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Development of Miconazole nitrate loaded micellar gel for improved topical delivery

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ABSTRACT

The objective of the present investigation was to develop micellar gel of miconazole nitrate using amphiphilic block co-polymer pluronic F-127. Miconazole nitrate was entrapped into the micelles at critical micelle concentration by dialysis method and F2 was selected as best formulation based on a micelle size of 82.6 nm, entrapment efficiency of 96% and cumulative drug permeation amounting to 86 ± 0.31%. The spherical micelles as revealed by TEM were incorporated into gel. The gel with a pH of 6.16 and viscosity of 6942 ± 1.1cp displayed cumulative in vitro drug permeation 86.5 ± 0.01%. CLSM images upholded the assumption that the higher delivery observed for micellar gel in porcine skin was primarily due to penetration pathway. Thus the prepared micelles loaded gel represented higher permeability in the treatment of deep seated infection.

Introduction

For effective treatment of deep seated fungal disease, the drug must be delivered in sufficient concentration to the site of infection via topical therapy. Various approaches have been used to enhance the therapeutic action. Creams, based on use of cyclodextrin complexes have been reported to improve oral and topical delivery of miconazole nitrate.[1-4] Novel approaches like solid lipid nano particles [5] vesicular systems(ethosomes) and submicron emulsions have also been developed for improved topical delivery [6,7] While creams require high concentration of active agents to be incorporated for effective therapy because of their low efficacy as delivery system, polymeric nanoparticles are liable to aggregate [8] and problems with ethosomes includes instability and high price. Several reports have described the use of micelles for topical delivery of drugs into the deep layers of the skin that have potential to circumvent the limitattion associated with listed systems. Topical administration of drug via micellar system proficiently ensures delivery of the therapeutic agent to the target compartment and reduces the risk of systemic side effects. Micellization of active pharmaceutical ingredient also increases the solubility and permeability of hydrophobic drugs.[9]

During last few years, supramolecular chemistry has resulted into development of many dynamic supramolecular systems, such as polymeric micelles. [10] Pluronics are block copolymers based on ethylene oxide and propylene oxide and can form polymeric micelles that are much stable than micelles prepared from surfactant due to low critical micelle concentration (CMC).[11] Above the CMC, monomers and micelles exist in dynamic equilibrium.[12] Polymeric micelles possess high stability both in vitro and in vivo and good biocompatibility, and can solubilize a broad variety of poorly soluble pharmaceuticals many of these drug-loaded micelles are currently at different stages of preclinical and clinical trials.# Block copolymers make up a large class of micelle forming molecules and include those containing polypeptide segments, which can be enzymatically degraded to natural metabolites and possess ordered conformations not found in conventional polymers.(ALEXANDRIDS 1994, Macro molecules) .

The objective of the present project was aimed to develop and characterize micellar formulation for MCZ using of Pluronic block co-polymer and to compare them with market formulation. Drug loaded micelles prepared

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by dialysis method were investigated for particle size distribution, shape and stability. The optimized formulation was incorporated into gel and skin penentration was investigated using confocal laser scanning microscopy.

Experimental

Materials

Miconazole nitrate was provided as gift sample from FDC Ltd., Mumbai, India; Pluronic F-127 and Pluronic F-68 were procured from BASF corporation, USA; methanol, sodium dihydrogen phosphate and sodium chloride obtained from s.d. Fine-Chemicals Ltd. (Mumbai, India); Rodamine B and carbopol 934 was provided by Central Drug House Ltd, New Delhi, India; Dialysis membrane provided by Qualigens Fine Chemicals, Mumbai, India. All other ingredients used were of analytical grade and were used without further purification.

Methods

Critical micelle concentration

Critical micelle concentration of pluronic F-127 and F-68 was determined by dye micellization method using rodamine B dye. Stock solution of rodamine B (1% w/v) in water was prepared. To 2 ml of the polymeric surfactant solutions of different concentrations in the range of 1-6 mg/ml 1 ml rodamine B solution was added. The samples were allowed to reach equilibrium by shaking for 24 h at 37 0C.[15] The absorbance of polymeric surfactant solution was measured at 554 nm using UV/VIS spectrophotometer (Shimadzu, PharmSpec 1700, Kyoto, Japan). The X-intercept of the linear fit for the plot of solubilized rodamine B versus polymer concentration was used to determine the critical micelle concentration.

Formulation design

MCZ loaded micelles formulations F1 to F6 were prepared by varying drug: pluronic ratio based on critical micelle concentration (Table 1) by dialysis method. Drug and pluronic were dissolved in ethanol (95%v/v), which is a good solvent of both. The solution was filtered through a poly (tetrafluoroethylene) filter and 10 ml of micelle solution was dialyzed using dialysis bag (MW 12-14 KDa), against double distilled water (200ml) stirred at 200 rpm on a magnetic stirrer. Subsequently equal volume of dialysate water was replaced at 2, 5 and 8 h. The resultant drug loaded micelles were sonicated and centrifuged at 2000 rpm to eliminate unloaded MCZ and aggregated micelles.[12]

Evaluation of MCZ loaded pluronic micelles

Entrapment efficiency

Entrapment efficiency (EE) was determined by dissolving 1mL polymeric micellar formulation in 5 mL ethanol to ensure complete micelle destruction and drug release thereof. The volume was made up to 10mL with phosphate buffer, pH 6.8. The MCZ content was evaluated spectrophotometrically at 272 nm and % EE was calculated using the following equation: %

EE = Amount of drug present in micelles /Total drug incorporated x 100 ……Eq. 1

Particle size

The size of pluronic micelles and polydispersity index measured were measured by Zetasizer (Malvern Instruments Ltd, Worcestershire, UK). The scattering angle was fixed at 173º and temperature was maintained at 25ºC. For analysis of particle size, each sample was diluted with double distilled water prior to particle size determination and determination was done using a clear disposable sizing cuvette.

Table 1. Formulation design and characterization of miconazole nitrate loaded micelles

Formulation code	Pluronic (CMC)	Drug : Pluronic	Particle size (nm)	Polydispersity index	Entrapment efficiency (%)	Cumulative Drug permeated (12 h)(%)	Flux (Jss) mg/cm2/ hr	r^2	Best fit Model
F1		0.5:2	99.2	0.204	91.0	69.4± 0.52	3.05	0.823	First order
F2	Pf 127 (2mg/ml)	1:2	82.6	0.189	96.0	78.6± 0.31	4.12	0.882	Zero order
F3		2:2	105.1	0.213	55.6	51.3± 0.12	2.20	0.992	Matrix
F4		1:3	120.5	0.219	90.8	35.1± 0.43	1.30	0.981	First order
F5	Pf 68 (3mg/ml)	2:3	128.3	0.223	94.4	86.1± 0.55	4.52	0.952	First order
F6		3:3	152.3	0.228	58.6	42.2± 0.61	1.90	0.963	Hixson-Crowell

Pure Drug Flux (Jss) = 0.8 mg/cm2/hr

In-vitro permeation

The in-vitro permeation of MCZ loaded micelles was carried out using fabricated Franz diffusion cell consisting of donor and receptor compartment using dialysis membrane (Hi Media Ltd., India). One millilitre of MCZ loaded micellar formulation was placed in donor compartment and the receptor compartment was filled with phosphate buffer, pH 6.8 constantly stirred using magnetic stirrer. Donor and receptor chambers were separated by dialysis membrane (MW 12-14 KDa), already soaked in receptor medium overnight prior to experiment. The temperature was maintained at 32 ± 1.0ºC to mimic skin conditions. One millilitre sample was withdrawn through the sampling port of the diffusion cell at 0, 1, 2, 3, 4, 6, 8, 10 and 12h time intervals and analyzed spectrophotometrically at 272 nm. An equal volume of fresh phosphate buffer, pH 6.8 was replaced into the receptor compartment after each sampling. Best polymeric micellar formulation was selected on the basis of highest entrapment efficiency, least particle size and superior permeation profile. The selected formulation was subjected to further characterization by transmission electron microscopy and differential scanning calorimetry and formulated as gel.

Transmission electron microscopy

One drop of the micellar suspension (F2) was spread on a 400- mesh carbon coated copper grid and the excess droplets were removed with filter paper. A drop of 4%w/v phosphotungstic acid solution was dropped into the grid. The negatively stained sample was air dried at room temperature, and examined at 1950X magnification. TEM images were obtained at an acceleration voltage of 80 KW.

Differential Scanning Calorimetry

Thermal behavior of pluronic F-127, MCZ loaded micelles and MCZ were analyzed using Differential scanning spectroscopy (NETZSCH DSC 200 F3 240-427-L, Japan). Accurately weighed sample(s) was placed in aluminum pan and sealed with a lid. Aluminum oxide was used as the reference sample. In the scanning process, a heating rate of 100C/min was applied in the temperature range from 100 to 2100C with a nitrogen purge of 0.2 mL/min.

Table 2. Table 2 Evaluation of micellar gel

Formulation code	pH	Viscosity (cp)	Cumulative Drug Permeation (%) ± SD	Flux (Jss) mg/cm2/hr	Best fit Model
G1	6.16±.05	6942±1.1	82.2 ± .01	4.72	zero
MF	6.30±.02	5872±2.1	56.1 ±.31	4.15	matrix

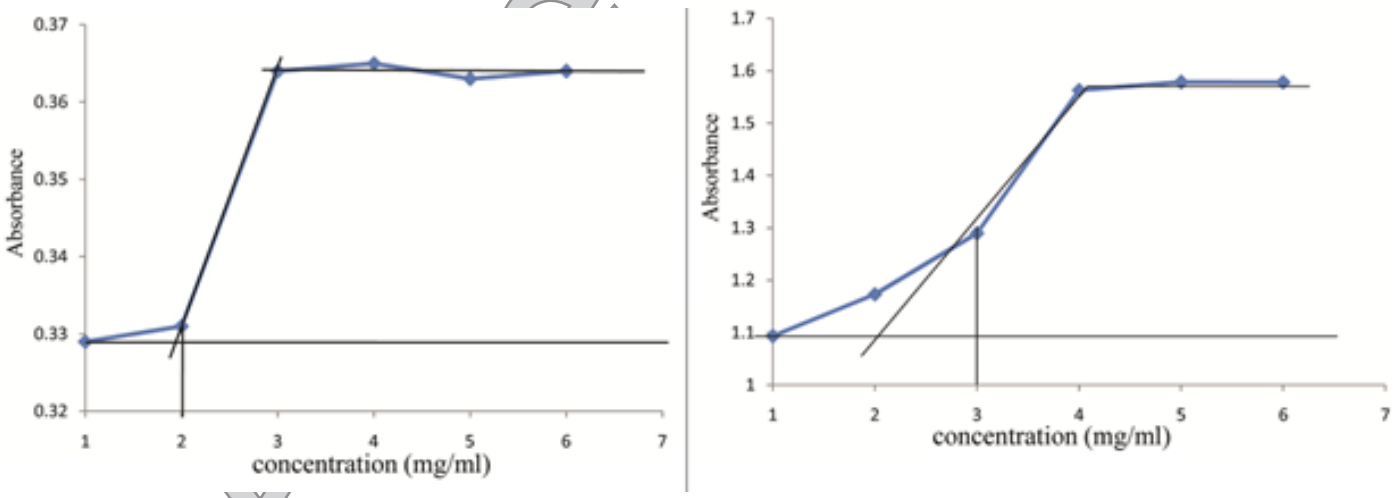

Figure 1 CMC determination of (A) Pf 127 and (B) PF 68

Stability of F2

Stability test of micelles consisted of visual control and analytical measurement of drug content. For this purpose MCZ loaded micelles suspension (F2) was placed in sealed vial at 25 ± 20C for 3 months. Drug content was determined at zero time and at the end of 3 months.

Preparation of micellar gel

Optimized formulation of MCZ loaded micelles (F2) was incorporated in micellar gel using dispersion method. [2] Gel base was prepared by dispersing Carbopol 934 in distilled water. Carbopol 934 was soaked in distilled water for 2 hr and stirred to obtain a homogeneous gel

base of strength 2% w/w. Formulation (F2) was subjected to cooling centrifugation (REMI Instrument Ltd, Vasai, India) at 2000 rpm for 20 min and the pellets obtained were incorporated into gel base to get 2 % w/w MCZ in the gel base.

EVALUATION OF MCZ MICELLAR GEL

pH and viscosity

The pH of pluronic micelles loaded gel was determined using Digital pH meter model 111 E(HICON, New Delhi, India) and the viscosity of gel was measured by Brookfield viscometer using T-bar spindle (spindle-C, S-95) at 50 rpm (Model DV-II+ Pro, Brookfield engineering laboratories, INC., USA).

Drug content

Weighed amount (100mg) of MCZ loaded gel was diluted using 5 ml of methanolic HCl. The resultant dispersion was vortexed and shaken for 10 min. The volume was made up to 10 ml with methanolic HCl and analyzed at 272 nm.

Ex-vivo permeation

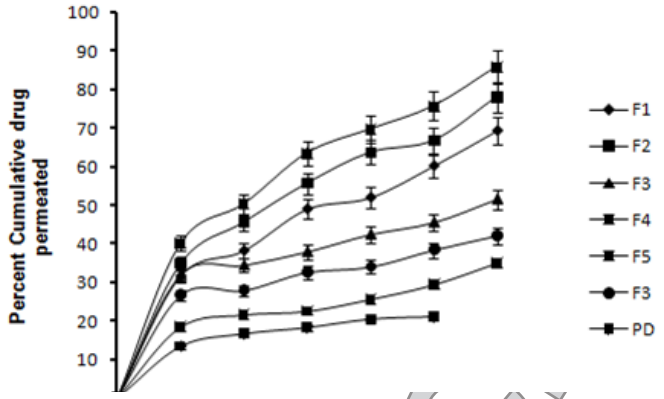

Figure 2 Comparative In vitro drug permeation profiles of MCZ from developed polymeric micelles (F1-F12) and pure drug suspension in STF pH 7.4 using Franz diffusion cell

Ex-vivo skin permeation of MCZ loaded micellar gel was carried out using modified Franz diffusion cell consisting of donor and receptor compartment sepearted by porcine ear skin, which was very similar to the human shoulder epidermal thickness. The follicular structure of pig skin also resembles that of human skin, with hairs and in fundibula extending deeply into the dermis. Averages of 20 hairs are present per 1cm of porcine ear skin as compared to 14 – 32 hairs (except the forehead area) in humans. Full thickness porcine skin samples were equilibrated in phosphate buffer, pH6.8 for 30 min and mounted on standard Franz diffusion cells (area = 2.0 ± 0.1 cm2); silicone grease was applied to ensure a watertight seal. The receptor compartment (volume 10 mL) was filled with phosphate buffer, pH6.8 magnetically stirred and maintained at 32 ± 1°C. One ml sample was withdrawn through the sampling port of the diffusion cell at 0, 1, 2, 3, 4, 6, 8, 10 and 12 h intervals and analyzed spectrophotometrically at 272 nm. An equal volume

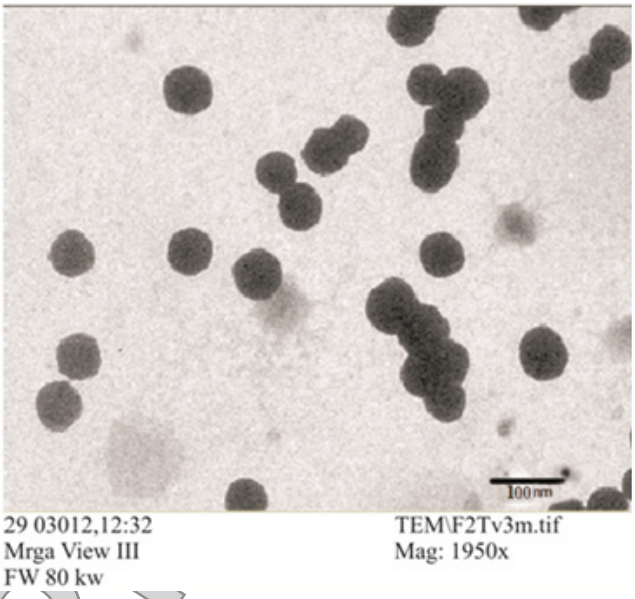

Figure 3 TEM image of optimized MCZ polymeric micelles (F5)

of fresh phosphate buffer, pH 6.8 was replaced into the receptor compartment after each sampling. The cumulative amount permeated the skin per unit surface area was plotted against time. The slope of linear portion of the plot was taken as being the steady-state flux.

Confocal laser scanning microscopy

Depth and mechanism of skin permeation of rodamine B loaded micelles were investigated by CLSM. Formulation G1 was replicated by replacing MCZ with rhodamine B as the flourescent marker and applied to the porcine ear skin 3h. In a comparative experiment MCZ incorporated in gel base was used as reference. Sections of the skin samples were prepared and examined with CLSM (Fluo view FV 1000, Olympus, Japan) at 100 X.

Microbiological Evaluation

It was determined on Candida albicans by the measurement of the mean diameter of growth inhibition zones in millimeter. In this method, nutrient agar plates were prepared and sterilized by autoclaving at 120ºC, 15 pounds pressure for 15 min. Then, about 30 ml nutrient agar media was inoculated with fungal strain i.e. Candida albicans (2 ml of inoculum to 100 ml of nutrient agar media) , poured in two sterilized petri plates and three wells of 5mm diameter were made via sterile cork borer in each petri plate. Then, accurately 0.2 ml each of test formulation (micelle loaded in situ gel (G1)), standard formulation (Miconazole nitrate pure drug suspension) were transferred to cups aseptically and labeled accordingly as test and standard. Along with preparation of test and standard a negative and positive control were also prepared which consist of uninoculated media and

media seeded with test organism but deprived of antifungal agent. The prepared petri plates were maintained at room temperature for 2 h to allow the diffusion of the solutions in to the medium and then incubated at 28°C for 48 h. The diameter of zone of inhibition surrounding each of the wells was recorded.

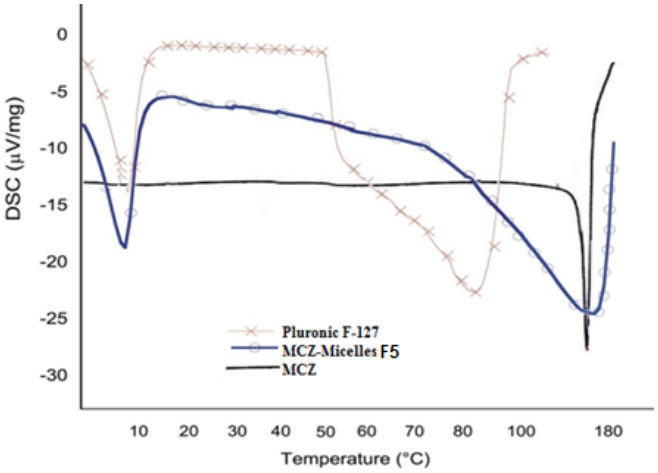

Figure 4 DSC thermogram of Pluronic F-127, MCZ-micelles (F5) and MCZ

RESULT AND DISCUSSION

Critical micelle concentration

Critical micelles concentration (CMC) was estimated to prove the potential of micelles formation of Pluronic co-polymer F-127 and F-68 in a given environment dye micellization method. An increase in the absorbance was observed as concentration of polymeric surfactant was increased until a sharp inflection due to micellization process was detected. The linear portion near the inflection point was extrapolated to the point where the absorbance matched that of the dye represented by the horizontal dashed line and this concentration was defined as the CMC. The CMC values of pluronic F127 and pluronic F-68 were thus determined as 2 and 3 mg/ml. The CMC of block copolymers is strongly dependent on the lengths of the blocks [24]. An increase in the length of the hydrophobic propylene oxide (PO) block elevates the net hydrophobicity of the Pluronic molecule and favors the segregation of the PO chains into the micelle core [21- 23]. The chain length of PO for pluronic F-68 is 28.97 and for pluronic F-127 it is 65.17. Correspondingly, pluronic F-68 had higher CMC value than pluronic 127. Conversely, an increase in the length of the ethylene oxide (EO) blocks elevates the probability of contacts of the PO units with the EO units within the core of the micelles. This effect decreases the hydrophobicity of the core and results in destabilization of the micelle. Therefore, the CMC increased as the hydrophilic EO block length increased [24, 25].

MCZ LOADED MICELLES

Entrapment efficiency

The entrapment efficiency was affected by the nature of polymeric surfactant used. Pf127 insignificantly (P> 0.05) displayed higher entrapment efficiency than Pf68 (Table 1). The effect of varying drug: polymeric surfactant ratio was indicated by an increase in entrapment efficiency as the drug content was increased from 0.5 to 1 keeping Pf127 constant probably due to sufficient availability of drug available for entrapment in the polymeric micelles. However, on further increase in the drug content that equalled to Pf127 content, the entrapment efficiency lowered down to 55.6 % suggesting lesser number of polymeric micelles available for entrapment. Similar pattern was observed for Pf68 also. However, the net total weight of drug: pluronic was maintained at higher level for Pf68 owing to its CMC value.

Particle size and Polydispersity index

The amount of polymeric surfactant and miconazole nitrate were critical parameters governing particle size that ranged between 82.6- 152.3 nm (Table 1). Particle size and uniform size distribution are the pivotal parameters for topical delivery and a size below 250 nm and PDI near 0.25 is considered as optimum. The mean particle size of Pf127 micelles (F1-F3) ranged from 82.6 nm to 105.1 nm while those of Pf 68 (F4-F6) ranged from 120.5 nm to 152.3 nm. The PDI value of all formulations was below 0.25 indicating uniformity in size distribution. Critical analysis of result revealed lower particle size and PDI for F1- F3 than F4 –F6 because the former were made with Pf127 that has EO chain length of 200 that is more than that of Pf68. The EO chain governs the micelle aggregation and its size [17]. In general, for a given PO block, the block copolymers with a higher EO block content are less aggregated and have a smaller core size [23]. Conclusively, F2 exhibited smallest particle size of 83.6 nm and least PDI of 0.189 among all the formulations.

In-vitro drug permeation

The In-vitro drug permeation profiles of F1- F3 (Fig. 6)

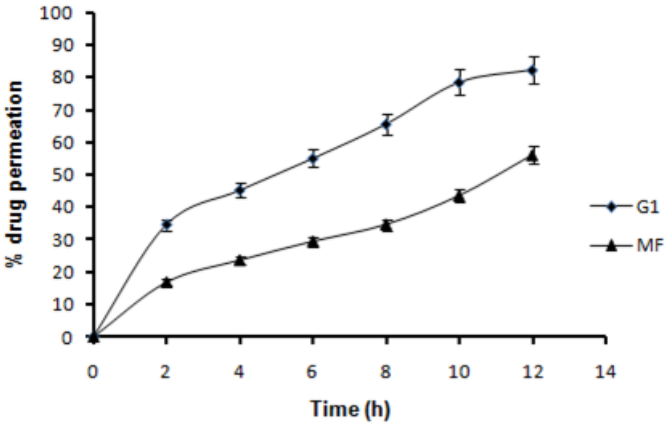

Figure 5 DSC thermogram of Pluronic F-127, MCZ-micelles (F5) and MCZ

in phosphate buffer, pH 6.8 were compared with the pure drug. Owing to its poor aqueous solubility (0.03% w/v) it displayed 20.46% permeation against its micellar formulations. Micellar formulations developed using Pf68 (F4 - F6) displayed slow and incomplete drug permeation

(51.6 ± 0.61 to 82 ± 0.55 %) in comparison to those developed using PF-127 (F1-F3). This variability in the drug permeation may be due to chemical structure and chain length of tri-block copolymer used. The PO chain length of Pf127 is longer as compared to Pf-68 hence the drug was higher and consequently the drug permeation. Maximum cumulative drug permeation (86 ± 0.31%) was observed for F5 at12 hr and least was recorded for F4 (35.1± 0.43). Model fitting of the permaetion data revealed variable orders with F5displaying first order as the best fit model (Table 1). The permeation flux was calculated from the slope of drug permeation profiles and determined as 1.30 – 4.52 mg/cm2/hr. The permeation flux from F5 was highest (4.52 mg/cm2/hr), almost four folds higher than PD (0.8 mg/cm2/hr). Hence based on highest percentage cumulative drug permeation (86 ± 0.31), least particle size (82.6 nm) and PDI value (0.189), highest entrapment efficiency (96%) F2 was considered as optimized formulation, characterized and was incorporated in gel (G1) for topical delivery.

Transmission Electron Microscopy

Transmission electron microscopic image of micelles indicated spherical shaped particles with apparently narrow size distribution (Fig 3). Clear boundaries suggested no aggregation and apparently the particles were less than 100nm, a size particularly suitable for topical delivery.

Differential Scanning Calorimetry

To study the binding strength of MCZ in the micelles DSC was performed. The thermogram of MCZ displayed a sharp endothermic peak at 170.35 0C and was used as reference peak (Fig 4). The DSC thermogram of Pf127 showed an endothermic peak at 10.310C due to the CMT and a broad endothermic peak with onset at its melting temperature of 57.1 0C that peaked at peak at 84.210C*. This broad peak is attributable to dehydration of the EO chains with increasing temperature. In case F5 (MCZ micelles) the CMT shifted to 8.80C, contrary to the shift in broad melting peak to 170.20C corresponding to MCZ. As explained in literature, high loading of a poorly soluble drug into micelles normally leads to crystallization of the loaded drug, whereas drug molecules are present in the molecularly dispersed state at low loading.35 Lidocaine and clonazepam are good examples showing crystallization in micelle cores at high loadings of about 30 wt%.36,37

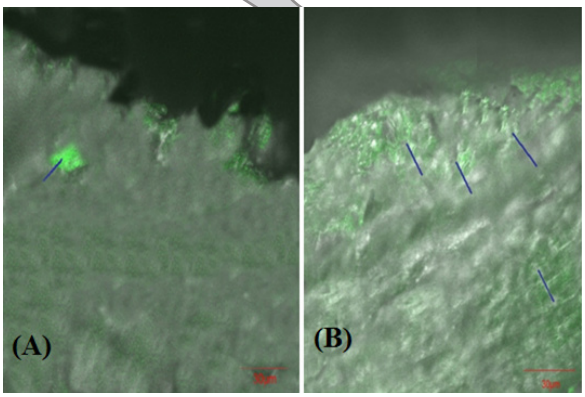

Figure 6 Confocal laser scanning microscopy (CSLM) of (A) rodamine loaded control gel and (B) micellar gel

Stability

Micelles structure is strongly affected by temperature and the temperature at which micelles are formed is known as critical micellization temperature (CMT). For most copolymers this value is 25-40 0C. If micelles were kept in refrigerator, temperature would fall lower than CMT, thus micelles would lose their intact structure and drug would precipitate. For this reason, stability test was performed at 25 0C. No sedimentation or phase separation was observed in three months stability test. The spectrophotometric spectrums stayed the same in 3 months, suggesting no chemical degradation accurse. Drug content of MCZ loaded pluronic micelles did not change showing high storage stability of F2.

MCZ loaded Micellar Gel

pH, viscosity and Drug Content

The micellar gel had a pH of 6.16 ± 0.05 owing to the gel base of carabopol. Carbopol 934 is made of carbomers that maintain the pH within the physiologically recommended pH range of 4.5 to 7. The drug content of G1 was 82.2 ± 0.1 and the viscosity amounted to 5872 ± 2.1 cp.

Ex-vivo drug permeation

The cumulative amount of MCZ permeated via porcine skin from micellar gel was 1.32 times than marketed formulation of MCZ in the experimental conditions. This may be attributed to high entrapment and loading efficiency of drug in micellar formulation. Moreover, the micellar gel showed zero order kinetics in contrast to the marketed product that displayed matrix kinetics (Table 3). For the formulations aimed at achieving permeation, target flux is important consideration. In present study, target flux for topical delivery was calculated as 60% of Css, where, Css is the concentration in plasma at steady state. Css of MCZ is reported to be 0.83µg/ml therefore target flux was calculated as 0.498µg/ml. The significance of the target flux is that it defines the minimum amount of the drug that should reach the site for the therapeutic efficacy. The micelles loaded gel displayed a steady state flux of 3.276 µg/cm²/hr that is higher than that of the target flux and hence can be considered as therapeutically efficacious system.

In vivo permeation by CLSM

CLSM study of G1 was performed to study the depth and mechanism of the rodamine B red dye loaded micelles through the porcine skin (Fig. 6). The confocal micrograph of the rodamine loaded control gel indicated slight change in the thickness of the stratum corneum, resulting in accumulation of rhodamine in the top most layers (Fig 6A). Contrastingly, enhanced permeation

of the marker into the deeper layer of epidermis was observed from micellar gel (Fig. 6B). Thus G1 containing F2 was judged as the optimized gel for effective topical delivery.

Microbiological Evaluation

The zone of inhibition produced by test (G1) measured (21.00 ± 3.60 mm) that was more than zone of inhibition produced by standard solution (17.33 ± 2.51 mm). After applying Student's t-test ($p < 0.05$) at 95% confidence interval, a significant difference in the antifungal activity of reference standard and micelle loaded in situ gel was observed. Thus, it was confirmed that antifungal activity of test was more than standard preparation i.e., Miconazole pure drug suspension. Hence, MCZ loaded micellar gel is more effective than standard preparation.

Conclusion

Miconazole nitrate was successfully entrapped into Pluronic F-127 micelles by the dialysis method. The micelles loaded gel represented higher permeability than the marketed product of miconazole nitrate indicating its higher capability in treatment of deep seated infection.

Conflict of Interests

Herewith the author(s) declare(s) that there is noconflict of interests regarding the publication of this article.

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