

Lawsonone co-crystals loaded antifungal gel for cutaneous candidiasis

ABSTRACT

Background: An attempt was made to improve the solubility and to achieve better penetration of antifungal agent lawsone a poorly water-soluble naphthoquinone, derivative via co-crystallization to treat cutaneous candidiasis. The co-crystals of lawsone were prepared using benzoic acid as co former by solvent crystallization method. The formulated co-crystals were assessed for different parameters namely, saturation solubility, thermal properties, crystalline nature, particle size and its antifungal activity against *Candida albicans*.

Results: Lawsonone co-crystals exhibited enhanced solubility of lawsone (9.57 ± 2.5 mg/ml and 0.523 ± 0.23 mg/ml respectively) and the particle size of the co-crystals were reduced to 560 ± 2.2 nm as compared to pure lawsone (2478 ± 1.5 nm) which further resulted in enhancement of antifungal activity. Lawsonone co-crystals were loaded in the xanthan-gel base for easy applicability to the skin and to achieve patient compliance. The prepared gel was evaluated in terms of spreadability, adhesiveness, *in vitro* diffusion, viscosity, and antifungal activity.

Conclusion: Lawsonone co-crystals loaded gel showed enhanced retention of drug in the skin as compared to plain lawsone gel. The antifungal potential of lawsone co-crystals loaded gel was at par with marketed Clotrimazole gel formulation. The short-term stability study carried out as per ICH guideline indicated that the formulation was stable. Lawsonone co-crystals loaded gel could be a potential approach over the synthetic antifungal agent for the treatment of cutaneous

KEYWORDS:Co-crystals, topical candidiasis, lawsone, benzoic acid, *Candida albicans*

Keywords: Co-crystals, topical candidiasis, lawsone, benzoic acid, *Candida albicans*

1. INTRODUCTION

Fungi forms an important part of commensal skin microbiota, but the study reports suggests that some of its species are pathogenic. Around 20–25% of the world, population is affected by fungal skin infections [1]. Many synthetic agents are available to treat cutaneous candidiasis, but the problem of growing microbial resistance and the side effects associated with them have limited their use [2]. Hence, the focus is changed from the synthetic treatment to the use of medicinal plants to treat the ailments of disease.

Lawsone (2-hydroxy-1,4-naphthoquinone) belongs to the family Lythraceae. Lawsone is reported to have an antidiabetic, immunomodulatory effect. It is also hepato protective and has antioxidant, antibacterial, antifungal, antiviral, antidermatophytic, tuberculostatic, cytotoxic, enzymes inhibitory, nematicidal, anticoagulant and wound healing effect [3]. According to Rahmoun and co-workers, lawsone exhibits strong fungitoxicity due to hydroxynaphthoquinone structure [2]. Many compounds with naphthoquinone structure have reported to have antifungal activity [4]. However, lawsone suffers from the disadvantage of poor solubility, poor permeability and low bioavailability, which limits its use. The formulation of lawsone co-crystals would definitely serve as a suitable approach to improve the biopharmaceutical

properties of lawsone. The functional groups present in lawsone namely C=O and O-H makes it a suitable candidate for forming cocrystals through hydrogen bonding [5]. Earlier microemulsion of lawsone for effective transdermal delivery [6] SMEDDS of lawsone loaded in hollow pessary for vaginal candidiasis [7], lawsone loaded niosomes) were formulated to improve the physicochemical properties of lawsone [8].

Pharmaceutical co-crystals are non-ionic supramolecular complexes serving as one of the best and easiest approach to improve the physicochemical and biopharmaceutical properties of active pharmaceutical ingredient (API) without actually changing its chemical composition [9]. The API along with the co-former forms the basic component of co-crystals. Any excipient or API when combined with a drug improves the efficacy of the drug is a co-former. Benzoic acid was used as a co-former to prepare the cocrystals of lawsone due to its nontoxic nature and the presence of carboxyl group in its structure, which can readily form co-crystal with different drug [10].

Hence, the current research work was intended to formulate lawsone co-crystals by simple solvent evaporation method to improve the physicochemical properties of lawsone as well as increase its antifungal activity. The lawsone co-crystals were further loaded in gel base, thus helping to achieve easy application of the formulation to the skin to treat cutaneous candidiasis. The increased solubility of lawsone due to development of its co-crystals reduced its dose needed for antifungal activity. In addition, it promoted increased dermal absorption with the highest percentage of drug retained into the skin at the target site. Thus, the co-crystals of lawsone-loaded gel improved the patient compliance by reducing the burden on synthetic antifungal agents as well as avoiding exposure to this synthetic drug.

2. METHODS

2.1 MATERIAL

Lawsone was purchased from Alfa Aesar, India. Benzoic acid was procured from Fisher scientific. Clotrimazole was procured from Merck Pharmaceuticals and Chemicals Pvt.Ltd. Ahmedabad, India and Sabouraud dextrose agar was purchased from Hi-media. All other chemicals used were of analytical grade.

2.2 METHOD

Lawsone co-crystals were prepared by solvent crystallization method. The co-former benzoic acid and lawsone (1:1) were dissolved in methanol followed by cooling the solution in an ice bath for a period of 8h. The crystals formed were filtered through the membrane and complete drying of the crystals was carried out at room temperature for 24 h. The dried crystals were packed in an airtight container and stored in a desiccator until, for further use. The solubility of drug was checked by increasing ratio of co-former.

2.2.1. Saturation solubility study

The saturation solubility of lawsone co-crystals was carried out as per the method suggested by Higuchi and Connors [11]. An excess quantity of lawsone co-crystals were placed in the vials containing 10ml of distilled water. The vials were stirred in incubator shaker (100 stir/min) for 24 h at room temperature. Thereafter, the solution was filtered using a membrane (0.45 μ m). The amount of the drug dissolved was analysed by UV spectrophotometer at the wavelength of 452nm. The study was performed in triplicate and the solubility was calculated in g/ml.

2.2.2. Antifungal assay

In vitro antifungal assay of lawsone and co-crystal against *Candida albicans* was carried out using cup plate method [12]. The test solutions were prepared in dimethyl sulfoxide. Sabouraud Dextrose agar plates were used for the study. The suspension of test organism (*Candida*

albicans) was freshly prepared in 1 mL distilled water with the turbidity of microbial suspension adjusted equivalent to 0.5Mc Farland. About (100mL) of the microbial suspension was then spread on Sabouraud Dextrose agar petri plates. After stabilization of culture, cups (6mm) diameter were bored on the agar plates using a sterile cork borer. About 1ml of co-former (500µg/ml), lawsone (500µg/ml) and co-crystal compound solution (500µg/ml) was transferred into each cavity. The plates were incubated at $37 \pm 5^\circ\text{C}$ and the zone of inhibition was measured at the end of 48 h using zone reader. The evaluation for the antifungal activity was repeated three times using three plates for each formulation.

2.2.3. Fourier Transform Infrared Spectroscopy

To investigate any possible interaction between the drug and excipients, Fourier transform infrared (FTIR) spectra of samples were recorded on FTIR instrument (Alpha Brucker, Germany). FTIR spectra of pure drug lawsone and the co-crystals of lawsone were scanned over a range of $4000\text{-}400\text{ cm}^{-1}$.

2.2.4. Drug content

Co-crystals (10mg) were dissolved in methanol (10 ml) and shaken for 15 min. This solution was filtered through membrane ($0.45\mu\text{m}$). The quantitative determination of lawsone in co-crystals was carried out using a linear model UV absorbance detector using double beam UV spectrophotometer (1800, Shimadzu, Japan) at 452 nm. The study was carried out in triplicate.

2.2.5. Mean Particle size

Particle size distribution of co-crystal was performed by using laser diffraction technique (Malvern instruments Ltd.) at 50X. An appropriate amount of co-crystals was dispersed in distilled water and the average particle size was estimated. The study was carried out in triplicate.

2.2.6. Differential Scanning Calorimeter

The thermal properties of lawsone and its co-crystal formulations were studied by using differential scanning calorimetry (Lab Mettler Star Sw 12.10). Approximately (3-5 mg) sample was hermetically sealed in an aluminium pan and heated at a constant rate of 10°C/min over a temperature range 25–300°C in an inert atmosphere maintained by filling nitrogen gas at a flow rate of 50mL/min.

2.2.7. Scanning electron microscopy

Scanning electron microscopy (JEOL oxford JSM 630A scanning electron microscope) was used to study the surface morphology of lawsone co-crystals at magnification 100X and 1000X. Prior to analysis, coating of the co-crystals with gold–palladium under an argon atmosphere at room temperature was done.

2.2.8. X-ray crystallographic studies

The crystalline properties of co-crystals were analysed by X-ray diffraction (XRD) using an X-ray diffractometer. The samples were exposed to monochromatized Cu K α radiation (1.542Å) and analysed at 2 θ between 5°-50°. The voltage and current used for analysis were 30kV and 30mA respectively [13].

2.2.9. Formulation of gel

The xanthan-gel base (2% w/w) was used for the formulation of gel. Xanthan gum (2g) was dispersed in 100 ml of distilled water and soaked for 4 h. The solution was then agitated for a specific period at 600 rpm until a uniform dispersion was obtained. Propylene glycol (5ml), methyl paraben (0.1g) and propyl paraben (0.05g) was added into distilled water. Finally, the appropriate amount of lawsone co-crystals were added to the gel base to get 2% w/w of lawsone calculated based on drug content.

2.3. Evaluation of co-crystal loaded topical gel

2.3.1. Physical evaluation

Formulations were evaluated for organoleptic characteristics, visual appearance, odour, color, texture, and washability [14].

2.3.2. Measurement of pH

The pH of lawsone loaded co-crystal gel was evaluated with digital pH meter (Systronics Instruments, India). The electrode was immersed in the gel and the readings were recorded using pH meter. The estimation was carried out for three times and observations were recorded as mean [15].

2.3.3. Measurement of viscosity

Viscosity measurements were carried using Brookfield viscometer (Brookfield Engineering Corporation, USA) by using spindle number 64 at 10 rpm. Prior to analysis, the gel was allowed to set in a beaker (50ml) and then the spindle groove was dipped and dial reading was determined after three minutes to calculate the viscosity of gel. The estimation was carried out for three times and observations were recorded as mean [16].

2.3.4. Drug content

Gel (10g) was dissolved in 100 ml of methanol and stirred for 30 min. The solution was allowed to stand for 24 h and filtered through membrane filter (0.45 μ m). The absorbance of the solution was measured spectrophotometrically at 452 nm. The Placebo gel solution was used as reference.

2.3.5. Texture profile analysis

Texture profile analysis of gel was performed using a CT3 Texture Analyzer (Brookfield) in compression mode by using spreadability accessory (TA-BT-KIT). Optimized gel formulation was filled into the female probe, taking care to avoid air pocket into the samples followed by inserting a conical analytical male probe (35 mm diameter of 45°) into each sample at a defined rate (1 mm/s) and to a defined depth (10 mm). At least two replicate analysis of samples were performed to calculate the spread ability, hardness and adhesiveness of the gel [16].

2.3.6. Homogeneity and grittiness

A small quantity of gel was pressed between the thumb and the index finger. The homogeneity of the gel was evaluated based on appearance of any grainy particles on fingers. In addition; the homogeneity can be detected by rubbing a small quantity of the gel on the skin of the dorsal side of the hand. In the same way, the grittiness of prepared gel was evaluated [17].

2.3.7. In vitro antifungal study

The antifungal activity of the lawsone loaded co-crystal gel (2% w/v) and marketed clotrimazole gel (2% w/v) was assessed by cup plate method to evaluate the effectiveness of lawsone loaded co-crystal gel in comparison to clotrimazole gel.

2.3.8. Ex-vivo diffusion study

Ex-vivo release study was carried out using Franz diffusion cell. Goatskin (used as membrane) was soaked in receptor media (acetate buffer, pH 6.8) for 24 h prior to the experiment. A specific quantity of gel -- Lawsone co-crystals loaded gel and plain lawsone gel without co-crystals were used individually to carry out the diffusion studies. The gels were applied on the surface of the goat skin placed on the donor compartment and the diffusion pattern of each gel was studied individually. The receptor compartment contained 20 ml of acetate buffer, pH 6.8 maintained at

a temperature of $37\pm 0.5^{\circ}\text{C}$ and agitated at 25 rpm. The amount of drug diffused in the receptor compartment was assessed by withdrawing specific amount of sample (2.5ml) at definite time interval and replacing it with the same volume of fresh acetate buffer to maintain the sink condition. The withdrawn samples were analysed by UV spectrophotometer with proper dilutions. In addition, the retention of higher amount of drug in the skin is important for killing the fungus. To evaluate the amount of drug retained in the skin, the goatskin was cut into the small pieces and soaked in 10ml of methanol for 24h. After 24h the solutions was centrifuge and then filtered through Whatmann filter paper. The sample was analysed by UV spectrophotometer and the % drug content (retained) in skin was calculated [18].

2.3.9. Stability study

The stability study of lawsone loaded co-crystal gel was performed as per International Conference on Harmonization (ICH) guideline. The formulation was kept in the stability chamber (Thermolab, India) at $25 \pm 2^{\circ}\text{C}/60 \pm 5\% \text{RH}$, and $40 \pm 2^{\circ}\text{C}/75 \pm 5\% \text{RH}$ for a period of 3 months. The samples were assessed for physical appearance, pH and drug content at specific time interval of 15, 30, 45, 60, 90 days.

3. RESULTS

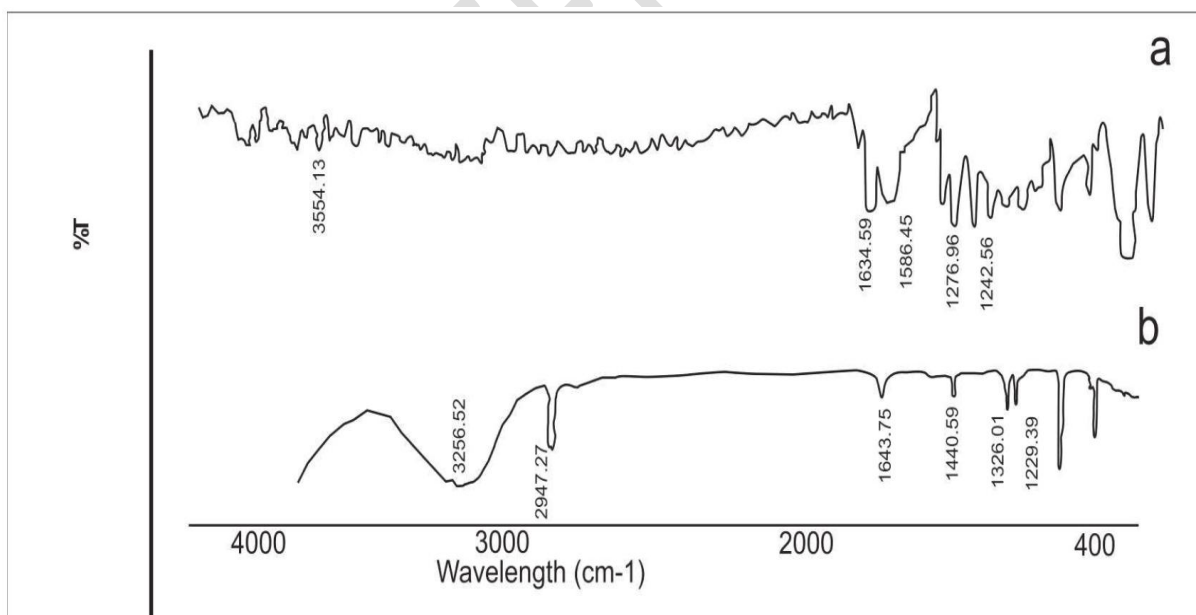
3.1. Saturation solubility study

The results of saturation solubility studies indicated that the prepared lawsone co-crystals have increased solubility than pure drug alone. The saturation solubility of F1 (co-crystal of lawsone with benzoic acid as co-former in the ratio of 1:1) exhibited enhanced solubility in water which was found to be $9.57\pm 2.5\text{mg/ml}$, whereas the pure drug exhibited solubility of $0.523\pm 0.23\text{mg/ml}$. Literature reveals that increase in the concentration of co-formers do have significant influence on the solubility of the drug due to the theory of solubility complexation

[19]. Therefore, the ratio of drug and co-formers (1:1) were solely selected for the formation of co-crystals.

3.2. Fourier Transform Infrared Spectroscopy

The samples were scanned in the region of 4000–400 cm^{-1} for FTIR studies. The IR spectrum of lawsone and the co-crystal was compared to confirm the formation of hydrogen bond between the two components. Pure lawsone showed characteristic peaks at 3554 cm^{-1} corresponding to phenolic O-H involving hydrogen bonding. The C = C stretching of benzene ring at 1586 cm^{-1} corresponds to aromatic group of lawsone. The acid C-O stretching at 1276 cm^{-1} and O-H bending & C-O stretching at 1242 cm^{-1} were also observed in the FTIR spectrum of pure lawsone as shown in Figure 1. In co crystal of lawsone a new peak appeared at 2947 cm^{-1} , the keto group was shifted to higher wavelength 1634 cm^{-1} indicating the formation of hydrogen



bond between pure lawsone and co-former.

Figure 1 Fourier transform infrared spectroscopy of lawsone, co-crystals formulation

3.3 Differential Scanning Calorimeter

The DSC thermogram of lawsone exhibited a sharp melting endotherm at 200.4°C. Lawsone co-crystals exhibited an endotherm peak at 229.58°C Figure 2.

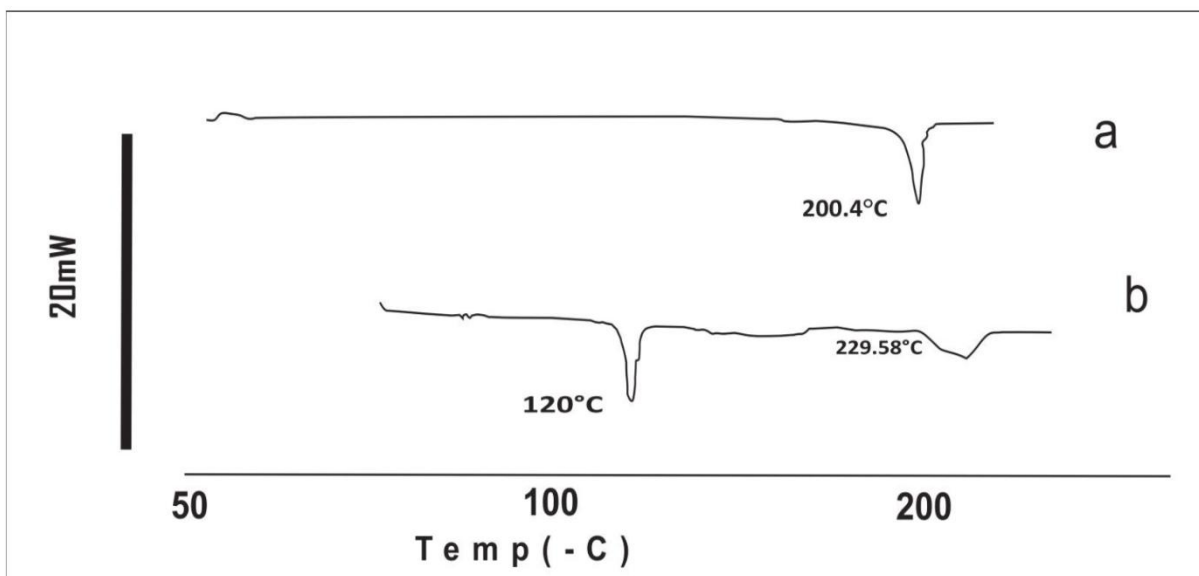


Figure 2 Differential scanning calorimetry of lawsone co-crystals

3.4 Scanning electron microscopy

The scanning electron microphotograph showed crystalline nature of co-crystals with a narrow particle size. Fig.3 (A) shows the co-crystals at 100x magnification and (B) at 1000x magnification.

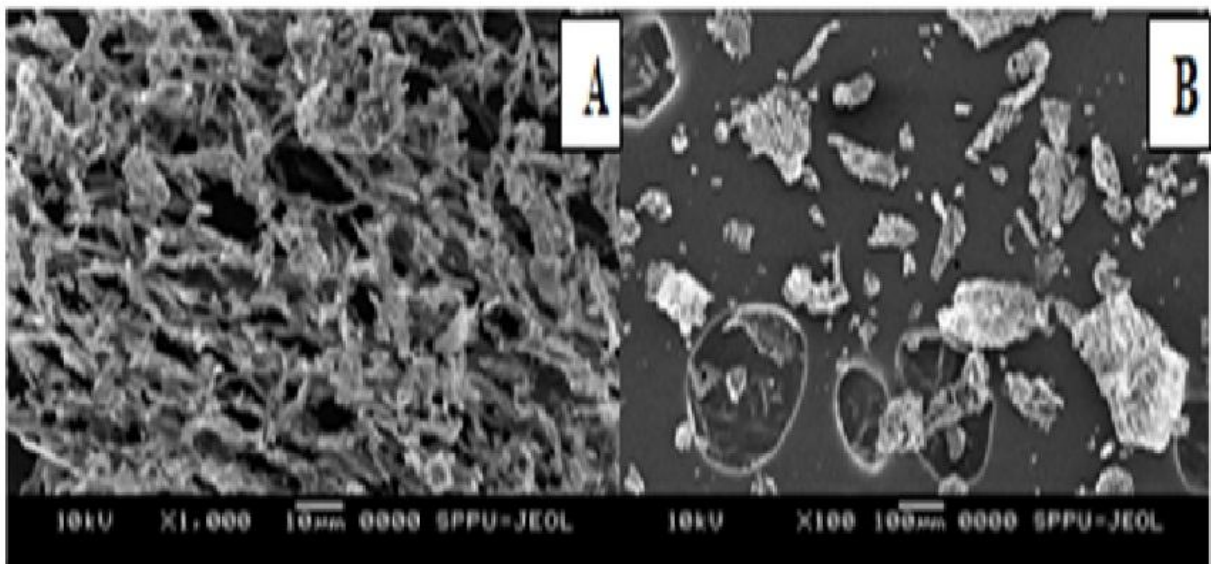


Figure 3 Scanning Electron Microscopy of co-crystals formulation

3.5 X-ray Diffraction

Powder XRD determines the crystallinity of compound. XRD studies of lawsone co-crystals exhibited sharp peaks indicating the crystalline nature of the co-crystals as shown in Fig.4

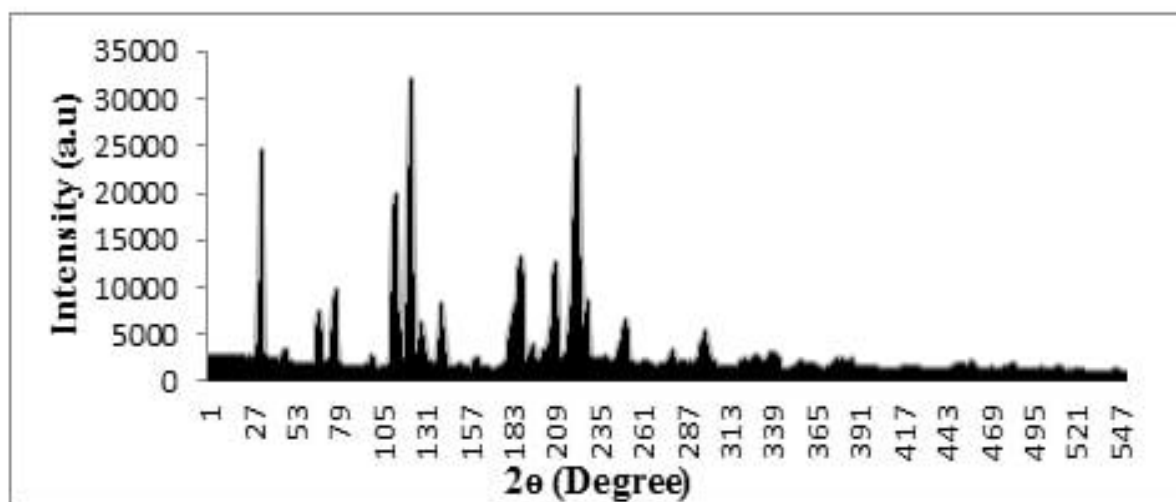


Figure 4 X-ray diffraction graph of co-crystals

3.6 Mean Particle size

The particle size of lawsone was $2478 \pm 1.5 \text{ nm}$ whereas prepared co-crystals (lawsone: benzoic acid) showed particle size of $560 \pm 2.2 \text{ nm}$. As compared to lawsone, the prepared co-crystals showed reduction in particle size. Fig.5

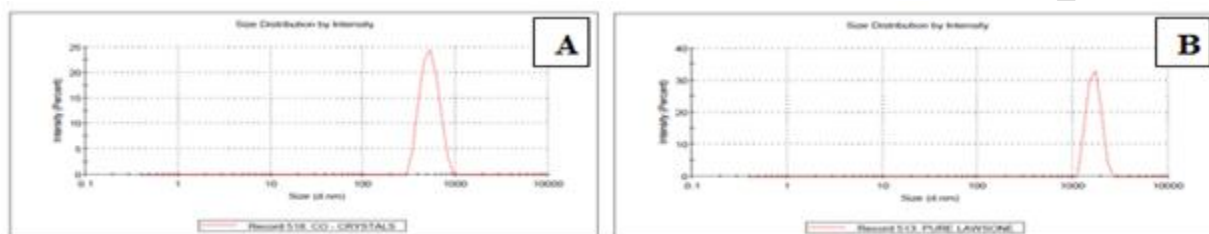


Figure 5 Particle size analysis A Lawsone co-crystal B Pure Lawsone

3.7 Antifungal assay

The pure drug lawsone, co-former benzoic acid and its co-crystals with lawsone were evaluated for its anti-fungal activity against *C. albicans*. The mean zone of inhibition was calculated which was found to be $30 \pm 0.5 \text{ mm}$ and $29 \pm 0.2 \text{ mm}$ for pure lawsone and benzoic acid respectively. The zone of inhibition of lawsone co-crystals was found to be $38 \pm 0.8 \text{ mm}$ Fig.6.

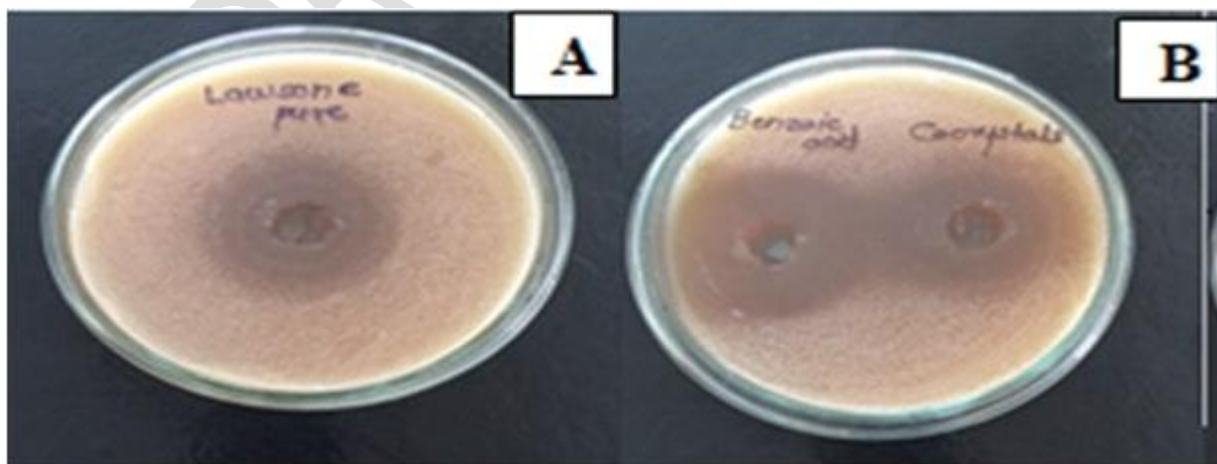


Figure 6 Zone of inhibition of (A) Pure lawsone, (B) Pure Benzoic acid and Lawsone co crystal

3.8 Evaluation of gel formulation

3.8.1 Physical evaluation

The pH of the gel was 6.4 which was nearer to the pH of skin (6.4) observed in the fungal infection caused by *C. albicans* thus avoiding irritation after application to the affected area of the skin [20]. The viscosity of the formulated gel was found to be (19,020 cps) which indicated easy applicability as well as retention of the gel on the affected part. The drug content of the gel was found to be 96.8%. Texture Profile Analysis studies of gel indicated spreadability of the gel with regard to its hardness. The gel formulation Fig. 7 showed the hardness (firmness) 110 g and adhesiveness 3.7 mJ.

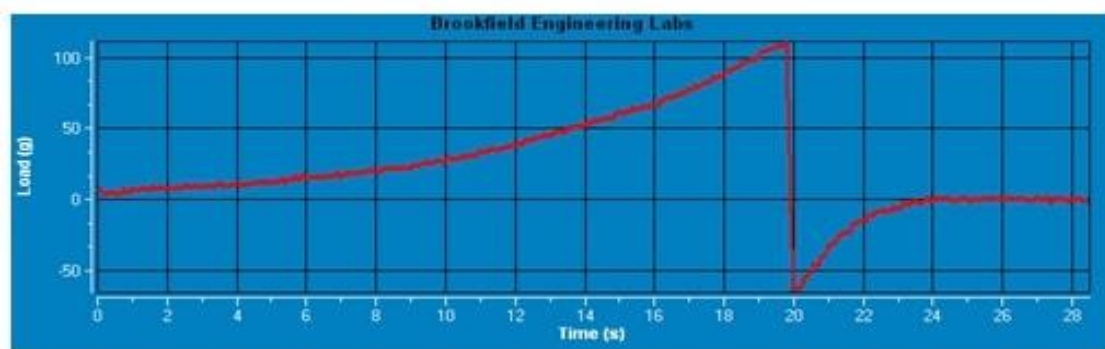


Figure 7 Texture profile analysis of lawsone loaded cocystal gel

3.8.2 In-vitro diffusion studies

In-vitro drug release studies of plain lawsone gel and lawsone co-crystals loaded gel was performed using Franz diffusion cell throughgoatskin as a membrane. The amount of drug diffused in the receptor compartment was 24.8 ± 0.4 % and 45.21 ± 0.34 % in 8 h for lawsone

cocrystal loaded gel and plain lawsone gel respectively Fig.8. The retention of drug in the skin was found maximum with lawsone co-crystal loaded gel ($70.2\pm 0.2\%$) as compared with plain lawsone gel ($35\pm 0.2\%$).

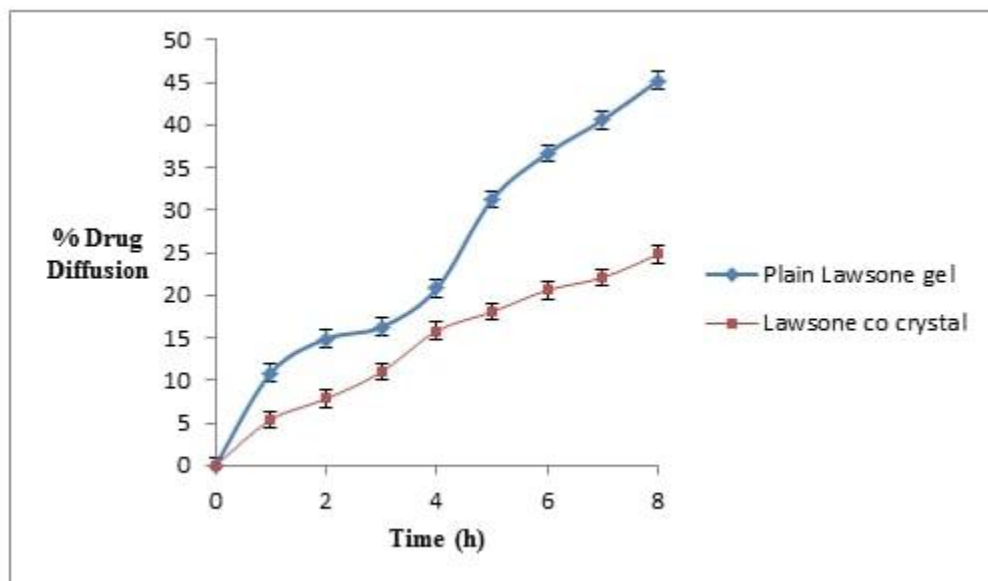


Figure 8 Drug diffusion of Lawsone loaded co-crystal gel and plain lawsone gel.

3.8.3 In-vitro antifungal study

Antifungal activity of lawsone co-crystals loaded gel and clotrimazole gel was evaluated by cup plate method. Zone of inhibition of lawsone co-crystals loaded gel was 29 ± 0.14 mm and marketed clotrimazole gel was 28 ± 0.1 mm respectively Fig.9.

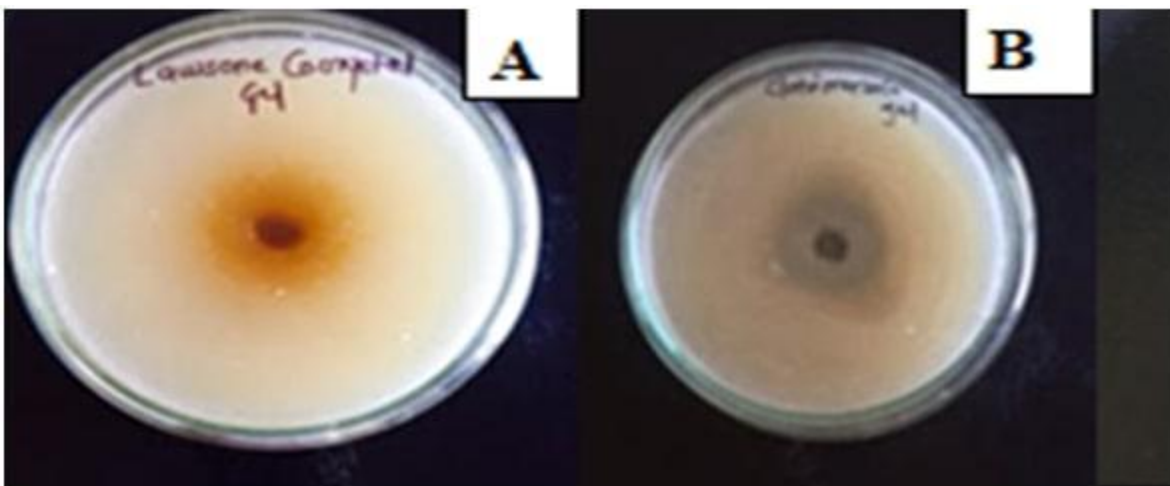


Figure 9 Zone of inhibition of A-, Lawsone co-crystals loaded gel formulation. B- Marketed Clotrimazole gel (n=3) \pm SD.

3.8.4 Stability Study

Lawsone cocrystal loaded gel was stable over a period of 3 months without any change in its physical appearance. No major change in pH (6.4–6.8) and drug content ($95.52 \pm 1.74\%$) at $25 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$ / $60 \pm 5\%$ RH and $40 \pm 0.5 \text{ }^\circ\text{C}$ / $75 \pm 5\%$ RH over a period of 3 months was observed.

4. DISCUSSION

The reason for the increased solubility of lawsone co-crystals could be attributed to the formation of metastable polymorphic form of the drug using benzoic acid as a co-former with respect to thermodynamics. The solubility was found to increase to 18.29 than pure drug alone. The enhanced rate of solubility in co-crystals is linked to supersaturation phenomenon wherein there is formation of supramolecular aggregates representing the amorphous form of the drug. The intermolecular interaction between the drug and the co former as observed in the FTIR spectrum caused the main functional groups of lawsone to shift to higher or lower wavelength indicating the formation of hydrogen bonds between pure lawsone and conformer confirming the formation of lawsone co crystals. A different melting point obtained for co-crystal than the pure drug

lawsone in the DSC studies also confirmed the formation of co-crystals. The rough nature of co-crystals with tight packing was observed in the XRD studies. This could also be one of the reasons for higher solubility of the co-crystals. The results of the antifungal study indicated that the co-crystals showed increased zone of inhibition than pure lawsone. The reason for this could be decrease particle size of the co-crystal, which resulted in better interaction of drug with *Candida albicans*.

Lawsone co crystal loaded gel was found to have good homogeneity and absence of grainy particle, which indicated no irritation to skin and revealed easy applicability of the gel on the skin. The high value of firmness and adhesiveness obtained from the texture profile analysis of the gel confirmed higher retention time of gel on the skin. The results of the *in-vitro* diffusion studies clearly indicate that lawsone-co-crystals loaded gel showed less permeation of the drug through the skin and maximum deposition of drug in the skin as compared to plain lawsone gel. To achieve effective dermal drug delivery, it is essential that high concentration of drug should be deposited in the affected area. The formulation of lawsone co-crystals caused significant difference in the permeation of plain lawsone and lawsone co-crystal. The reason for this could be the process of co-crystallization; co crystals interact differently with the proteins of the skin that are responsible for the drug transport through the skin as compared to plain lawsone. Additionally, the intermolecular interaction between the drug and the co-former could be one of the reasons that can cause this difference in the permeation of drug through the skin. Also, the incorporation of the co-crystals in the gel base have resulted in higher retention of drug in the skin due to increased viscosity of the gel base. Thus, co-crystals could be a promising approach to deliver the drug into the affected area of the skin to treat superficial fungal infection.

The antifungal activity of lawsone cocrystal loaded gel was at par to marketed clotrimazole gel. The smaller particle size of the co-crystals resulted in the better diffusion of drug through the agar medium. In addition, the larger surface area of the lawsone co-crystals resulted in the increase in solubility and greater amount of drug localization in the surrounding area.

5. CONCLUSION

Lawsone co-crystals were formulated via solvent crystallization method using benzoic acid as a co-former. Lawsone co-crystals further loaded in gel exhibited enhanced solubility, better skin retention and antifungal activity than plain lawsone. The results of the stability studies indicated the stable characteristics of the formulation. Thus, lawsone co-crystal loaded gel was formulated successfully to treat cutaneous candidiasis and could be considered as a better substitute to the currently available synthetic antifungal agents.

ABBREVIATIONS

ICH International Council for Harmonization

API Active Pharmaceutical Ingredient

FTIR Fourier Transform Infrared Spectroscopy

XRD X-Ray Diffraction

DSC Differential scanning calorimetry

IR Infrared spectroscopy

ETHICAL APPROVAL

Not applicable

CONSENT FOR PUBLICATION

Not applicable

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Ameen M., Epidemiology of superficial fungal infections. Clinics in Dermatology. 2010; 28(2): 197-201.
2. Rahmoun N, Boucherit-Otmani, Z, Boucherit K, Benabdallah M, Choukchou Braham N. Antifungal activity of the Algerian *Lawsonia inermis* (henna), Pharmaceutical Biology, 2013;51(1):131-135.
3. Brahmeshwari G, Surekha M, Saini K. Antifungal activity of naphthothiazoles derived from Lawsone (*Lawsonia inermis*). Afr. J. Biotechnol., 2012; 11(78):14405-14409.
4. Morello A, Pavani M, Garbarino JA, Chamy MC, Frey C, Mancilla, J, et al. Effects and mode of action of 1,4-naphthoquinones isolated from *Calceolaria sessilis* on tumoral cells and *Trypanosoma* parasites. Comp Biochem. Physiol. C. Toxicol. Pharmacol., 1995;112,(2):119-128.
5. Pallipurath A, Skelton JM, Delori A, Duffy C, Erxleben A, Jones W. Crystalline adducts of the Lawsone molecule (2-hydroxy-1,4-naphthaquinone): optical properties and computational modeling. Cryst.Eng.Comm., 2015;17:7684-7692.
6. Aslam A, Samee F, Zaman M, Mufti AUR, Rehman AU. Formulation and Assessment of semi-solid carrier incorporated with herbal extract of *Lawsonia Inermis*. Acta Pol Pharm.,2017;74(2):497-504.
7. Pandit A, Kedar A, Koyate K. Hollow pessary loaded with lawsone via self-microemulsifying drug delivery system for vaginal candidiasis. J Drug Deliv Sci Technol. 2020;60(7):101955.
8. Barani M, Mirzaei M, Torkzadeh-Mahani M, Nematollahi MH. Lawsone-loaded Niosome and its antitumor activity in MCF-7 breast Cancer cell line: a Nano-herbal treatment for Cancer. Daru.2018; 26(1):11-17.

9. Jignasa S. Co-crystallization: An approach to improve the performance characteristics of active pharmaceutical ingredients. *Asian J.Pharm.* 2015;9(3): 147.
10. Huang Y, Zhou L, Yang W, Li Y, Yang Y., et al. Preparation of Theophylline-Benzoic Acid Cocrystal and On-Line Monitoring of Co-crystallization Process in Solution by Raman Spectroscopy. *Crystals*, 2019; 9(329):1-13.
11. Higuchi T, Connors KA. Phase solubility techniques. *Adv. Anal. Chem. Instrum.*, 1965; 4:117–212.
12. Kamble RN, Bothiraja C, Mehta PP, Varghese V. Synthesis, solid state characterization and antifungal activity of ketoconazole cocrystals. *J. Pharm. Investig.*, 2018; 48:541–549.
13. Wavikar P, Vavia P. Nanolipidgel for Enhanced Skin Deposition, and Improved Antifungal Activity. *AAPS Pharm.Sci.Tech.*, 2013;14(1):1-12.
14. Shetgaonkar T, Charyulu RN. Microsponge Drug Delivery of Terbinafine Hydrochloride for Topical Application. *Int. J. Pharm. Sci. Rev. Res.*,2015;33(1):48-54.
15. Gulati N, Tomar N, Nagaich, U., Miconazole Microsponges based topical delivery system for diaper dermatitis: (2016);1-11.
16. Pandit A, Pol V, Kulkarni V Xyloglucan Based In Situ Gel of Lidocaine HCl for the Treatment of Periodontosis. *Journal of Pharmaceutics.* (2016);1-9.
17. Thakur V, Prashar B, Arora S, Formulation, and in vitro Evaluation of Gel for Topical Delivery of Antifungal Agent Fluconazole Using Different Penetration Enhancers. *Drugs invent. Today.* 2012;4(8):414-419.
18. Amrutiya N, Bajaj A, Madan M. Development of microsponges for topical delivery of mupirocin. *AAPS Pharm. Sci. Tech.*, 2009; 10(2):402-409.

19. Bavishi D, Borkhataria C. Spring, and parachute: How co-crystals enhance solubility. *Prog. Cryst. Growth Charact. Mater.* 2016; 62:1-8.
20. Chang JO, Yu K, Kong H, Kim E, Jang D, Nam K, Kim CK. Prolonged antifungal effects of clotrimazole-containing mucoadhesive thermosensitive gels on vaginitis. *J. Control Release*, 2002; 82:39-50.

UNDR PEER REVIEW