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Formulation and Evaluation of Mycophenolate Mofetil Loaded Niosomal Suspension

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ABSTRACT

The aim of the present work was to prepare and evaluate niosomal suspension containing mycophenolate mofetil, cholesterol, and surfactants such as tween 20 and span 80 by sonication method for controlled release of the drug. FTIR suggests that there is no drug interaction between drug and excipient. Formulations with different ratios of surfactant and cholesterol were prepared. Several physicochemical characteristics of niosomes such as morphology, vesicle size determination, drug release profile were investigated. The preformulation study parameters for the drug mycophenolate mofetil were studied. They were melting point, solubility, FTIR. All the result was found within the standard value. The evaluation study like pH, viscosity, microscopic study, zeta potential, drug content, drug entrapment, and dissolution study was studied for all the formulations containing various surfactants concentration. The pH value of the entire sample was within the range. The viscosity of the entire sample was determined and the highest viscosity found in F3 formulation. There was a clear view of vesicles when examined through the microscope. The zeta potential study showed that F3 formulation has the least value. By analyzing the drug contents in all the formulation the least drug content was in F2 formulation and the highest value was for F3 formulation. The drug entrapment was done by centrifugation and the highest value was found in the F3 formulation. The dissolution study of all the formulation was conducted in 6 hours which ranges from 49%-54%. The high dissolution rate was found in the F3 formulation about 54% drug is diffused. The lowest dissolution rate was found in F2 formulation about 49% of drug diffused. F3 was found to be the best formulation which was subjected to scanning electron microscopic study.

INTRODUCTION

Niosomes are microscopic lamellar structures formed on an admixture of non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media. Niosomes are a promising vehicle for drug delivery and being non-ionic, Niosomes are unilamellar or multilamellar vesicles formed depending on their method of preparation[1]. A heart transplant, or a cardiac transplant, is a surgical transplant procedure performed on patients with end-stage heart failure or severe coronary artery disease when other medical or surgical treatments have failed. But, the human body considers the transplanted heart as a foreign organ and body immune system starts to reject the organ.[2,3] As a result, the cascade of immunostimulatory reactions takes place. Hence, the patient may have to take the immunosuppressant for a certain period or lifelong. Immunosuppressant posses serious side effects, and hence to be used carefully[4,5]. Mycophenolate mofetil is an immunosuppressant that has been used for organ transplantations such as liver, kidney, heart, etc. Reduction in the dose of the Mycophenolate mofetil can be achieved by loading into novel drug delivery systems such as niosomes, liposomes, nanoparticles, etc. Niosomal drug delivery system has two distinct advantages: they are made up of non-ionic surfactants and cholesterol, which reduces the interactions with the drug, it can reduce the dose of the drug and controlled delivery of the drug can be achieved [6]. Hence, niosomes are a good platform for the delivery of immunosuppressant drugs. The present study aims to formulate and evaluate 4 formulations of niosomal suspension loaded with mycophenolate mofetil with different concentrations of surfactants (Span 20 and tween 80). The objective of the study is to reduce the dose of the mycophenolate mofetil and to decrease the frequency of the administration by achieving controlled delivery of the drug and overall improvement in the bioavailability of the drug.[7,8].

MATERIALS AND METHODS

2.1 MATERIALS USED:

Mycophenolate was obtained as a gift sample from Biocon industry, Banglore. Cholesterol, span 80 and tween 20 were obtained from Yarrowchem products, Mumbai.

2.2 PREFORMULATION STUDIES

2.2.1 Determination of melting point

Powder the crystalline substance, take a capillary tube and seal one end by heating. Fill the capillary tube with the drug. Now tap the sealed end of the capillary tube on the porous plate gently. Attach the capillary tube to thermometer using the thread. The temperature at which drug starts to melt was noted, which is the melting point of the drug[9].

2.2.2 Determination of λmax

Take 5mg of drug and dilute to 50ml with phosphate buffer pH 6.8. λmax was determined by using UV spectrophotometer[10].

2.2.4 Determination of the Calibration curve

Weigh accurately 5mg of mycophenolate mofetil and transfer to a 50ml volumetric flask and adjust the volume with phosphate buffer of pH6.8(stock 1). Stock 1 solution was diluted to get a series of concentration 0.5-2.5µg/ml. The absorbance of these solution measured at 257nm in UV spectrophotometer by taking pH 6.8phosphate buffer graph was plotted by taking concentration on X-axis and absorbance on the Y-axis to obtain a standard calibration curve[11,12].

2.2.5 Solubility study of the drug

Solubility of drug was determined in 5ml of 0.1n HCL, methanol, water, ethanol, phosphate buffer 6.8 and 7.4[13].

2.2.6 FTIR Studies

FTIR of the drug and the drug and cholesterol was determined by using Shimadzu FTIR spectrophotometer by producing an optical signal with all the IR frequencies encoded into it.

2.3 Formulation of Mycophenolate mofetil loaded niosomal suspension:

Table 1: Composition of mycophenolate mofetil niosomal suspension:-

Ingredients	F1	F2	F3	F4
Mycophenolate mofetil	500mg	500mg	500mg	500mg
Cholesterol	300mg	200mg	300mg	200mg
Tween 20	25ml (5%)	25ml (10%)	–	–
Span 80	–	–	25ml (5%)	25ml (10%)
Phosphate buffer 6.8	25ml	25ml	25ml	25ml

The mixture of vesicles forming ingredients like surfactant and cholesterol are dissolved in chloroform in a round-bottom flask. The organic solvent is removed at room temperature(20°C) using rotary evaporator leaving a thin layer of solid mixture deposited on the wall of the flask. The dried surfactant film can be rehydrated with an aqueous phase at 0 to 60°C with gentle agitation. This process forms typical multilamellar niosomes. Later, 1% acacia solution (3ml) was added as a suspending agent to obtain the final formulation [14,15].

EVALUATION OF MYCOPHENOLATE MOFETIL LOADED NIOSOMAL SUSPEN SION

4.6.1 Determination of pH

The pH of the suspension was determined by using the digital pH meter. pH was determined in triplicate and the average of the result was noted[16].

4.6.2Determination of Viscosity

The viscosity of the samples was determined at 25^0c using Brookfield Synchro-lectic viscometer; model LVF (Brookfield Laboratories, Massachusetts) at 30 revolution/min (spindle≠4)[17].

4.6.3 Microscopic study

A little amount of niosomal suspension was taken in a glass slide and the particles were identified with the 100X lens of the optical microscope and photograph was taken [18].

4.6.4 Determination of zeta potential

1 ml of the sample was taken in the cuvette and the zeta potential was determined by Marvlen Zetasizer [19].

4.6.5 Determination of drug content

5ml of the niosomal suspension was accurately measured and transferred into 10ml flask and volume made up to 10ml using phosphate buffer of pH of 6.8. Further, from the above suspension 10ml was withdrawn and added to the 10ml standard flask and make up to 10ml with the phosphate buffer 6.8 pH. Absorbance was measured using UV-Visible double beam spectrometer at λmax 257nm. Drug content was calculated by comparing the absorbance with a standard curve [20,21].

4.6.6 Determination of drug entrapment

Mycophenolate mofetil niosomal suspension was centrifuged at 15700×g for 90 min at 4^0C using the refrigerated centrifuge to separate niosomes from the non-entrapped drug. The concentration of the free drug in the supernatant was determined by measuring absorbance at 257nm with a UV spectrometer. The percentage of drug entrapment in niosomes was calculated. The process was repeated in triplicate and the average of the result was taken [22].

$$\% \text{ drug entrapment} = \frac{total\ drug - drug\ in\ supernatant}{total\ drug} \times 100$$

4.6.7 Dissolution study

The *in-vitro* dissolution test for the niosomal suspension was conducted in USP Type II dissolution apparatus. 900ml media was filled in the beaker and the temperature was maintained at 37.5±5^0C and the speed of paddle was set to 50rpm. 5ml of aliquot was collected at the time interval of 1 hour for 6 hours. Fresh media were replaced to the beaker to maintain steady-state concentration. The aliquots collected were diluted suitably with pH 6.8 phosphate buffer and absorbance was taken at 257nm in UV Visible spectrometer [23,24].

RESULTS AND DISCUSSION:

Table 2: Determination of Melting Point:

SI No:	Melting point
1	96
2	96.2
3	96
Average	96

Standard Plot of Mycophenolate Mofetil

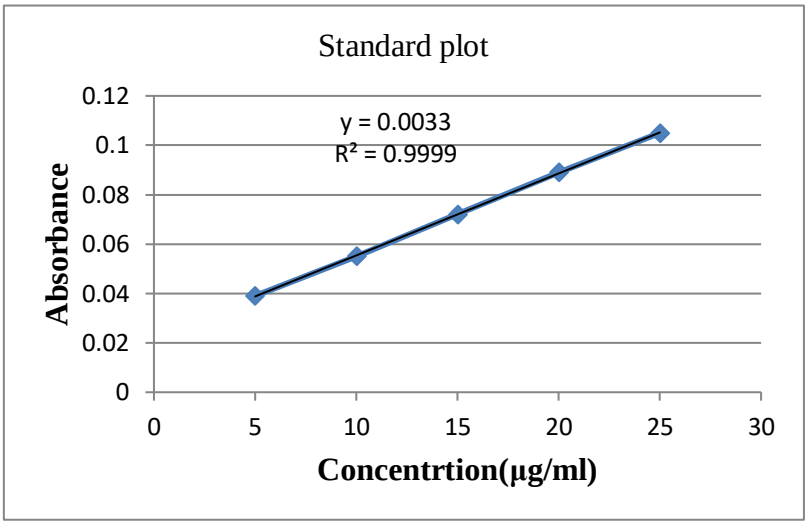

Fig:1 Standard plot of Mycophenolate mofetil

5.3 Solubility study

Table 3: Solubility of the drug in various solution

Sample	Solubility
0.1N HCl	Highly soluble
Methanol	Soluble
Water	Slightly soluble
Ethanol	Sparingly soluble
Phosphate buffer of pH 6.8	Slightly soluble
Phosphate buffer of pH 7.4	Slightly soluble

FTIR STUDY

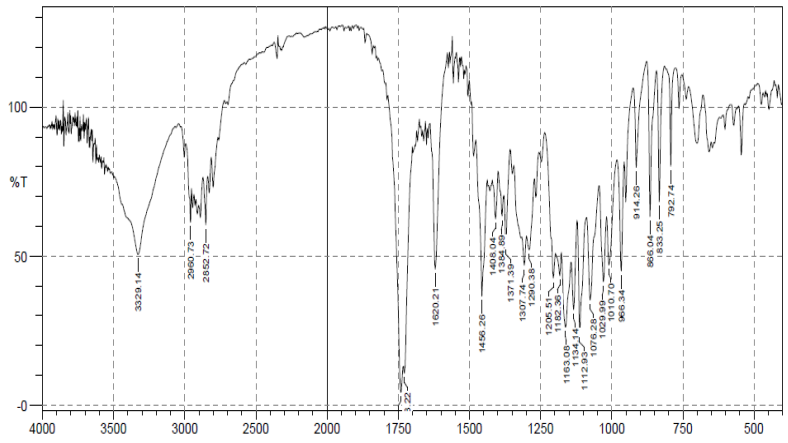

Fig 2: FTIR Spectrum of Mycophenolate Mofetil Pure

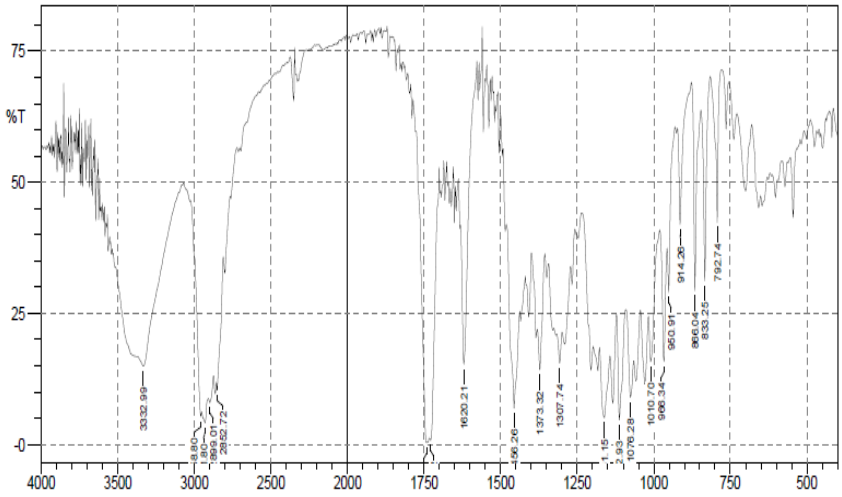

Fig. 3: FTIR spectrum of Mycophenolate Mofetil+Excipients

All the important peaks are present in the FTIR spectra of the drug and excipients. The results of the study indicate FT-IR spectrum of Drug and excipients did not differ with major peaks of mycophenolate mofetil.ie; all the major peaks of the drug appeared on the blend indicate that there is no possible interaction between drug and Excipients.

EVALUATION STUDY RESULTS

Table 4: Results of evaluation studies

PARAMETERS	FORMULATION CODE			
	F1	F2	F3	F4
Ph	6.3	6.69	6.69	6.7
Viscosity (CP)	47.51	36.42	49.32	40.19
Particle Size (nm)	272.1	601.3	165.2	305.7
Zeta Potential (mV)	4.28	8.92	1.78	8.76
Drug Content (%)	96.5	90	98.3	94.9
Entrapment efficiency (%)	83.6±0.9	79.7±1.2	85.8±1.84	79.8±1.6
In-vitro dissolution study (6 hrs) (%)	52.36	49.62	54	50.04

Inference: 300 mg of cholesterol among with 5% Span surfactant concentration has showed improvement in the formulation quality as the particle size was 165.2 nm, entrapment efficiency of 85.8% and 98.3% of drug content.

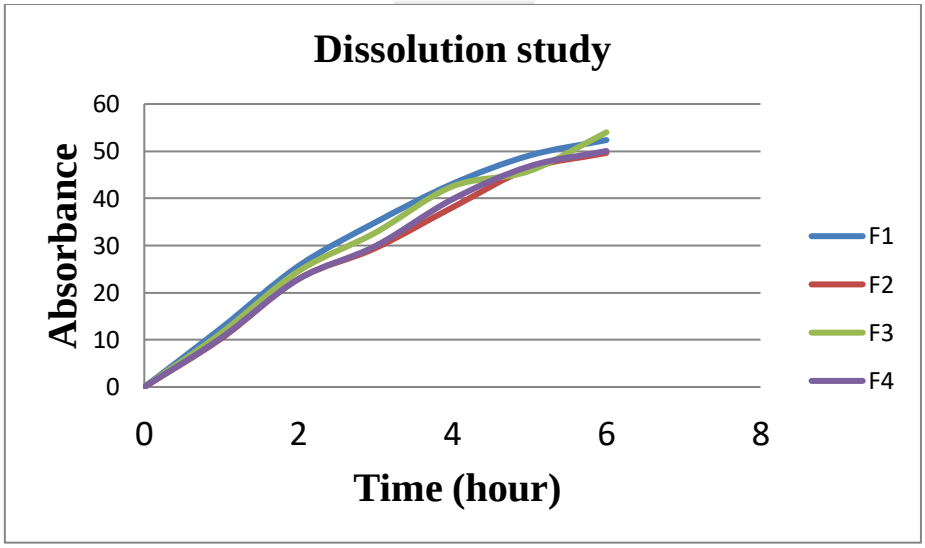

Fig 4: *In-vitro* Dissolution graph of mycophenolate mofetil niosomal suspension

From the evaluation studies, F3 formulation was found to be best with 98.3% drug content, 165.2 nm particle size and 54%release of the drug in 6 hours.

Inference: Drug release study was conducted for 6 hours and the maximum release was found in F3 formulation.

OPTICAL MICROSCOPIC STUDY RESULT OF F3 FORMULATION

Fig 5: Microscopic view of F3 formulation

Particle size distribution and zeta potential study of F3 formulation

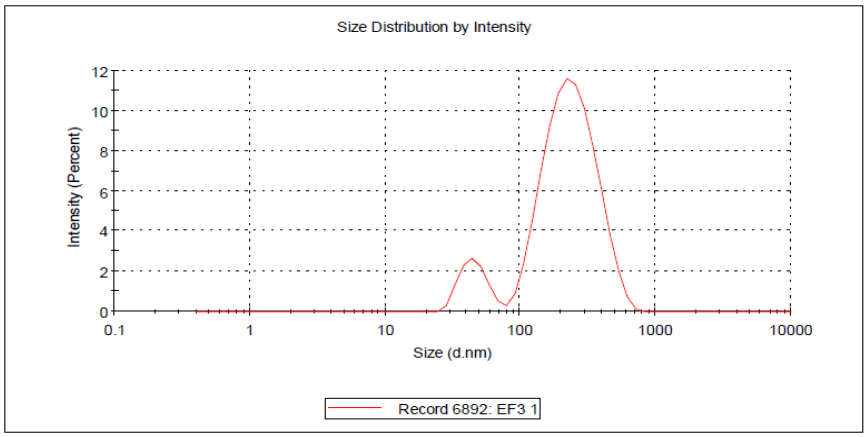

Fig 6: Particle size distribution graph of F3

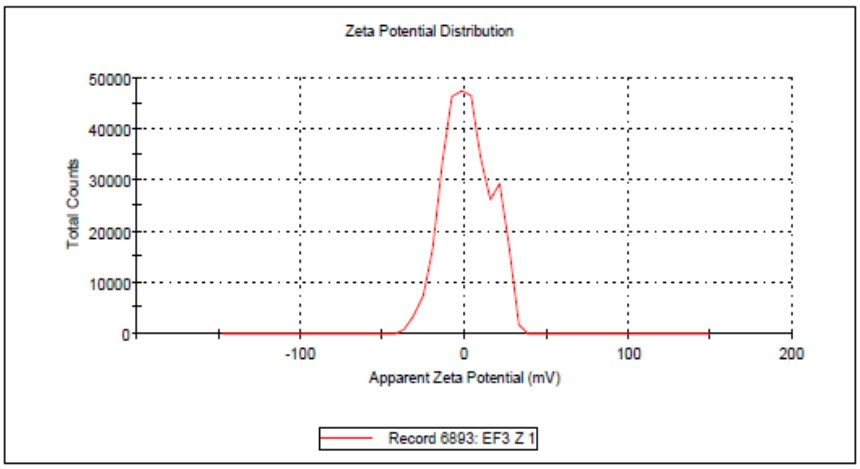

Fig 7: Zeta potential graph of F3 formulation

SCANNING ELECTRON MICROSCOPY OF F3 FORMULATION

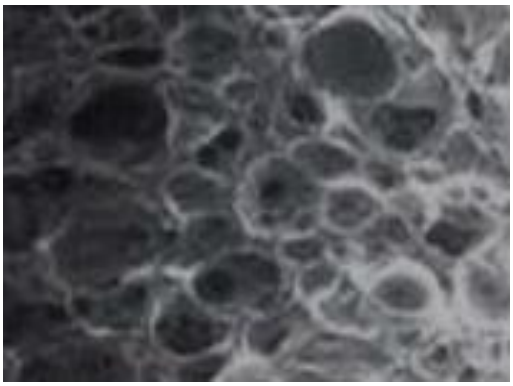

Fig 8: Scanning electron microscopic image of F3 formulation

CONCLUSION:

A niosomal suspension containing mycophenolate mofetil was successfully developed using thin film hydration method and evaluated. Mycophenolate mofetil niosomal suspension was prepared with different concentration of tween 20 and span 80. The mycophenolate mofetil niosomal suspension formulated by thin film hydration method was good quality with regards to drug content, drug entrapment, dissolution study. The suspension with least percentage of span 80(5%) shows the higher dissolution rate and efficiency among all other formulations. The formulation F3 showed better formulation among the other formulation. The formulation might suitable for large scale production.

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