in the formation of highly dispersed hydrophilic particles. This resulting structure is capable of protecting the desired loaded biomolecule from degradation. As a result, Nanogels are a versatile structure for drug encapsulation as well as drug controlled release at the target site.[3]

Nanogels were demonstrated to be a promising structure for systemic drug release, the design of multifunctional nanocarriers such as controlled drug release at the target site during the first decade of their development. Because of the large surface area and adjustable size of Nanogels, these molecules can incorporate a variety of molecules.[4]

Fungi are the most common microbial agents responsible for the prevalence of skin diseases globally. Pathogenic fungi dwell in hair, nails, epidermis, and mucosa causing superficial fungal infections. The three most common superficial infections are Dermatophytosis (tinea or ringworm), Pityriasis versicolor, and Candidiasis. [5]

There are several antifungal medications that are previously generated and proven themselves to be effective in killing the superficial fungi, but they have failed in providing the required therapeutic effect due to their poor aqueous solubility and permeability. As a result, fresh and more advanced antifungal therapeutic alternatives are desperately needed. Griseofulvin is a heterocyclic benzofuran found in the Penicillium. It belongs to BCS class II medication.[6,7]

Topical management of fungal infections has various benefits like fewer side effects, site-specific administration, high patient compliance, and effective cure. Antifungal treatment tactics have become increasingly complex and expensive due to a lack of effective medications and a rising proportion of drug resistance.[7] There have been a lot of recent findings on topical formulations of griseofulvin to increase penetration through skin by dissolving the agent in a variety of vehicles, use of new carrier systems, and more recently, by using penetration enhancers. The topical delivery of antifungal agents into the skin is improved by new drug delivery technologies such as vesicular carriers, colloidal systems, and nanoparticles. Therefore, it seems that the pharmacy world will benefit from the hydrophilicity, flexibility, versatility, high water absorptivity, and biocompatibility of these particles and all the advantages of the NPs, mainly long life span in circulation and the possibility of being actively or passively targeted to the desired biophase. [8]

Material and method

SR.NO	CHEMICAL NAME
1	Sodium alginate
3	Carbopol 940, glycerol, oleic acid
4	Triethanolamine
5	Hydroxyl methyl propyl cellulose
6	Methyl paraben
7	Propylene glycol
8	Eudragit S-100

Table 1: materials used

List of equipment used

SR.NO.	EQUIPMENT	MANUFACTURER
1	PH. METRE	(Systronics MK VI, Mumbai).
2	FTIR	(Shimadzu DR-8031, Japan)
3	DIGITAL BALANCE	Excell
4	UV	UV1800 SHIMADZU JAPAN
5	SONICATOR BATH	Leela Sonic

Table: 2

METHODOLOGY

Appearance

Griseofulvin appears as white to pale cream-colored crystalline powder. Odourless or almost odourless, Tasteless.

Homogeneity

The NEG homogeneity was inspected visually by placing it in a settled position in a container. It was of uniform consistency and homogeneous, with no aggregates. Homogeneity by visual examination done for all developed nanogel has remained to set in the beaker. Nanogels were tested for physical properties and presence of any particle.[9]

Estimation of melting point

For determination of melting point USP method was followed. Small quantity of drug was placed into a sealed capillary tube. The tube was placed in the melting point apparatus. The temperature in the apparatus was gradually increased and the observation of temperature was noted at which drug started to melt and the temperature when the entire drug gets melted. [10,11]

Result

Observed Melting point of drug: 218-220 °C

Preparation of Griseofulvin nanogel

Accurately weighed quantity of Drug(griseofulvin), Eudragit S-100 (polymer), and Tween-80 as stabilizer are dissolved in glycerol while stirring to prepare transparent drug solution. Prepared aqueous phase containing HPMC K15M or Carbopol-940 dissolved in water with continuous stirring and heat. Mixing the penetration enhancer Oleic acid or propylene glycol with sodium alginate. These drug containing phase is sonicated on Ultra sonic bath. The drug phase is added drop by drop into the aqueous phase during homogenization to form emulsion. The emulsion converted into nanodroplets by homogenizer which formed O/W emulsion. Homogenization was continued for one hour. Triethanolamine added to form the gel and to maintain pH with continuous stirring to nanogel and methyl paraben as preservative. Different Batches was prepared at highest rpm (5000- 8000) using homogenizer respectively.

Actual drug content

Precisely weighed equivalent quantity (10 mg) of nanogel containing drug was kept in 100 ml of phosphate buffer pH 7.4 solution for an hour with continuous stirring. Filtered samples were further analysed at 273 nm next to blank using UV-visible spectrophotometer. [12,13]

Actual Drug content (%) = (Nact/Nms) ×100

Where Nact =actual Griseofulvin content in weighed quantity of nanogel, Nms = weighed quantity of nanogel

Result

10mg of formulated nanogel was diluted with pH 7.4 phosphate buffer saline and the mixture was filtered through the membrane filter.

Obtained drug content is 98.93% - 101.56%

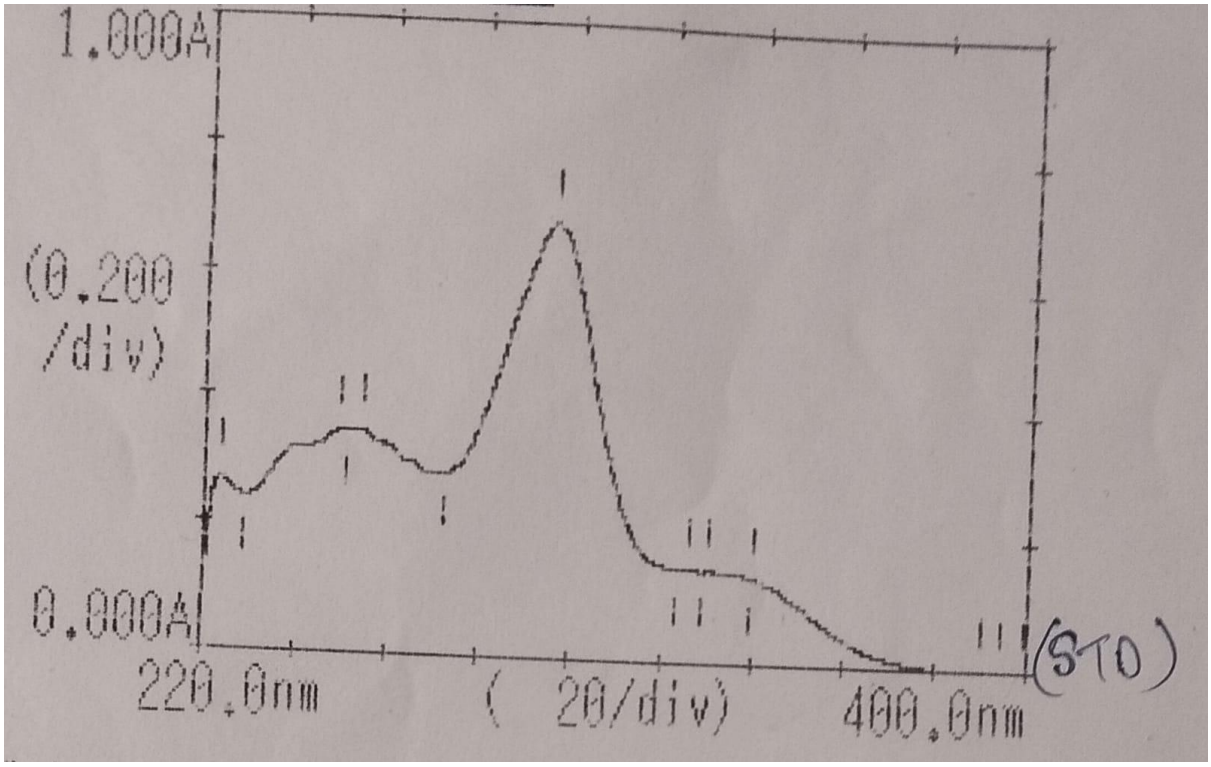

Figure: 1 (UV Peak of Griseofulvin)

Entrapment efficiency (EE)

Nanogel was liquefied insolvent using a magnetic stirrer for 30 min. The resultant dispersion was centrifuged (3-18 K, Sigma) at 10,000 rpm for 10 min and the drug content present in the supernatant was analyzed at λmax 293 nm using UV spectroscopy (UV-1800, Shimadzu, Japan). The entrapment efficiency was calculated using under mentioned equation.[14]

EE (%) = Weight of drug in nanogel − Free drug Weight of drug in nanogel ∗ 100

RESULT

Entrapment efficiency also is considered an important parameter as its understanding prevents the wastage of the drug (Singh et al., 2021). The entrapment efficiency is found to be 85.24%. During the optimization process, good entrapment efficiency was achieved starting from 37% to 89%. From the results obtained it was found that an increase in the concentration of oleic acid and sonication time increased the entrapping capacity. Griseofulvin nanogel showed a drug content of 90.12 ± 4.6% w/v. It was also noticed that on increasing the lipid portion relative to the drug, the amount of drug entrapped into the lipid matrix and hence entrapment efficiency increased significantly. However, on an increasing amount of liquid lipids, the solubility of drugs and hence, the efficiency of drug entrapment increased comprehensively.

In-vitro drug release

Franz diffusion cell of 20 ml capacity was used for the in-vitro drug release study with Phosphate buffer solution of pH of 7.4 in the receptor compartment and 0.5 gm prepared gel in the donor compartment. The gel was mixed using a magnetic stirrer and the temperature was maintained at 37 ˚C. The sample was withdrawn at regular intervals, and the sink condition was maintained with phosphate buffer solution. UV spectroscopy was used to analyse the samples at 293nm. [15,16]

Result

The initial rapid in-vitro drug release might be due to surface release followed by a slow release from the solid lipid core of NLCs. The release profile of the optimized NLC, dispersion, and topical marketed product of investigated drug were studied comparatively as represented in Fig. 1 and was observed that there was a relatively faster release than the NLC gel and marketed formulation. The drug release from NLCs loaded topical gel was higher (80 ± 0.87%) and faster than that of commercial cream (50.2 ± 0.69%) which might be due to the involvement of oils that enhance the solubilization, and drug release. There was an initial burst release noted which might be attributed to entrapment of griseofulvin superficially as a result of sudden cooling from high temperature.

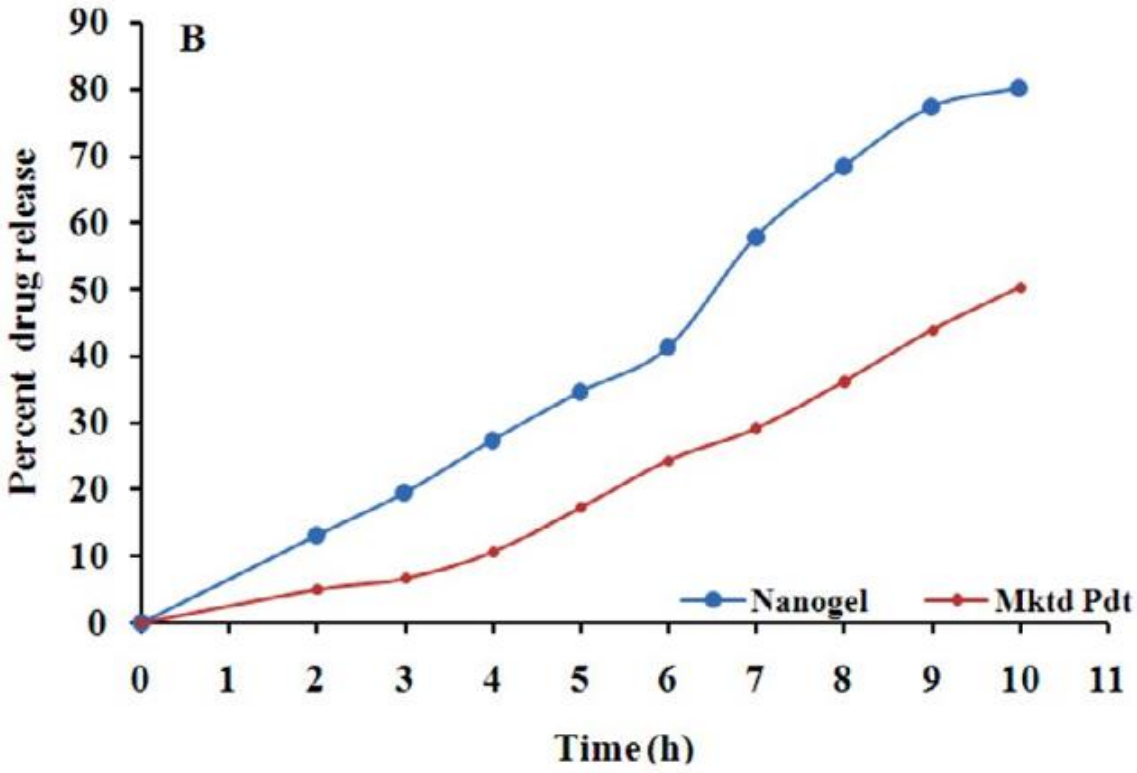

Figure: 2

Stability Study

NLCs Stability studies were performed for one month by exposing the finished product to storage conditions at a temperature of 25 ± 20C and relative humidity of 60% and refrigerated temperature of 6 0C in a well- sealed amber colored glass bottle and kept in a stability chamber. After storing the samples in temperatures, they were checked for any physical changes mainly like aggregation or separation by visual observation. [17,18]

Result

Storage stability tests were performed for an improved formula for NLCs loaded with antifungal gel at a temperature of approximately 25 ± 2°C and in a refrigerator close to 4 ± 2°C. Initially, visual experiments did not show aggregation or separation during the study period.

The results obtained at room temperature clearly showed that the particle size and polydispersity index at the end of the month study reached 113.60 ± 4.15 nm and 0.188 ± 0.02, respectively. But the EE decreased to -40.64 ± 0.51 mV and 86 ± 4.89%, respectively. However, in frozen conditions, small changes were observed in medium size particles and encapsulation efficiency, while PS and PDI appear to increase slightly at 243.80 ± 5.25 nm and 0.263 ± 0.03, respectively. This suggested that the nanogel stored in the refrigerator or room temperature was stable and did not mix or blend during storage in both previous conditions.

FTIR Spectroscopy

Chemical characterization of Griseofulvin, PEG 6000 and microwave irradiated solid dispersion in terms of functional group, structure, and interaction b/w drug & polymer was evaluated by an FT-IR spectrophotometer. FT-IR spectra were obtained in the range of 400-4000 cm $^{-1}$ [19]

Result

The powdered mixture of Griseofulvin and KBr was taken in a sampler and the spectrum was recorded using FTIR spectrophotometer. The principle peaks were obtained at wave number 2418 cm-1 for C=C stretching.

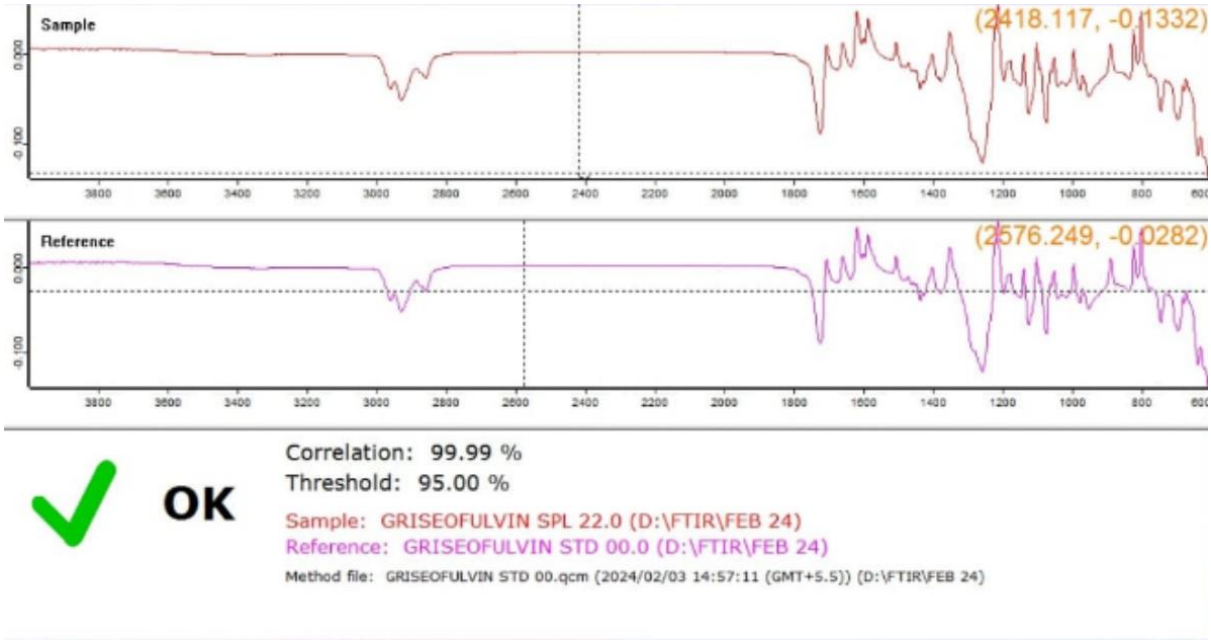

Figure: 3 (FTIR of Griseofulvin)

Conclusion

The study concludes the effect of improved stability of NLCs on increasing the liquid lipid to solid lipid ratio was least when the ratio of solid lipid was increased. The sonication time and ratio of liquid and solid lipids affect the particle size. The lipid peroxidation test of NLCs showed reduced lipid peroxidation after treatment with griseofulvin- loaded nanogel indicating better control of fungal infection than the marketed formulation which might be due to the significant decrease in the level of malondialdehyde.

A significant improvement was shown in a group that received griseofulvin loaded nanogel as compared to standard treatment suggesting that the formulation of griseofulvin-loaded nanogel increased the antioxidant efficacy of the drug and therefore enhancement of the antifungal efficacy of the drug in nanogel formulation. The research proved the therapeutic potential of griseofulvin- loaded nanogel for topical delivery against superficial infections as per preclinical investigations. It revealed the prospective of the nanogel in the treatment of superficial infections like ringworm and tinea pedis consequently, it could be an effective alternative for currently existing products.

REFERENCES

1. He Q, Liu J, Liang J, Liu X, Li W, Liu Z, et al. Towards improvements for penetrating the blood– brain barrier—recent progress from a material and pharmaceutical perspective. Cells, 2018; 7(4): 24

2. Neamtu I, Rusu AG, Diaconu A, Nita LE, Chiriac AP. Basic concepts and recent advances in nanogels as carriers for medical applications. Drug Deliv, 2017; 24(1): 539–57

3. Jensen LB, Griger J, Naeye B, Varkouhi AK, Raemdonck K, Schiffelers R, et al. Comparison of polymeric siRNA nanocarriers in a murine LPS-activated macrophage cell line: gene silencing, toxicity and off-target gene expression. Pharm Res, 2012; 29(3): 669–82

4. Jain N, Jain R, Thakur N, Gupta BP, Jain DK, Banveer J, et al. Nanotechnology: a safe and effective drug delivery system. Asian J Pharm Clin Res, 2010; 3(3): 159–65.

5 Arida, A. I., Al-Tabakha, M. M., & Hamoury, H. A. J. (2007). Improving the high variable bioavailability of griseofulvin by SEDDS. Chemical and Pharmaceutical Bulletin, 55 (12), 1713–1719. 10.1248/cpb.55.1713

6. Arnold, T. M., Dotson, E., Sarosi, G. A., & Hage, C. A. (2010). Traditional and emerg- ing antifungal therapies. Proceedings of the American Thoracic Society, 7 (3), 222–228. 10.1513/pats.200906-048al

7. Schmitt F, Lagopoulos L, Käuper P, et al. Chitosan-based nanogels for selective delivery of photosensitizers to macrophages and improved retention in and therapy of articular joints. Journal of Controlled Release. 2010; 144:242-50.

8. R. Gref, C. Amiel, K. Molinard, New self assembled nanogels based on host guest interactions: characterization and drug loading. Journal of control release, 2006; 111: 316-324.

9. Tomar S, Singhal T. Pre-formulation studies of niosomal gel of prednisolone and azithromycin for topical drug delivery system. J Innov Pharm Biol Sci 2015;2 Suppl 3:312-21.

10. Kumar S, Talegaonker S, Negi LM, Khan ZI. Design and development of ciclopirox topical nanoemulsion Gel for the treatment of subungal onycomycosis. Indian J Pharm Educ Res 2012;46 Suppl 4:303-10.

11. El-Feky GS: In-vitro in-vivo evaluation of oral indomethacin-hp-β-cyclodextrin loaded chitosan nanoparticles. International Journal of Pharmacy and Pharmaceutical Sciences 2013; 5(4).

12. Anasuya Patil, Pranoti kontamwar. Formulation and Evaluation of Antifungal nanogel for topical drug delivery system. Asian Journal of Pharmaceutical and Clinical Research, 2021;14(10):127-134. 10.22159/ajpcr.2021.v14i10.42436.

13. Muniraj S N, Yogananda R, Nagaraja T S, Bharathi D R. Preparation and Characterization of Nanogel Drug Delivey System Containing Clotrimazole an anti fungal drug. Indo American Journal of Pharmaceutical Research, 2020;10(7):24-31. 10.528/zenodo.3970394.
14. Hu, F.-Q., Jiang, S.-P., Du, Y.-Z., Yuan, H., Ye, Y.-Q., & Zeng, S. (2006). Preparation and characteristics of monostearin nanostructured lipid carriers. International Journal of Pharmaceutics, 314 (1), 83–89. 10.1016/j.ijpharm.2006.01.040
15. Sanad, R. A., AbdelMalak, N. S., & Badawi, A. A. (2010). Formulation of a novel oxybenzone-loaded nanostructured lipid carriers (NLCs). AAPS Pharm SciTech, 11 (4), 1684–1694 doi.org/10.1208/s12249-010-9553-2.
16. Chen, Y., Zhou, L., Yuan, L., Zhang, Z., Liu, X., & Wu, Q. (2012). Formulation, characterization, and evaluation of in vitro skin permeation and in vivo pharmacodynamics of surface-charged tripterine-loaded nanostructured lipid carriers. International Journal of Nanomedicine, 7 , 3023. 10.2147/ijn.s32476.
17. Anwar, W., Dawaba, H. M., Afouna, M. I., Samy, A. M., Rashed, M. H., & Abde- laziz, A. E. (2020). Enhancing the oral bioavailability of Candesartan Cilexetil loaded nanostructured lipid carriers: In vitro characterization and absorption in rats after oral administration. Pharmaceutics, 12 (11), 1047. 10.3390/pharmaceutics12111047 .
18. Patil SM, Patrick E, Maibach HI. Animal, human, and in vitro test methods for predicting skin irritation. In: Marzulli FN, Maibach HI, editors. Dermatotoxicology. 5th ed., Ch. 31. New York: Taylor and Francis; 1996. p. 411-36.
19. Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, et al. PubChem in 2021: New data content and improved web interfaces. Naproxen Sodium Res 2019;47:D1388-95.