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FORMULATION DEVELOPMENT AND IN-VITRO EVALUTION OF OXICONAZOLE NITRATE MICROSPONGE DRUG DELIVERY SYSTEM OF ANTIFUNGAL DRUG

Mr. VILAS S. BHAGAT[1], Dr. SURESH KUMAR DEV[2], Mr. Akhil Mangal[3],

FACULTY OF PHARMACY PACIFIC ACADEMY OF HIGHER EDUCATION AND RESEARCH UNIVERSITY UDAIPUR.

ADESH INSTITUTE OF PHARMACY AND BIOMEDICAL SCIENCE (ADESH UNIVERSITY)

ABSTRACT

In this study, Oxiconazole nitrate microsponge were prepared by quasi-emulsion solvent diffusion method. The microsponges thus prepared, were evaluated for production yield, loading efficiency, particle size analysis, SEM, IR, DSC, PXRD, characterization of pore structure, in-vitro release study of microsponges, and stability. Then, gels loaded with microsponge and plain drug were formulated and evaluated for rheology, diffusion, skin irritation and antifungal activity. Antifungal activity of Oxiconazole nitrate containing microspongic gels were compared with marketed formulations. The microsponges showed good production yield, loading efficiency and particle size. All characteristic peaks of the drugs were concordant with IR spectra of pure drugs. PXRD and DSC studies revealed amorphization of drugs. SEM images showed that styrene microsponges prepared by suspension polymerization were finely spherical and uniform, while Eudragit microsponges prepared by quasi-emulsion solvent diffusion method were comparatively less spherical. According to intrusion and extrusion curves, majority of the pores present in Eudragit microsponges were spherical type, whereas the pores of styrene microsponges were mainly cylindrical-hole type. BET multipoint adsorption isotherm studies revealed that the percent of porosity of styrene microsponges is comparatively higher than Eudragit based microsponges. During the storage of drug-entrapped microsponges at 40±2°C and 75±5% RH for 6 months, surface morphology and release of drug showed no notable changes. Viscosity determination of gel showed that gels loaded with microsponge are more viscous than gel loaded with plain drug. The controlled drug release was observed with all the microspongic gels. Antifungal activity of gels containing microsponge entrapped Oxiconazole nitrate showed that antifungal activity of drugs was retained even after entrapment in microsponges and it was higher as compared with the gel containing free drug and marketed formulation. Gels containing drugs entrapped in microsponges showed reduced skin irritation when compared

with gels containing free drug and marketed formulations. Hence, the present work concluded that MDS has a great potential in topical delivery of Oxiconazole nitrate, with added advantage of reduction in irritation profile due to the controlled release and possible enhancement in the activity due to amorphization of the drug.

Key words: Micro sponge Drug delivery system, Oxiconazole nitrate, anti-fungal activity, quasi emulsification technique.

INTRODUCTION

Microsponge delivery systems are uniform, spherical, porous polymeric microspheres having myriad of interconnected voids of particle size range 5- 300µm. These microsponges have the capacity to entrap a wide range of active ingredients such as emollients, fragrances, essential oils, sunscreens and anti- infective, etc. and then release them onto skin over a time and in response to trigger [1-2]. Micropores within the spheres comprise a total pore density of approximately 1mL/g, and pore length 10 ft for extensive drug retention. Further, these porous microspheres with active ingredients can be incorporated in to formulations such as creams, lotions and powders[3]. Microsponges consisting of non-collapsible structures with porous surface through which active ingredients are released in a controlled manner. When applied to the skin, the MDS releases its active ingredient on a time mode and also in response to other stimuli (rubbing, temperature, pH, etc). MDS technology is being used in cosmetics, over-the-counter (OTC) skin care, sunscreens and prescription products. Their high degree of cross-linking results in particles that are insoluble, inert and of sufficient strength to stand up to the high shear commonly used in manufacturing of creams, lotions, and powders. Their characteristic feature is the capacity to adsorb or 'load' a high degree of active materials into the particle and on to its surface. Its large capacity for entrapment of actives, up to three times its weight, differentiates microsponge products from other types of dermatological delivery systems. The active payload is protected in the formulation by the microsponge particle; it is delivered to skin via controlled diffusion [4]. The microsponge technology was developed by Won in 1987, and the original patents were assigned to Advanced Polymer Systems, Inc.

The Micro-sponge delivery system comprised of polymeric bead having network of pores with an active ingredient held within was developed to provide controlled release of the active ingredient whose final target is skin itself. The system was employed for the improvement of performance of topically applied drugs[5-6]. This system can prevent excessive accumulation of ingredients within the epidermis and the dermis. The Microsponge systems are based on microscopic, polymer-based microspheres that can bind, suspend or entrap a wide variety of substances and then be incorporated into a formulated product, such as a gel, cream, liquid or powder. A single Microsponge is as tiny as a particle of talcum powder, measuring less than one-thousand of an inch in diameter.

EXPERIMENTAL

Preformulation Studies

Preformulation testing is the first step in the rational development of dosage forms of a drug. It can be defined as an investigation of physical and chemical properties of drug substance, alone and when combined with excipients. The overall objective of preformulation testing is to generate information useful to the formulator in

developing stable and bioavailable dosage forms, which can be mass-produced.A thorough understanding of physicochemical properties may ultimately provide a rationale for formulation design or support the need for molecular modification or merely confirm that there are no significant barriers to the compounds development.

Characterization of Oxiconazole Nitrate Pure Drug

Spectroscopic Studies

UV spectroscopy: (Determination of λ max)

The stock solutions (100µg/mL) of the drugs were prepared in methanol Oxiconazole nitrate. The stock solutions were appropriately diluted with the respective solvents to obtain a concentration of 20µg /mL. The UV spectrum was recorded in the range of 200-400 nm on Schimadzu 1700 UV spectrophotometer to find the λ_{max}.

IR Spectroscopy

The spectrum was recorded in the wavelength region of 4000 to 400 cm^{-1}. A dry sample of the drug and potassium bromide were mixed uniformly and filled into the die cavity of sample holder and an IR spectrum was recorded using diffuse reflectance FTIR spectrophotometer.

Construction of Calibration Curve for Drugs

The stock solution (100 µg/mL) was prepared by dissolving 10 mg of the drug in methanol Oxiconazole nitrate in a 100 mL volumetric flask. From the stock solution, solutions containing 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg/mL of the drugs were prepared by appropriate dilutions. Absorbance of these solutions were measured at 211 nm for Oxiconazole nitrate against respective blank solvents.

DRUG-EXCIPIENT COMPATIBILITY STUDIES

Drug-excipients compatibility studies were carried out for one month. The drug with excipients Eudragit RS 100 & PVA were subjected to storage at room temperature and elevated temperature at 45°C/ 75% RH in stability chamber for one month. After 7, 14, 21 and 30 days the samples were taken to check the following parameter.

FORMULATION DEVELOPMENT OFMICROSPONGES

Free Radical Polymerization Reactions: Fundamentals

It is possible to form addition polymers from monomers containing C=C double bonds; many of these compounds polymerize spontaneously unless polymerization is actively inhibited. The simplest way to catalyze the polymerization reaction that leads to an addition polymer is to add a source of a free radical to the monomer. The term free radical is used to describe a family of very reactive, short-lived components of a reaction that contain one or more unpaired electrons. In the presence of a free radical, addition polymers form by a chain reaction mechanism that contains chain initiation, chain propagation, and chain termination steps.

Quasi-emulsion Solvent Diffusion method: (Eudragit microsponges)

To prepare the inner phase, Eudragit RS 100 was dissolved in 3 mL of methanol and triethylcitrate (TEC) was added at an amount of 20% of the polymer in order to facilitate the plasticity. The drug was then added to the solution and dissolved under ultrasonication at 35°C. The inner phase was poured into the PVA (72000) solution in 200 mL of water (outer phase). The resultant mixture was stirred for 60 min, and filtered to separate the microsponges. The microsponges were washed and dried at 40°C for 24h. [7] Seven different ratios of drug to Eudragit RS 100 (1:1, 3:1, 5:1, 7:1, 9:1, 11:1 and 13:1) were employed to determine the effects of drug : polymer

ratio on physical characteristics and dissolution properties of microsponges. Agitation speed employed was 500 rpm using three blade propeller stirrers.

Table no.01 : Microsponge formulations using Eudragit RS100

Constituents	**Oxiconazole nitrate Microsponges**						
	F17	F18	F19	F20	F21	F22	F23
Inner phase							
Oxiconazole nitrate	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Eudragit RS 100 (g)	2.5	0.83	0.50	0.36	0.28	0.23	0.19
Methanol (mL)	3	3	3	3	3	3	3
Outer phase							
Distilled water (mL)	200	200	200	200	200	200	200
PVA 72000 (mg)	50	50	50	50	50	50	50

EVALUATION OFMICROSPONGES

Determination of Production Yield and Loading Efficiency

The production yield of the microparticles was determined by calculating accurately the initial weight of the raw materials and the last weight of the microsponge obtained (Kilicarslan M., 2003). The loading efficiency (%) of the microsponges can be calculated

Particle Size Analysis

Particle size analysis of prepared microsponges was carried by using Malvern Particle Size Analyzer Hydro 2000 MU (A). Microsponges were dispersed in double distilled water before running sample in the instrument, to ensure that the light scattering signal, as indicated by particles count per second, was within instrument's sensitivity range.

Scanning Electron Microscopy

For morphology and surface topography, prepared microsponges were coated with platinum at room temperature so that the surface morphology of the microsponges could be studied by SEM.The SEM, a member of the same family of imaging is the most widely used of all electron beam tools [8] . The SEM employs a focused beam of

electrons, with energies typically in the range from a few hundred eV to about 30 keV, which is rastered across the surface of a sample in a rectangular scan pattern. Signals emitted under this electron irradiation are collected, amplified, and then used to modulate the brightness of a suitable display device which is being scanned in synchronism with probe beam.

Infrared Spectroscopy

FTIR spectroscopy was conducted using Perkin Elmer, Spectrum 100 FT-IR spectrometer. Spectrum was recorded in the wavelength region of 4000 to 400 cm^{-1}. The procedure consisted of dispersing a sample in excess of potassium bromide nearly at the ratio 1:100, mixed well, after which the mixture was kept into the sample holder for analysis.

Differential Scanning Calorimetry(DSC)

Thermograms of pure Eudragit RS 100, pure oxiconazole nitrate and drug entrapped microsponges were obtained using DSC instrument, Differential Scanning Calorimetry (SDT-2960); TA4000, Mettler, Japan. Indium standard was used to calibrate the DSC temperature and enthalpy scale. The powder sample of microsponges was hermetically kept in the aluminum pan and heated at constant rate 5°C/min, over temperature range of 10^0 C to 250°C. An inert atmosphere was maintained by purging nitrogen at the flow rate of 100 mL/min.

Powder X-ray Diffraction Studies

PXRD is one of the most widely attempted quantification techniques because of its simplicity and it measures differences in periodicities of atoms/molecules in a powder sample [9]. PXRD patterns of crystalline forms show strong diffraction peaks, whereas amorphous ones exhibit diffuse and halo diffraction patterns.

Characterization of Pore Structure

Physical and chemical gas adsorption and mercury intrusion porosimetry (MIP) are the most widely used techniques to characterize powders and solid materials. With nitrogen gas adsorption, depending on the equipment used pore diameter range of 0.3–300 nm, i.e. mesopores and macropores, are determined. Low-pressure mercury porosimetry determines macropores (pore diameter14– 200 µm), and high-pressure porosimetry mesopores and macropores (pore diameter 3 nm–14 µm), depending on the equipment.

Mercury intrusion porosimetry (MIP)

Porosity parameters of microsponges such as intrusion–extrusion isotherms, pore size distribution, total pore surface area, average pore diameters, shape and morphology of the pores, bulk and apparent density can be determined by using mercury intrusion porosimetry. Incremental intrusion volumes can be plotted against pore diameters that represented pore size distributions. The pore diameter of microsponges can be calculated by using Washburn equation [10].

***In-vitro* Release Study of Microsponges**

Accurately weighed loaded microsponges (5 mg) were placed in 50 ml of ethanol/methanol in 100 ml glass bottles. The later were horizontally shaken at 37°C at predetermined time intervals. Aliquot samples were

withdrawn (replaced with fresh medium) and analysed UV spectrophotometrically at 211 nm for Oxiconazole nitrate. The contents of drugs were calculated at different time intervals up to 6hrs.

Stability Profile of Microsponge Formulation

In any, rationale design and evaluation of dosage forms for drugs, the stability of the active component is the major criteria in determining their acceptance or rejection. During the stability studies the product is exposed to normal conditions of temperature and humidity. However, the studies take a longer time and hence it would be convenient to carry out the accelerated stability studies where the product is stored under extreme conditions of temperature. To assess the drug and formulation stability, stability studies were done according to ICH and WHO guidelines. Optimized formulation sealed in aluminum packaging coated inside with polyethylene, and various replicates were kept in the humidity chamber maintained at 40±2°C and 75±5% RH for 6 months. The samples were analyzed for the physical changes and *in-vitro* release profile at an interval of 1 month for 6months.

FORMULATION OF GEL LOADED WITH MICROSPONGES AND PLAIN DRUG

Table no. 02 : Composition of gels

Ingredients	**Quantity (% w/w)**
Drug (free or entrapped, equivalent to)	Oxiconazole nitrate : 1
Propylene glycol	40
Methanol	8
Menthol	0.04
Methyl paraben	0.18
Sodium metabisulphite	0.10
Disodium edentate	0.10
Carbopol 934	1.00
Triethanolamine	q. s.
Purified water q. s. to make	100

A clear dispersion of carbopol was prepared in water using moderate agitation. Intermittent sprinkling of carbopol prevents lump formation resulting in clear homogenous dispersion. Drug or drug containing

microsponge formulation was dispersed in propylene glycol and methanol. Various ingredients viz. paraben, sodium metabisulphite and disodium edetate were dissolved in water and added to the drug solvent system. Triethanolamine was used to neutralize and adjusted to final weight with water. Gels prepared were degassed by ultrasonication [11].

EVALUATION OF GEL LOADED WITH MICROSPONGES AND PLAIN DRUG

Determination of Viscosity

Viscosity of the formulated gels was determined by Brookfield Viscometer using Spindle type 93/T-C.

Drug Diffusion from Microspongic Gels

The *in-vitro* measurement of drug permeation through cellophane membrane was performed in Franz Diffusion cell [12]. 1 g of gels containing free or entrapped drug were placed in the donor compartment, while the receptor compartment contained 12 mL of the receptor phase. Aliquots of 0.5 mL samples were withdrawn at suitable intervals from the receptor compartment and the drug was assayed spectrophotometrically.

Safety Considerations (Draize Skin Irritation Testing)

The irritation potential of the gels containing free drug and drugs entrapped in microsponges were evaluated in comparison to marketed gel by carrying out the Draize patch test on rabbits [13]. Animal care and handling throughout the experimental procedure was performed in accordance to the CPCSEA guidelines. The experimental protocol was approved by the Institutional Animal Ethical Committee. White New Zealand rabbits weighing 2.5-3 kg were obtained and acclimatized before the beginning of the study.

Antifungal Activity of Ketoconazole and Oxiconazole nitrate Gels

The antifungal activity of Ketoconazole and Oxiconazole nitrate from the optimum formula (microspongic-gels) as well as the free Ketoconazole and Oxiconazole nitrate and marketed formulations of the same were determined using *Candida albicans* as a representative fungus, adopting the cup plate method. The mean inhibition zone was calculated for each plate, and this value was taken as an indicator for the antifungal activity.

RESULT AND DISUSSION

PREFORMULATIONSTUDY

Characterization of Oxiconazole nitrate Pure Drug Oxiconazole nitrate

Sr. No.	Characters	Specification	Result
1.	Description	Nearly white crystalline powder	Nearly white crystalline powder
2.	Melting point	137-138°C	137-138°C
3.	Solubility	Soluble in methanol; sparingly soluble in ethanol, chloroform, and acetone; and very slightly soluble in water	Soluble in methanol; sparingly soluble in ethanol, chloroform, and acetone; and very slightly soluble in water

SPECTROSCOPIC STUDIES

UV Spectroscopy: (Determination of λmax)

The UV spectrum of Oxiconazole nitrate in methanol were scanned and λ_{max}. Was 211 nm.

IR Spectroscopy: IR Spectra of Oxiconazole nitrate in their pure form was recorded. Results are depicted in Table no. 03

Table no. 03 : IR spectrum interpretation of Oxiconazole nitrate

Functional group	Wave number observed (cm^{-1})
C-H (methylene, CH_2)	2959
C=N	1474, 1455
N-O (of cis isomer)	1330 – 1384
C-H (aromatic)	3139 – 3059

CONSTRUCTION OF CALIBRATION CURVE

Calibration curve of Oxiconazole nitrate

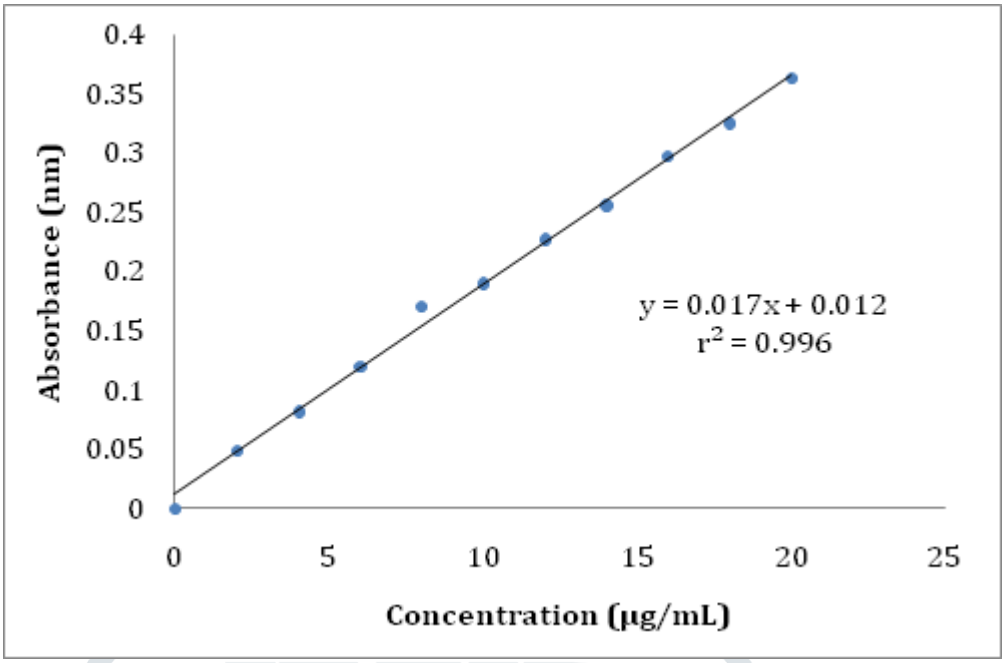


Figure no. 01: Calibration curve of Oxiconazole nitrate

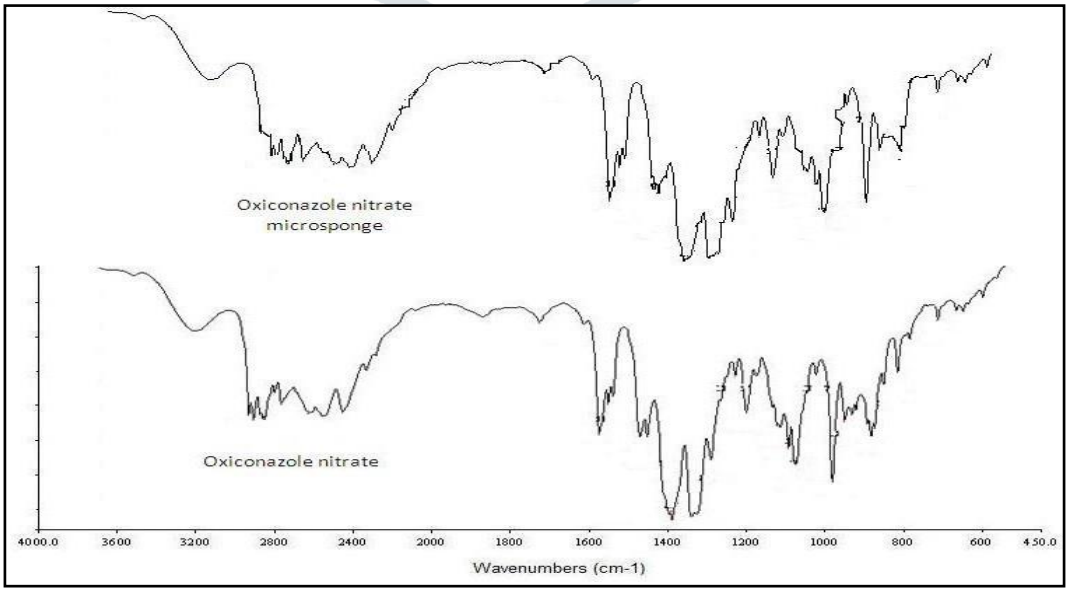
DRUG-EXCIPIENT COMPATIBILITYSTUDIES Physical Change

No physical changes such as discoloration; change in texture etc were observed during compatibility study.

FTIR Study

FTIR spectra of all the three 'pure drugs' and 'drug entrapped microsponges' were compared to study incompatibility of drugs with excipients and reaction conditions. Principal peaks of microsponge-entrapped drugs were compared with peaks of pure drugs to know about whether they are concordant with each other. Overlay FTIR spectra of pure and entrapped drugs are shown in Figure no. 02 .

Figure no. 02: Compatibility study of Oxiconazole nitrate by IR



Principle peaks of drugs were observed retained; broadening of peaks may be due to overlapping of peaks of polymer system and drug in microsponge formulation.

EVALUATION OF MICROSPONGES

Production Yield

Table no. 04 : Production yield of Oxiconazole nitrate microsponge

Formulation code	Production yield (%)
F17	72.45±1.36
F18	75.35±2.14
F19	78.21±2.84
F20	83.17±2.31
F21	86.45±1.38
F22	89.01±2.12
F23	91.47±2.81

***Each value is average of three separate determinations ±SD

production yield of Oxiconazole nitrate microspongeswere between 72.45 to 91.47 % (Table no.04). In case of Eudragit RS 100 microsponges, it was revealed that, by increasing drug: polymer ratio there is increase in the production yield of the microsponges.

Drug Loading Efficiency

Table no. 05 : Drug loading efficiency of Salicylic acid microsponge formulations

Formulation code	Drug Loading efficiency (%)
F1	86.17±1.13
F2	85.74±0.18
F3	84.38±1.24
F4	86.76±2.03
F5	87.94±1.28
F6	85.42±0.68
F7	87.71±0.47
F8	88.73±2.17
F9	85.03±0.19

***Each value is average of three separate determinations ±SD

Table no. 06 : Drug loading efficiency of Oxiconazole nitrate microsponge formulations

Formulation code	Drug Loading efficiency (%)
F17	52.65±0.28
F18	65.69±2.84
F19	70.86±1.08
F20	73.84±1.84
F21	78.49±0.37
F22	81.59±1.86
F23	84.57±1.89

***Each value is average of three separate determinations ±SD

The loading efficiency was found to be high 52.65 to 84.57 % in Oxiconazole nitrate microsponges. In case of Eudragit RS 100 microsponges, it was found that as drug: polymer ratio increases, drug loading efficiency also increases, whereas in case of Salicylic acid microsponges, it was found that, there was no significant effect of concentration of divinylbenzene on production yield and drug loading efficiency.

Scanning Electron Microscopy

SEM images showed that Eudragit RS 100 microsponges prepared by quasi-emulsion solvent diffusion method (Oxiconazole nitrate) were comparatively less spherical. .

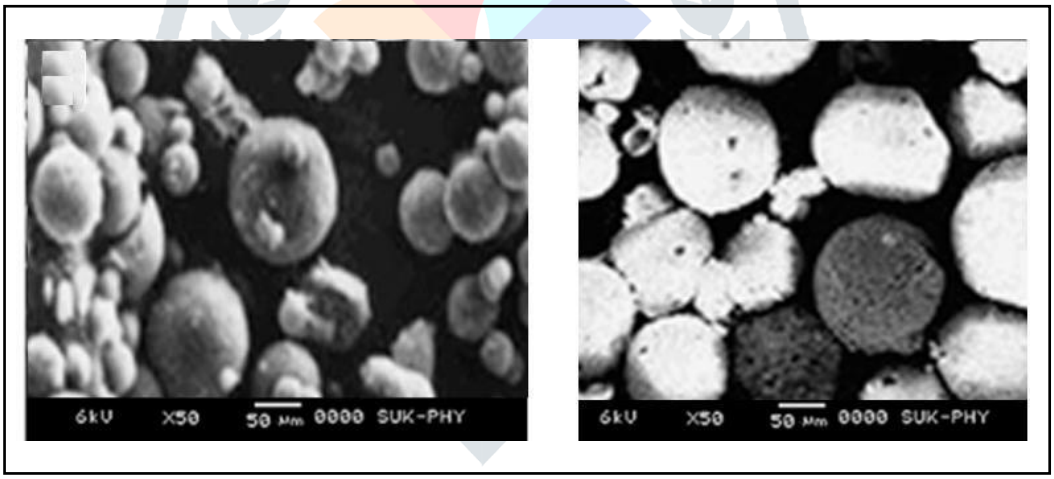


Figure no. 02 : SEM Photographs of Oxiconazole nitrate microsponges

Particle Size Analysis

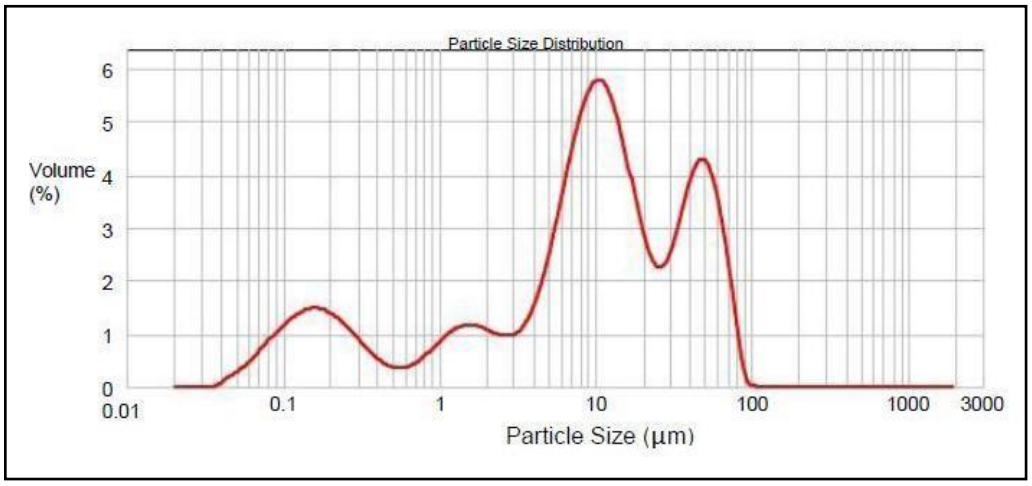


Figure no. 03 :Particle size distribution of Oxiconazole nitrate microsponges (Mean particle size10.11µm)

Free-flowing powders with fine aesthetic attributes are possible to obtain by controlling the size of particles during both the polymerization methods. Representative of the particle size distribution of Oxiconazole nitrate microsponge) is shown in Figure no. 03 The mean particle size of Oxiconazole nitrate was found to be 10.11 µm

Infrared Spectroscopy

FTIR spectra of Oxiconazole nitrate, Eudragit RS 100 and microsponges prepared by Eudragit method (F23) and overlay spectra are, as shown in Figure no. 04

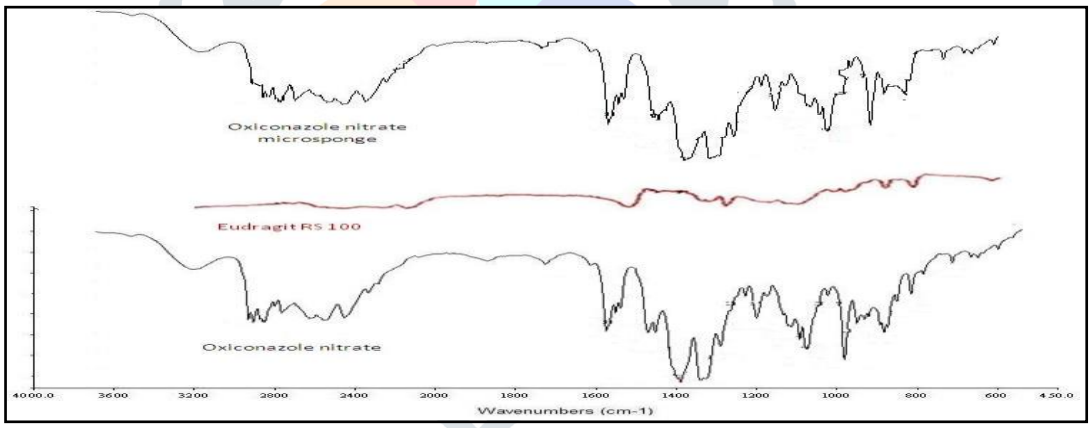


Figure no. 04: Overlay FTIR Spectra of: Oxiconazole nitrate, Eudragit RS 100 and Eudragit microsponges containing Oxiconazole nitrate

All characteristic peaks of drugs in the IR spectra of F23 formulations were observed to be concordant with respective pure drugs. Eudragit RS100 also showed an ester C=O stretching peak. These results showed that there was no chemical interaction or changes during microsponge preparation.

Differential Scanning Calorimetry(DSC)

The results of DSC were observed for the integrity of the drug in microsponge formulation prepared by the entrapment process.

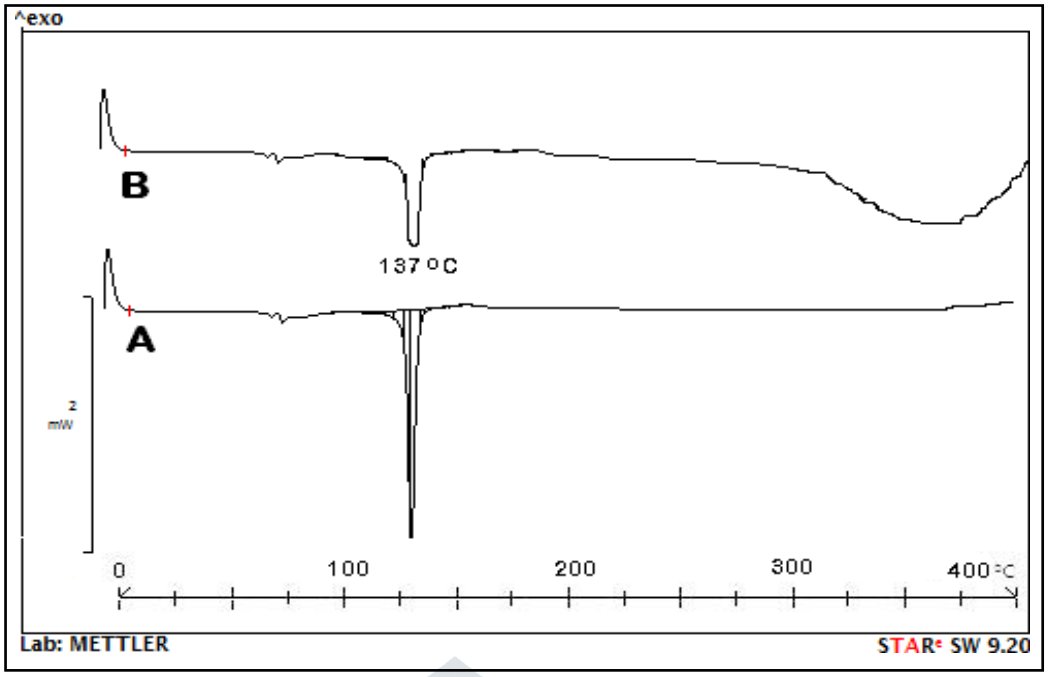


Figure no. 05 : Overlay DSC Thermograms of A: Pure Oxiconazole nitrate, B: Eudragit microsponges containing Oxiconazole nitrate

In the DSC curves of F23 formulations, characteristic peaks of, Oxiconazole nitrate and Eudragit RS 100 were seen. The thermograms of F23 formulation showed that there was no interaction between the drugs and the polymer.

Powder X-ray Diffraction Studies

X-ray powder diffraction study was done in order to get information on whether process conditions have triggered polymorphic modifications in the drug. Oxiconazole nitrate microsponges (F23) and overlay patterns are as shown in Figure no. 06.

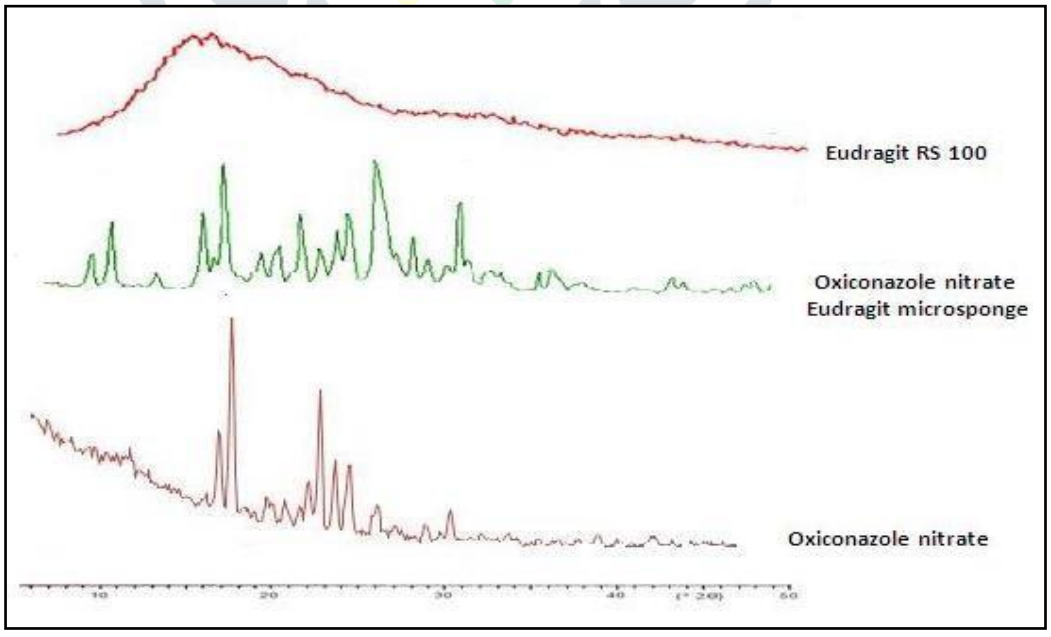


Figure no. 06: Overlay PXRD of: Oxiconazole nitrate, Oxiconazole nitrate microsponge and Eudragit RS

100

Overlain DSC thermograms, PXRDs of microspongic drugs, pure drugs and polymers revealed that drug peaks are retained but with decrease height, this is due to decreased quantity of drug in sample shared by polymers and may be due to partial amorphization of drugs.

***In-vitro* Release Study of Microsponge**

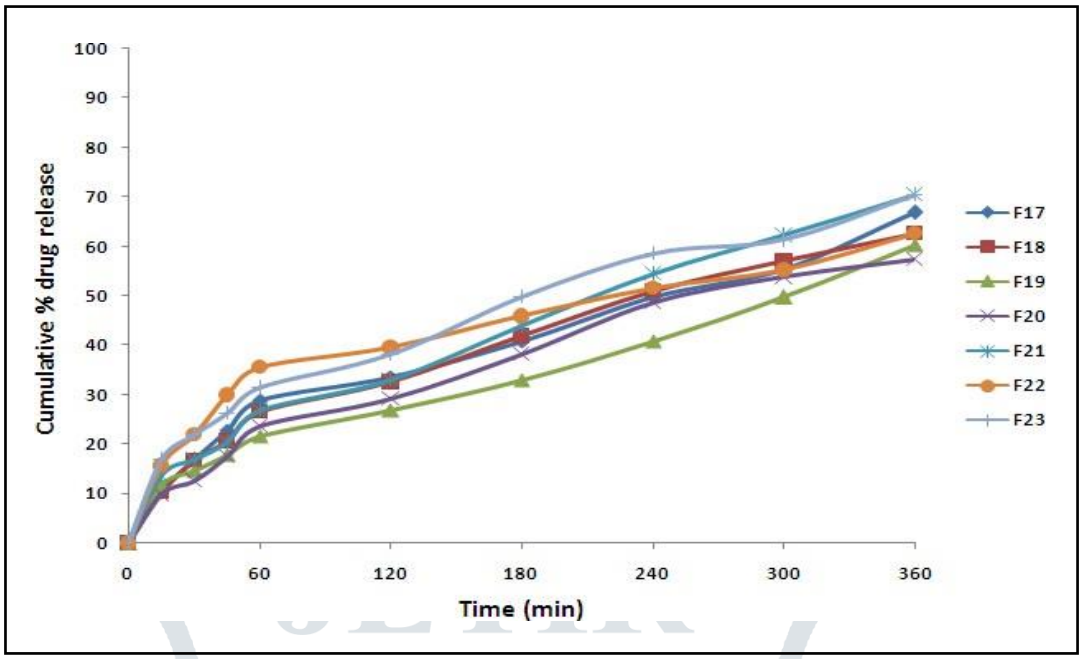


Figure no. 07 : In-vitro drug release profiles of Oxiconazole nitrate microsponge formulations

The drug release profiles of the Oxiconazole nitrate microsponge formulations are illustrated in Figure no. 07. Drug release from Oxiconazole nitrate microsponge was found to range from 57.42 % to 70.55 % for all the formulations.

Stability Profile of Microsponge Formulation

The stability studies of MDS formulation revealed that no significant changes were observed in the physical parameters, when stored at temperature and humidity conditions of 40 ± 2°C and 75 ± 5% RH to access their long-term stability. The samples were withdrawn and retested for drug content at an interval of 30 days up to 6 months.

Table no. 07 : Drug release profile of formulation F23 before and after stability

Sampling Interval	**Drug release (%) after 360 min***
	F23
0 month	70.42±0.81
1 month	69.26±1.69
2 month	70.39±0.27
3 month	70.31±1.81

4 month	69.14±1.38
5 month	70.17±0.57
6 month	70.21±0.29

*Each value is average of three separate determinations ±SD

The results indicated that no significant reduction in percentage drug release was observed over a period of 6 months; therefore no evidence of degradation of drug was observed.

EVALUATION OF GEL LOADED WITH MICROSPONGES AND PLAIN DRUG

Determination of Viscosity

Table no. 08: Viscosity of different gel formulations

Gel formulation	Viscosity (cps)
Gel containing F23 microsponge	351650±1.80
Gel containing free Oxiconazole nitrate	211584±1.30

*Each value is average of three separate determinations ±SD

Results of viscosity determination of gel showed that gel loaded with microsponge is more viscous than gel loaded with plain drug.

Drug Diffusion from Microspongic Gels

Percentage of drug released from diffusion studies during 6 hrs. from gels containing free drug, drug entrapped in microsponges (F23) are shown in Table no. 09 and in Figure no. 08

Table no.09 : Cumulative % drug released from gels loaded with pure drug and microsponge entrapped drug.

Time in minute	Cumulative % drug released	
	Free Oxiconazole nitrate	F23
0	0	0
30	2.12±1.28	3.41±1.01
60	4.27±1.94	5.38±1.32
90	6.27±1.05	11.47±0.93
120	9.84±1.32	13.41±1.71

150	12.71±0.74	15.48±1.37
180	14.64±0.39	15.97±1.28
210	17.96±0.26	16.89±1.03
240	19.38±1.85	17.85±1.35
270	22.41±1.06	19.38±1.28
300	25.31±1.71	21.61±0.94
330	27.84±1.06	23.54±0.34
360	31.59±1.02	25.96±0.63

*Each value is average of three separate determinations ±SD

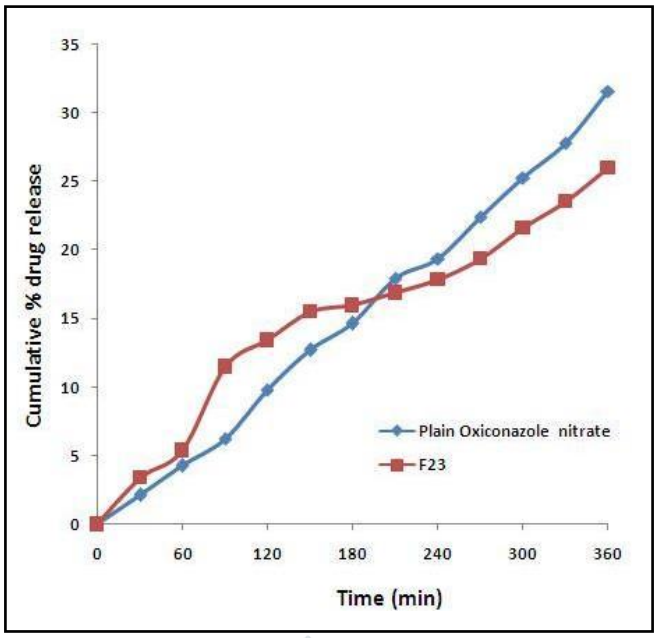


Figure no. 08 : Drug release Vs Time plot of gels containing plain Oxiconazole nitrate and drug entrapped in F23 microsponges

Table no. 10 : Kinetic study

Sr. No.	**Kinetic model**	**F23**
1.	First order	0.893
2.	**Higuchi**	**0.969**
3.	Korsemayer Peppas	0.959
4.	Hixon Crowell	0.969
5.	Zero order	0.962

Safety Considerations (Draize Skin Irritation Test)

Figure no. 09 shows photographs of primary skin irritation studies where Site 1 indicates positive control; Site 2 indicates test drug entrapped in the microsponges; Site 3 indicates marketed product and Site 4 indicates negative control

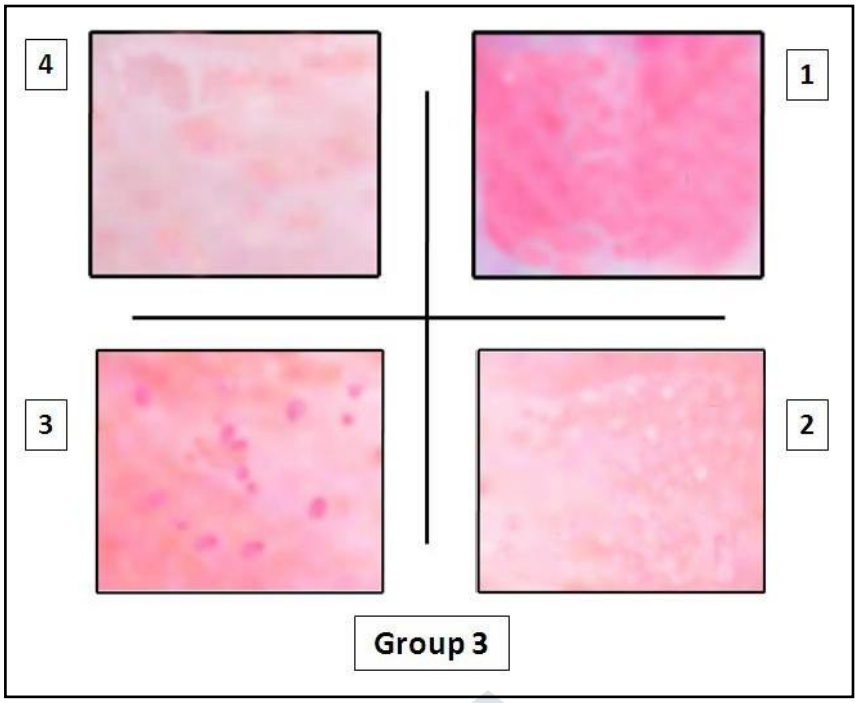


Figure no. 09 : Photographs of skin irritation studies carried out on New Zealand rabbits: Group 3: Oxiconazole nitrate microsponge gel

Standard Calibration Curve of Oxiconazole nitrate using Cup Plate Method

Table no. 11 : Zone of inhibitions for standard Oxiconazole nitrate for calibration curve

Concentration of Oxiconazole nitrate (µg/mL)	**Zone of inhibition in mm**					**Mean ± SD**
500	10.1	11.5	10.6	10.3	10.5	10.1±0.54
750	13.5	14.6	13.4	13.5	14.8	13.4±0.68
1000	16.4	16.9	16.5	16.4	16.7	16.4±0.22
1250	19.2	18.7	18.7	19.6	18.6	18.6±0.43
1500	21.4	21.4	21.3	21.7	21.5	21.3±0.15
1750	24.6	24.5	24.6	23.3	24.8	23.3±0.60
2000	27.8	26.8	27.5	27.5	26.9	26.8±0.43

CONCLUSION

Quasi-emulsion solvent diffusion is now a days the preferred method to prepare porous microparticles. Eudragit RS100 microsponges containing Oxiconazole nitrate was successfully prepared by while those prepared by quasi-emulsion solvent diffusion method were comparatively less spherical. According to

intrusion and extrusion curves, the majority of the pores present in Eudragit RS100 microsponges were spherical type,. The microsponges differ from regular microspheres with their highly porous surface. This characteristic gives property to release the drug at a faster rate through the pores. Due to smaller pore diameter, the Eudragit Rs 100 microsponges showed less and slower drug release as compared with the styrene microsponge formulations, in the in-vitro release studies. Release from all the microsponges followed zero order reaction kinetics. Viscosity determination of gel showed that gels loaded with microsponge were more viscous than gel loaded with plain drug. The controlled drug release was observed with all the microspongic gels. Drug release from all the gels was best fitted in Higuchi model. Gels containing microsponge entrapped Ketoconazole and Oxiconazole nitrate showed that antifungal activity of drugs were retained and it was higher as compared with gels containing free drug and marketed formulations. During storage at 40±2 °C and 75±5% RH for 6 months, no notable changes in surface morphology and release of drugs were observed. It was observed that the gels containing free drug (marketed product) shows more irritation than the gels containing drug entrapped in MDS.

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