International Journal of Research and Review
DOI: https://doi.org/10.52403/ijrr.20211059
Vol.8; Issue: 10; October 2021
Website: www.ijrrjournal.com
Original Research Article *E-ISSN: 2349-9788; P-ISSN: 2454-2237*

Development and Characterization of Metronidazole Loaded Microsponges for the Management of Diabetic Foot

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ABSTRACT

The objective of present work was formulation and evaluation of Metronidazole loaded microsponges for the management of diabetic foot ulcer via topical application and to reduce side effects. The microsponges were prepared by quasi-emulsion solvent diffusion method using different concentrations of Ethyl cellulose and Poly vinyl alcohol. The prepared microsponges were evaluated for particle size analysis, SEM, % production yield, % drug entrapment efficiency, in-vitro drug release studies, DSC and antimicrobial studies. FTIR studies shown that there was no interaction between drug and polymers. The optimum sustained release of drug around a period of 12hrs was shown by formulation F8. The n value of optimized formulation indicated that the drug release followed zero order kinetics. It was confirmed from the stability studies that the optimized formulation remained stable at 45±2 °C and 70±5% relative humidity.

Keywords: Microsponges, Metronidazole, Diabetic Foot, Quasi-emulsion solvent diffusion, Sustained release, Scanning electron microscopy, Differential scanning calorimetry.

INTRODUCTION

The application of drug via skin to directly treat or cure the skin disorders is called topical delivery. Topical delivery systems are generally used for local skin infection or in places where other routes of the drug administration fail. These dosage forms are mostly applied to a small area anywhere in the body through ophthalmic, rectal, vaginal and skin as route. Skin is the largest and most accessible organ of human body Controlled drug delivery has wide and increased application in pharmaceutical industry. For topical delivery, drugs having lipophilic character are mostly suitable. These systems make the drug enter into the body and reach the area where it is needed. For providing local or systemic effects topical dosage forms are applied on to the skin.

As compared to the conventional system topical route favours safe and effective delivery of drugs with smaller doses. Drugs via skin reach the desired area in optimum concentration, thereby reduces the chance of side effects which leads to increased bioavailability and increased patient compliance.

Transdermal Drug Delivery System (TDDS) are defined as self-contained, discrete dosage forms which when applied to the intact skin, deliver the drug through the skin at a controlled rate to the systemic circulation. The dosage forms which are designed to deliver a therapeutically effective amount of drug across a patient's skin are called TDDS. The main objective of transdermal drug delivery system is to deliver drugs into systemic circulation through skin at predetermined rate with minimal inter and intra patient variation. The one which delivers the drug at a predetermined rate, for locally or systemically, for a specified period of time is referred to as controlled delivery.

Controlled drug delivery systems provide the maintenance of drug levels within a desired range, fewer administrations, optimal use of the drug in question, and increased patient compliance. Currently many novel drug delivery systems are available such as organogel, emulgels, microsponges. hydrogels, liposomes, niosomes, etc.

In recent years, in order to modify and control the release behaviour of the drugs, a considerable priority has been given to develop novel Microsponge based drug delivery systems. Microsponges are porous, polymeric microspheres that are used mostly for topical use and have recently been used for oral administration. In this research work the Microsponges approach will be used to overcome the problems with the conventional topical / transdermal drug delivery systems. The polymeric delivery systems composed of porous microspheres which can enhance the stability, reduce side effects and modify drug release favourably are defined as microsponges. Mechanism of action highlights the importance of formulation. Microsponges are skilful to absorb skin secretion thereby they can reduce the moisture and prevent the infection and growth of bacteria at the site of action. Metronidazole [64][65] is the drug of choice for anaerobic infection in diabetic foot ulcers (DFU) for a majority of clinicians. The present study is conducting to establish that the Metronidazole loaded microsponges are really making a difference in the management of DFU.

MATERIALS & METHODS

Metronidazole (MTZ) was obtained as a gift sample from Balaji Drug Company, Gujarat. Poly Vinyl Alcohol (PVA) was purchased from Yarrow Chem, Mumbai. Ethyl cellulose and Dichloromethane were kindly given as a gift sample by Balaji drug company, Gujarat. All the chemicals used were of analytical grade and were used as received.

Methods

Preparation of Metronidazole loaded microsponges by Quasi-emulsion solvent diffusion method [42]. The microsponges of respective composition as shown in table 1 were formulated using Ethyl cellulose as a polymer, Poly Vinyl Alcohol as a stabilizer.

Table: 1 Composition of Metronidazole loaded microsponges

COMPONENTS	F1	F2	F3	F4	F5	F6	F7	F8
Drug(g)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Ethyl cellulose(g)	2	3	4	5	2	3	4	5
Dichloromethane(ml)	10	10	10	10	10	10	10	10
PVA(g)	0.3	0.3	0.3	0.3	0.7	0.7	0.7	0.7

Fig 1: Preparation of microsponges by quasi emulsion solvent diffusion method

The microsponges loaded with Metronidazole were prepared by quasi emulsion solvent diffusion method. For that mainly two phases were considered, an internal phase and an external phase. The internal phase consists of accurately weighed amount of Metronidazole and Ethyl cellulose dissolved in Dichloromethane. The external phase consists of polyvinyl alcohol dissolved in warm water. PVA was used as an emulsifying or stabilizing agent. The internal phase was gradually added to external phase and stirred mechanically at 500rpm for 2 hours at room temperature to remove the solvent Dichloromethane from

the mixture. Microsponges formed were filtered and dried at room temperature and stored in a tightly closed container

Evaluation of Metronidazole loaded microsponges

PREFORMULATION STUDIES

Preformulation studies such as determination of organoleptic characteristics, solubility, melting point, determination of λ_{max} and incompatibility study using FT-IR spectroscopy were conducted.

Determination of organoleptic properties

The physical appearance of the drug was observed and compared with the Pharmacopoeial specifications.

Determination of melting point

Melting point was determined by capillary method. Fine powder of Metronidazole was filled in glass capillary tube (previously sealed at one end). The capillary tube was inserted into the melting point apparatus and observed the temperature at which drug started to melt by using the thermometer which was already immersed into the liquid paraffin in the apparatus. The practically obtained melting point of Metronidazole was compared with that of theoretical melting point of the same.

Solubility

The solubility of Metronidazole was determined in various solvents such as water, Dichloromethane, Ethanol and Acetone. For those small increments of Metronidazole were added to 10ml of solvent (water, ethanol, dichloromethane, and acetone) in a 25ml stoppered flask with vigorous shaking. The solution was visually observed and if the solution was clear and no undissolved particles were observed, again another increment of Metronidazole was added and the procedure was continued until undissolved Metronidazole was found.

Compatibility studies

FT-IR Spectroscopy of Metronidazole

The pure drug was scanned from 4000-500cm^{-1} in FT-IR spectrophotometer. The FT-IR spectrum of the obtained sample of drug and polymer were compared with the standard functional group frequencies.

Compatibility between drug and polymer

FT-IR spectroscopy was carried out to check the compatibility between drug and polymer. The compatibility between the drug, polymer were evaluated using FTIR peak matching method.

PREPARATION OF STANDARD CALIBRATION CURVE

Preparation of standard calibration curve of Metronidazole

Weighed accurately 50mg of pure Metronidazole & made upto 50ml with 0.1 N HCL (stock solution A). Taken 10.0 ml of the above solution & diluted further to 50.0 ml with 0.1 N HCL (stock solution B). Again taken 10 ml of stock solution B and made upto 100 ml with 0.1 N HCl. Pipetted out 0.2ml, 0.4ml, 0.6ml, 0.8ml,1.0mlof the solution & diluted to 10.0 ml in separate 10ml volumetric flask to make 2,4,6,8,10µg/ml concentration solutions. The absorbance was measured at 277 nm and standard calibration curve was plotted.

CHARACTERIZATION OF METRONIDAZOLE LOADED MICROSPONGES

(1) Physical properties

The prepared Metronidazole loaded microsponge formulations were inspected visually for their colour and appearance.

(2) Particle size analysis

Determination of the average particle size of Metronidazole loaded microsponges was determined with an optical microscope using a calibrated ocular and stage micrometer [42]. A minute quantity of microsponges was spread on a clean glass slide with a drop of liquid paraffin and a cover slip was placed on it. The average particle size was calculated by measuring

100 particles of each batch using the equation:

$dav = \sum nd / \sum n$

Where, dav is the average diameter of particles (µm), n is number of particles per group, and d is the middle value (µm).

Fig 2: Particle size analysis of microsponges using optical microscope

(3) Scanning electron microscopy

For the evaluation of surface morphology of microsponges, the sample was analyzed in scanning electron microscope. The samples were randomly scanned and photomicrographs were taken at the acceleration voltage of 20Kv.From the resulting image, average particle size was determined.

(4) Production yield (%)

Percentage yield can be determined by calculating the initial weight of raw materials and the finally obtained weight of microsponges. Percentage yield can be calculated by using the formula [22]:

Production yield=(practical mass of microsponges)/(Theoretical mass[drug&polymer])*100

(5) Drug content estimation and Entrapment efficiency

Samples of drug loaded microsponges (100mg) were dissolved in 10ml phosphate buffer pH 7.4 under sonication for 20min at 25 °C. The samples were filtered using 0.45µm membrane filter and analyzed for Metronidazole content spectrophotometrically using UV-VIS double beam spectrophotometer at 277 nm. The actual drug content and entrapment efficiency were calculated as given below [22]:

Percentage drug content=(Actual drug content)/(Drug added in microsponges)*100

Percentge entrapment efficiency=(Actual drug in microsponges)/(Theoretical drug content)*100

(6) Differential Scanning Calorimetry (DSC)

DSC studies were carried out using Simultaneous Thermal Analyser STA 8000 instrument equipped with an intercooler. Indium and zinc standards were used to calibrate the DSC temperature and enthalpy scale. The samples were hermetically sealed in aluminum crucibles and heated at a constant rate of 10°C/min over a temperature range of 25–300°C. Inert atmosphere was maintained by purging Nitrogen gas at flow rate of 50 mL/min.

(7) In- vitro drug release studies

Fig 3: In-vitro drug release study using USP Type I Apparatus

In-vitro drug release study was carried out in USP dissolution test apparatus. A quantity of microsponges equivalent to 100 mg of Metronidazole microsponges was kept in basket type apparatus and immersed in 900ml of phosphate buffer (pH 7.4) in 1000 ml

dissolution flask and temperature was maintained at 37±0.5ºC throughout the study. At predetermined time intervals 2 ml of samples was withdrawn by means of a syringe fitted with prefilter and same was replaced into the dissolution flask with phosphate buffer pH 7.4. The absorbance of sample was measured at 276 nm after required dilution with the fresh medium (pH.7.4). All the studies were conducted in triplicate.

(9) Kinetics of In-vitro drug release

The results obtained from in-vitro release studies were attempted to be fitted into various mathematical models as follows:

1. Cumulative percent drug released Vs Time (Zero order kinetics)
2. Log cumulative percent drug retained Vs. Time (First order kinetics
3. Cumulative percent released Vs Square root of Time (Higuchi model)
4. Log cumulative percent drug released Vs Log Time (Korsemeyer –Peppas model)

Kinetic Models

Zero Order Kinetics

It describes the system in which the drug release rate is independent of its concentration.

$Q_t = Q_0 + K_{0t}$

Qt is the amount of drug released at time 't' and

K_0 is the zero- order release rate constant expressed in units of concentration/time. To study the release kinetics, cumulative amount of drug released Vs time. Zero order kinetics can be used to describe the drug dissolution of modified release pharmaceutical dosage forms, matrix tablets with low soluble drugs in coated forms, osmotic systems etc.

First order Kinetics

It describes the drug release from the systems in which the release rate is concentration dependent.

$\log Q_t = \log Q_0 + kt/2.303$

Where, Q_t is the amount of drug released in time 't'

Q_0 is the initial amount of drug

K is the first order release constant

The data obtained from in vitro drug release studies were plotted as log cumulative percentage of drug remaining Vs time. First order kinetics can be used to describe the drug dissolution in pharmaceutical dosage forms such as those containing water-soluble drugs in porous matrices.

Higuchi model

It describes the fraction of drug release from a matrix is proportional to the square root of time. $Q = K_2 t^{1/2}$

Q is the percentage of drug released at time 't' and

K_2 is the Higuchi dissolution constant

The data obtained from in vitro drug release studies were plotted as percentage cumulative drug released Vs square root of time. Higuchi model can be used to describe the drug dissolution from modified release pharmaceutical dosage forms and matrix tablets with water soluble drugs and also to low water-soluble drugs incorporated to solid/semisolid polymer matrix.

Korsmeyer-Peppas model

It describes the drug release from the polymeric system in which release deviates from Fickian diffusion, as expressed in following equation.

$Q = Kt_n$

Q is the percentage of drug released at time 't'

K is the release rate constant and

'n' is the diffusion release exponent indicative of the mechanism of drug release.

To study the release kinetics, the data obtained from in vitro drug release studies were plotted as log percentage cumulative drug release Vs time. Non-Fickian diffusion refers to combination of both diffusion and erosion-controlled rate release

(II)MICROBIAL STUDIES

The organisms used in the study were Staphylococcus aureus and E.coli.

Disk Diffusion Method

An antimicrobial assay was performed by using the Kirby-Bauer disk diffusion agar plate method.Agar plate were prepared by pouring freshly prepared agar medium to the sterilized petridishes after autoclaving. The microbial suspension of Staphylococcus aureus and E.coli were applied onto the solidified agar by using sterile cotton swabs and allowed to dry for 10 minutes.Formulated drug loaded microsponges impregnated discs were aseptically transferred onto the inoculated agar plates and left to be inoculated for 2 days.The clear zones of inhibition around the test sample disc were shown for any indication of antimicrobial activity.All assays were carried out in triplicate.

(III)STABILITY STUDY

In any rational drug design or evaluation of dosage forms, the stability of the active component was a major criterion in determining their acceptance or rejection.For stability testing the formulation (F8) was stored at accelerated condition in aluminum foils for 3 months. The samples were withdrawn after end of 1st month, 2nd month and 3rd month. The samples were analyzed for its drug content and in vitro drug release.

RESULTS AND DISCUSSION

Determination of organoleptic characters

Table 2 :Organoleptic properties of drug sample

SAMPLE	COLOUR	ODOUR
Metronidazole	White to yellowish crystalline powder	Odourless

Determination of melting point

The experimental value of melting point of Metronidazole sample was in good agreement with the official value(159-163),thus indicating the purity of sample

Table 3:Melting point of Metronidazole

SAMPLE	MELTING POINT OBSERVED
Metronidazole	160 °C

Solubility studies

Solubility of Metronidazole in various solvents like dichloromethane, acetone, ethanol and water were studied and found that it was freely soluble in dichloromethane and slightly soluble in acetone, ethanol and water.

Table 4: Solubility of Metronidazole

SOLVENTS	AMOUNT OF MTZ DISSOLVED IN 10 ml	VALUES IN mg/ml	Solubility
Dichloromethane	0.08	0.008	Freely soluble
Acetone	0.06	0.006	Slightly soluble
Ethanol	0.05	0.005	Slightly soluble
Water	1	0.1	Slightly soluble

COMPATIBILITY STUDIES

FTIR spectroscopy

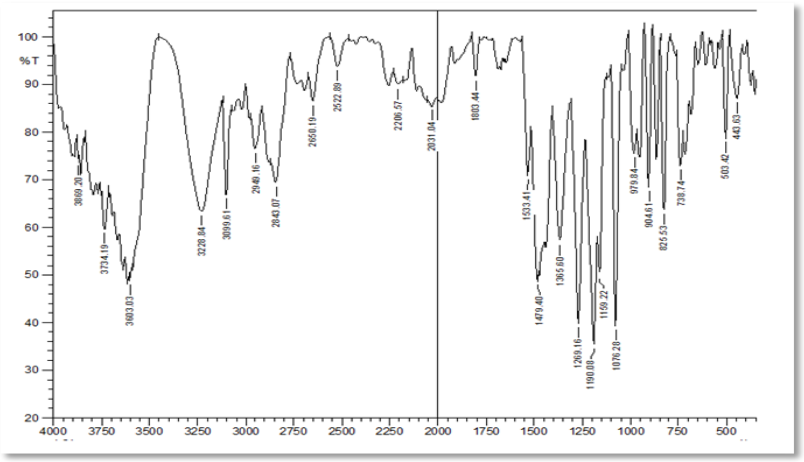


Figure 4:The FT-IR spectrum of Metronidazole

Table 5: IR frequencies of Metronidazole

Functional groups	Standard IR peaks	Observed peaks
N=O	1550-1350	1533.41
=C-H strecth	3100-3000	3099.61
-CH_3 bending	1475-1365	1365.60
-CH_2 bending	1465	1479.40
-C=C alkene	1600&1465	1533.41

The FTIR spectrum of Metronidazole is shown in Figure 4, which complies with standard functional group frequencies.

2 Compatibility between drug and polymer

The FTIR spectrum of combination of Metronidazole with excipients are shown in figure 5.

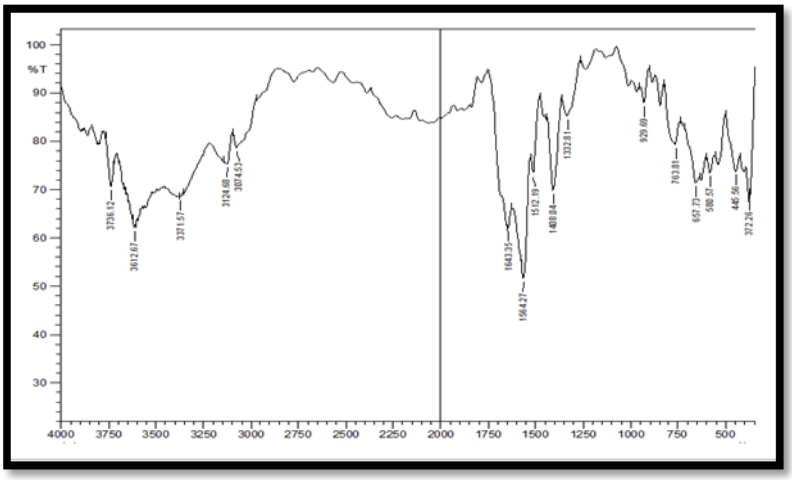


Figure 5 :FTIR spectrum of physical mixture of drug & polymers

Table 6: IR frequencies of Metrnodizaole with other excipients

Functional groups	Standard IR peaks	MTZ observed peaks	Observed IR peaks
N=O	1550-1350	1533.41	1512.19
=C-H stretch	3100-3000	3099.61	3074.53
-CH_3 bending	1475-1365	1365.60	1332.81
-CH_2 bending	1465	1479.40	1408.04
C=C alkene	1600&1475	1533.41	1408.04

After the compatibility study of Metrnonidazole with excipients,the IR spectra of pure drug and drug-excipient physical mixture were analyzed.The peaks analyzed in the Table 6 indicate that,most characteristic frequencies of functional group of Metronidazole which are N=O,=C-H stretch ,-CH_3 bending and C=C were found unchanged.This shows that the Metronidazole remained unaffected by the excipients used.No new complexes were observed as well.So it could be concluded that there was no interaction between drug and excipients used.

PREPARATION OF STANDARD CALIBRATION CURVE OF METRONIDAZOLE

Standard calibration curve of Metrnonidazole was determined in Hydrochloric acid by measuring the absorbance of the standard solutions at 277 nm using double beam UV spectrophotometer.

Table7: Absorbance of Metronidazole standard solutions at 277 nm

Concentration (µg/ml)	Absorbance (nm)
2	0.082
4	0.175
6	0.257
8	0.342
10	0.481

Figure 6 shows standard calibration curve of Metronidazole with slope and regression coefficient and intercept of 0.9986 and 0.0434 respectively. It was found that the solutions show linearity (R2=0.9986) in the range of 2-6 µg/ml at ʎmax 276 nm and obeys Beer Lambertʾs law.

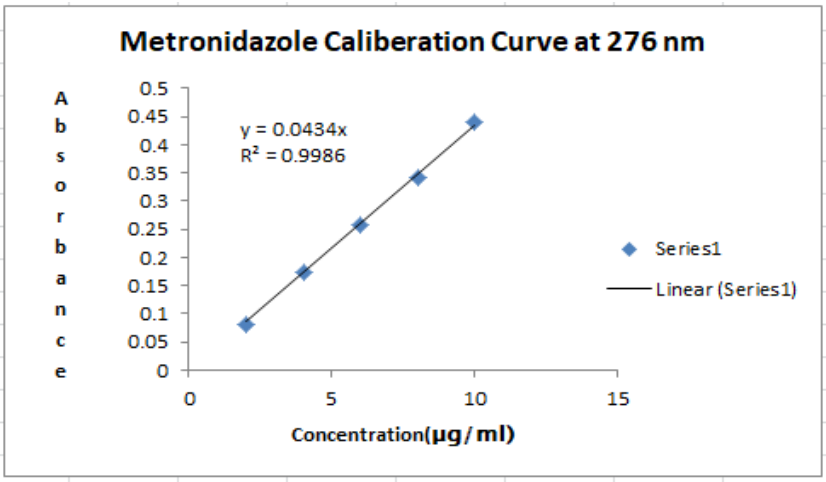


Figure 6 :Standard calibration curve of Metronidazole at 276nm

FORMULATION OF METRONIDAZOLE LOADED MICROSPONGES

Metronidazole loaded microsponges were prepared by quasi-emulsion solvent diffusion method at varying concentrations of polymer and emulsifire as shown in table 1 .

CHARACTERISATION AND EVALUATION OF METRONIDAZOLE LOADED MICROSPONGES

Physical properties

Table 8: Colour and appearance of prepared microsponge formulations

FORMULATION CODE	COLOUR	APPEARANCE
F1	White	Spherical,free flowing
F2	White	Spherical,free flowing
F3	White	Spherical,free flowing
F4	White	Spherical,free flowing
F5	White	Spherical,free flowing
F6	White	Spherical,free flowing
F7	White	Spherical, free flowing
F8	White	Spherical , free flowing

All the prepared Metronidazole micropsonge formulations were white in colour,free-flowing in nature and had rigid spherical structure. The concentration of emulsifying agent or external phase has a major role to play in the formation of microsponges.Minimum concentration of external phase is required.Insufficient concentration of emulsifying agent produces unstable microsponges.

Particle size analysis

The particle size of microsponges was determined using an optical microscope.The mean particle size of Metronidazole loaded microsponges ranged from 11.51 to 20.82 µm as shown in Table 9.It was found that,when concentration of polymer increases, the mean particle size of the microsponge also increases.This may be attributed to the hugher viscosity of the internal phase ,thus increasing the chances of formation of bigger particles and faster diffusion of the solvent.

Table 9:Mean particle size of MTZ microsponges

FORMULATION	MEAN PARTICLE SIZE (µm)
F1	11.51µm
F2	13.32µm
F3	15.64µm
F4	17.52µm
F5	16.79µm
F6	17.83µm
F7	19.7µm
F8	20.82µm

Scanning electron microscopy

The surface morphology of the optimized microsponge formulationF8 was investigated by scanning electron microscopy (SEM). The SEM image is shown in figure 8.The SEM images showed that the surface of prepared microsponges was spherical in shape and uniform in size and its surface was porous in nature.The pores were induced by the diffusion of the volatile solvent(dichloromethane) from the surface of the microparticles. No intact crystal of drug was seen visually. Based on

SEM studies, the mean particle size of microsponges was found to be 20 μm.

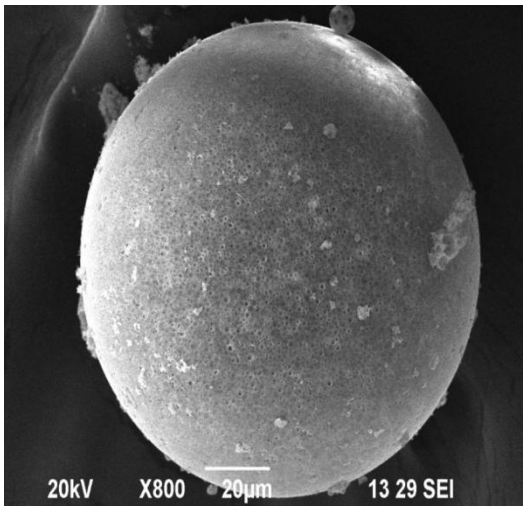


Fig 8: SEM images of MTZ loaded microsponges

Production yield(%)

The production yield of Metronidazole loaded microsponges was found to be in the range of 44.7-94% as reported in Table.When the concentration of polymer added was increased ,the production yield of microsponges was also found to be increased.This may be due to the higher amount of polymer,thus resulting in an increase in total mass of the microsponges.

Table 10: Percentage production yield of microsponge formulations

FORMULATION	PRODUCTION YIELD(%) mean±SD
F1	44.7%
F2	49.37%
F3	46%
F4	51.05%
F5	89%
F6	92%
F7	90%
F8	94%

Drug content (%)

The percentage drug content of drug loaded microsponges was found to be in the range of 90-98.6% as shown in table 5.11. From that it was found that the drug remained in entrapped form in microsponges and was uniformly distributed.

Table 11:Percentage drug content of prepared microsponges

FORMULATION	DRUG CONTENT(%) mean±SD
F1	90.6%
F2	92%
F3	93.3%
F4	94.6%
F5	90%
F6	93%
F7	97%
F8	98.6%

Drug entrapment efficiency

The percentage drug entrapment efficiency of Metronidazole loaded microsponge formulations ranged from 60-98.6% as shown in Table 12.The results of drug entrapment efficiency(%) showed that with increase in polymer concentration ,the drug entrapment efficiency(%) also increased.The increase in drug entrapment efficiency with increase in polymer concentration may be due to the sufficient amount of polymer being available for the drug to be entrapped.

Table12: Percentage drug entrapment efficiency of microsponge formulations

FORMULATION	DRUG ENTRAPMENT EFFICIENCY(%)
F1	60
F2	62
F3	82
F4	86
F5	70
F6	93.33
F7	72
F8	98.66

Differential scanning calorimetry

In DSC studies,dispersed in polymer showed the same thermal behaviour as a pure compound.In the thermogram,the endothermic peak was observed at 160℃ which does not correspond to the melting point of the pure drug.During formulation of microsponges the drug was entrapped inside the microsponges and was not available for showing any exothermic peak.Hence,no endothermic peak near to the melting point of the drug was observed confirming the entrapment of drug in microsponges.This indicates that the physical properties of Metronidazole were altered during formulation of microsponges using Ethyl cellulose.

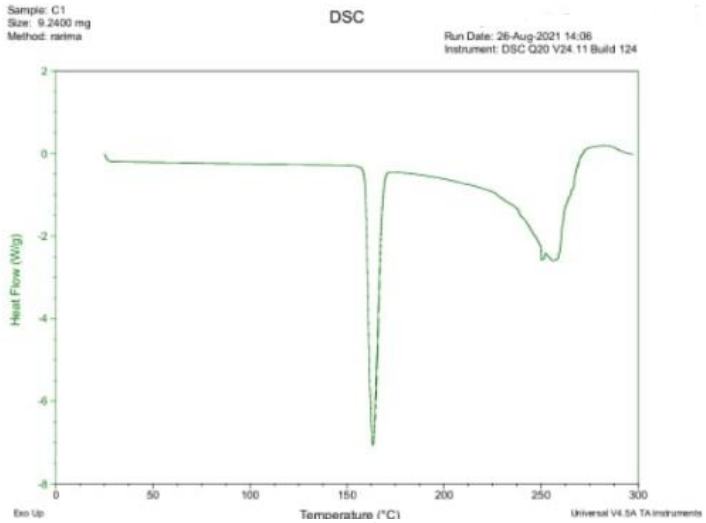


Fig 9:DSC Thermogram of pure Metronidazole

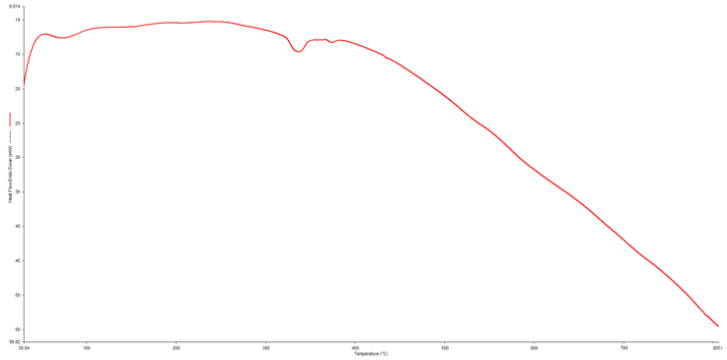


Fig 10:DSC Thermogram of optimized formulation F8

Invitro drug release study

The in-vitro drug release studies were carried out using USP Type I apparatus for 12hrs

Table 13: Percentage cumulative drug release data for formulations F1-F8

TIME (hrs)	F1 (%CDR)	F2 (%CDR)	F3 (%CDR)	F4 (%CDR)	F5 (%CDR)	F6 (%CDR)	F7 (%CDR)	F8 (%CDR)
0	0	0	0	0	0	0	0	0
0.25	19.92	17.64	19.92	17.23	19.51	19.92	18.89	19.51
1	26.158	23.25	26.15	24.70	22.83	24.49	24.70	21.79
2	33.00	31.97	33.00	28.23	28.23	28.23	27.81	25.74
3	38.40	38.61	38.40	35.08	32.80	32.80	32.38	30.10
4	42.76	42.76	42.76	45.88	40.27	40.27	42.97	43.38
5	48.99	51.07	48.99	53.14	43.59	43.59	46.29	47.12
6	59.16	59.37	59.16	61.03	51.69	51.69	54.80	53.14
7	67.67	63.73	67.67	63.11	59.99	59.99	59.58	61.03
8	75.77	68.09	74.52	71.62	64.97	65.39	66.22	66.43
9	80.75	79.92	80.75	79.92	79.71	76.60	71.00	76.39
10	85.53	84.70	85.53	84.70	83.87	86.15	85.53	88.43
11	87.19	88.43	87.19	87.19	88.43	88.43	90.51	95.91
12	92.38	93.83	94.04	94.87	95.08	96.53	97.98	99.85

From the in-vitro drug release data of MTZ microsponges, it was observed that the percentage cumulative drug release of MTZ decreased as the concentration of ethyl cellulose was increased.The increase in the ethyl cellulose concentration leads to the

increased density of polymer matrix of microsponges which results in an increased diffusion path length.This may decrease the overall drug release from the polymer matrix.The optimum controlled release of drug was shown by formulationF8.F8 released 99.85% of the drug in 12 hrs.Among all the formuations ,the least % cumulative drug release of 92.38% was shown by F3.F3 contains microsponges formed using low concentration of emulsifying agent compared to F8. Therefore F1to F4 showed a decrease in drug release compared to F8.

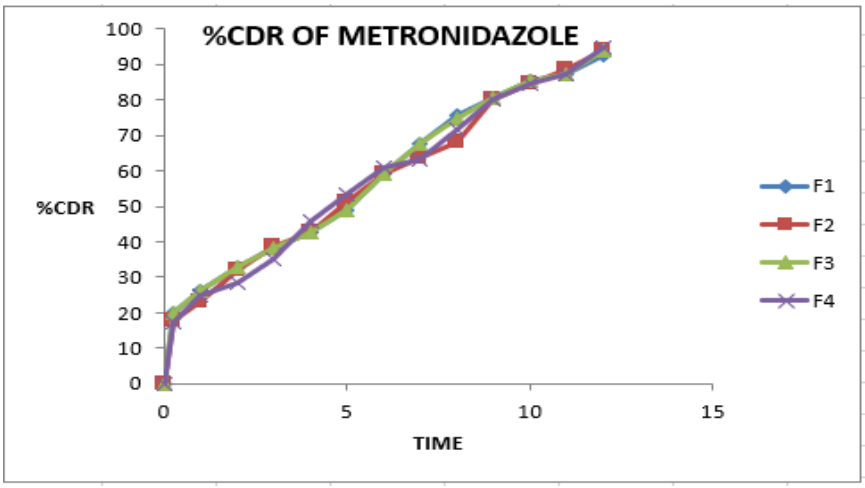


Fig11:Comparison of percentage cumulative drug release profile of formulations F1-F4.

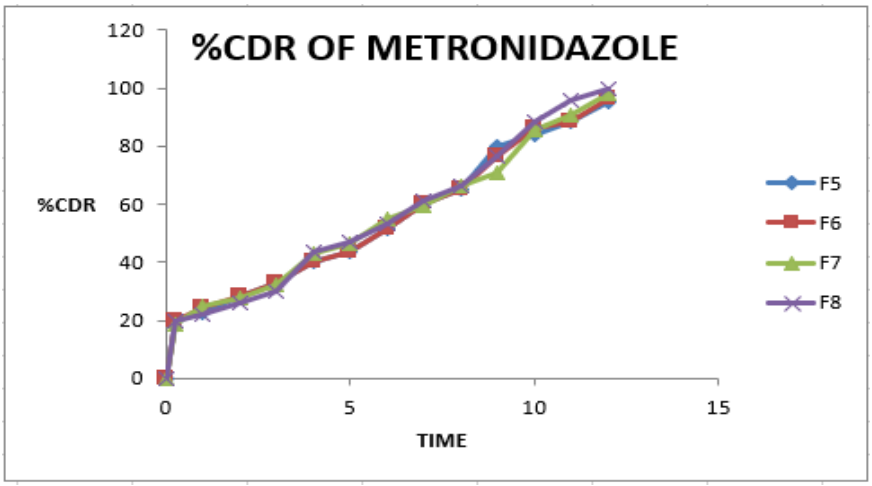


Fig 12Comparison of percentage cumulative drug release profile of formulations F5-F8

Kinetics of In vitro drug release

Table 14: Kinetic study of MTZ microsponges

Formulation	Drug release kinetics				
	Zero order R^2	First order R^2	Higuchi R^2	Peppas R^2	n
F1	0.9602	0.957	0.978	0.037	0.202
F2	0.973	0.926	0.987	0.630	0.575
F3	0.966	0.936	0.981	0.639	0.581
F4	0.972	0.917	0.985	0.625	0.59
F5	0.977	0.874	0.986	0.555	0.571
F6	0.976	0.834	0.985	0.516	0.595
F7	0.976	0.772	0.986	0.469	0.637
F8	0.980	0.628	0.987	0.347	0.664

The invitro drug release data of all the MTZ microsponges was subjected to goodness of fit test by linear regression analysis according to zero order and first order kinetic equations,Higuchi's and Korsmeyer-Peppas models to ascertain the mechanism of drug

release.The results of linear regression analysis including regression coefficients are summarized in Table 14

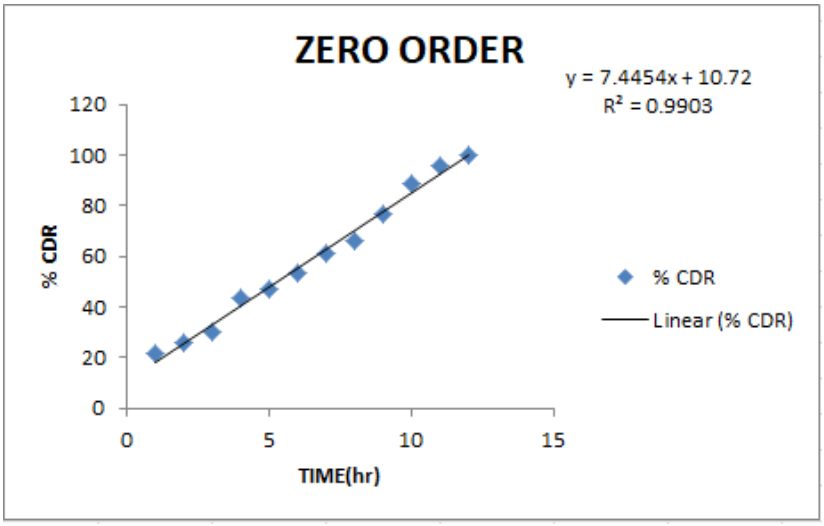


Fig13:Zero order release kinetics profile of optimised formulation F8

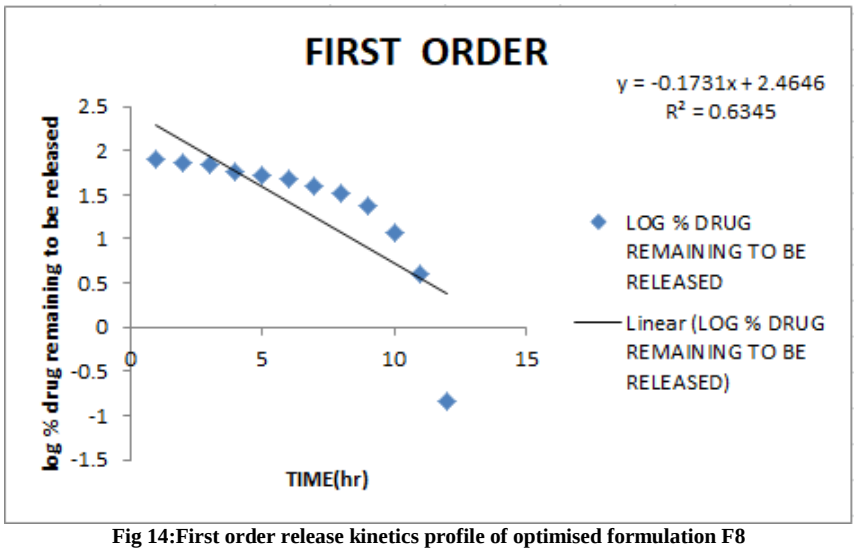


Fig 14:First order release kinetics profile of optimised formulation F8

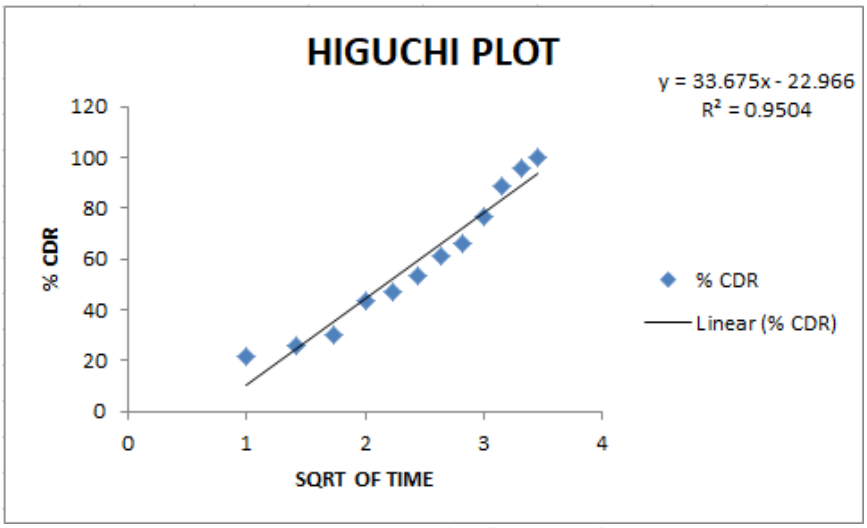


Fig15:Higuchi release kinetics profile of optimized formulation F8

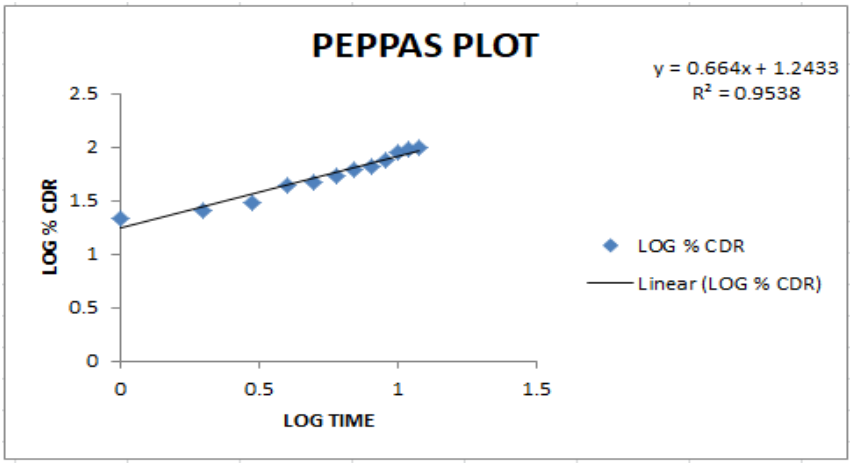
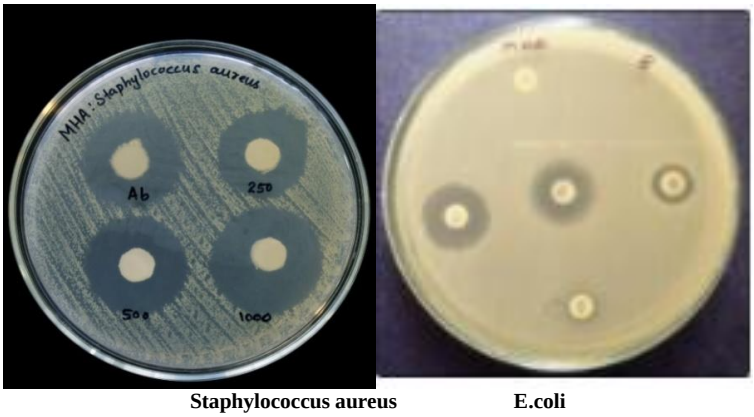


Fig16:Peppas release kinetics profile of optimized formulation F8

From the above data it was found that all formulations F1,F2,F3,F4,F5,F6,F7 and F8 followed zero order kinetics with R^2 values 0.96,0.97,0.96,0.97,0.97,0.97,0.98 respectively.To ascertain the drug release mechanism,the In-vitro drug release data were also subjected to Korsmeyer-Peppas plot.The 'n' values of optimised microsponge formulation F8(n=0.98) suggests that the drug was released by zero order kinetics with anomalous(non-Fickian) release.Non-Fickian diffusion refers to combination of both diffusion and erosion controlled rate release..All other formulations also followed case II non-Fickian mechanism.

Evaluation of antibacterial activity by disk diffusion method

The antimicrobial activity of optimized microsponge formulation F8 was carried out using Kirby-Bauer disk diffusion agar plate method. Staphylococcus aureus and E.coli were used as test organism.From the evaluation of bacterial activity,it was observed that the optimized formulation showed a clear zone of inhibition around the sample disc.



Staphylococcus aureus **E.coli**

Stability studies

The formulation F8 was observed after specified period stability studies as per ICH guidelines .The formulations was monitored for drug content and In-Vitro drug released profile and results were represented in Table 15 and percentage drug released profile was shown.

Table 15: Data of stability studies of formulation (F8)

Characteristics	Before stabilty studies	After stability studies (Day 45)	After stability studies (Day 90)
Physical appearance	Spherical,free flowing	No change	No change
Drug content(%)	98.6	98.59	98.48
In-vitro drug released	99.85	99.79	99.69

The results obtained from the stability studies showed that the optimized formulation F8 remain stable at 40°C. There was no change in appearnace. From the stability studies it was confirmed that the optimized formulation of MTZ microsponges are stable at 40°C and 75% relative humidity.

CONCLUSION

Metronidazole loaded microsponges were prepared by quasi-emulsion solvent diffusion method at varying concentrations of polymer and emulsifier. Characterization studies were conducted and found they complied with the standards. Standard calibration curve of Metronidazole was plotted. The ʎmax was observed at 276nm and found it obeys Beer Lambert's law. Drug polymer compatibility studies were conducted and found there was no incompatibility. Microsponges were evaluated for physical properties, production yield, particle size, SEM, DSC, drug content and entrapment efficiency, In vitro drug release studies stability and microbial studies. All the prepared MTZ loaded microsponges were white in colour, free flowing in nature and had a rigid structure. The percentage production yield of microsponge formulations was found to be in the range of 44-94%. The highest production yield was given by F8 and it was found that % production yield increased with increasing polymer concentration. The mean particle size of all microsponge formulations were found in the range of11.51-20.82 µm. Based on SEM studies the mean particle size of microsponges was found to be 20µm.From DSC studies, it was confirmed that drug was entrapped into microsponges. The drug entrapment efficiency (%) of MTZ loaded microsponge formulations ranged from 60-98%and showed that with increase in polymer concentration, the drug entrapment efficiency (%) was also increased. The % drug content of drug loaded microsponges was found to be in the range of 90-98%. From the in vitro drug release studies, it was found that Formulation F8 was more efficient to give sustained drug release which released 99.85% drug at the end of 12 hrs. The optimum sustained release of drug around a period of 12hrs was shown by formulation F8. All formulations followed zero order kinetics. The 'n' value of optimized formulation F8 indicated that the drug release follows zero order kinetics and Non Fickian diffusion. From the evaluation of bacterial activity, it was observed that the optimized formulation showed a clear zone of inhibition around the sample disc. It was confirmed from the stability studies that the optimized formulation remained stable at 40°C and 75% relative humidity. Based on the above evaluation studies, it could be concluded that, the formulation F8was considered as optimized formulation and it was safe and effective for topical use for Diabetic Foot Ulcer and shows a sustained release without side effects.

ACKNOWLEDGEMENTS

Firstly, I would like to give all the credit, gratitude and thanks to the great God for making each and every task related to my dissertation possible and successful and also his infinite blessings and love always guided me throughout my studies and life. It is a great pleasure to acknowledge my deepest thanks and gratitude to my esteemed guide, Prof.Dr. Umesh Kumar Sharma, Principal& HOD, Mar Dioscorus College of Pharmacy, Alathara, Sreekariyam, Trivandrum for his guidance, support, constructive criticism and never diminishing encouragement during the progress of my thesis work. Without his critical advice and deep-rooted knowledge, this work would not have been a reality. It is a great honour to work under his supervision. I take this opportunity to acknowledge my sincere thanks to Karunya Institute of Nanosciences & Technology, Coimbatore, University of Kerala, Kariavattom, Trivandrum and Biogenix Research Centre, Poojapura for their technical support for carrying out SEM, DSC and Antimicrobial study. I also thank Smt. Bessy, associate professor,

Women's College, Vazhuthacaud for providing facilities for carrying out FTIR.

Conflict of Interest: None

Source of Funding: None

Ethical Approval: Approved

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How to cite this article: Sujathan P, Sharma UK. Development and characterization of metronidazole loaded microsponges for the management of diabetic foot. *International Journal of Research and Review*. 2021; 8(10): 440-457. DOI: *https://doi.org/10.52403/ijrr.20211059*

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