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RESEARCH ARTICLE

Formulation and Evaluation of Meloxicam Microspheres Using Eudragit S-100

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ABSTRACT

In the present study, an attempt was made to formulate and evaluate microspheres of Eudragit S-100 for the colon targeted delivery of meloxicam for management of rheumatoid arthritis. To achieve these objectives different formulations of microspheres were prepared by emulsion solvent evaporation method by varying the polymer, surfactant as well as cross linking agent concentration. The prepared microspheres were evaluated for surface morphology, entrapment efficiency, Fourier transform infrared spectroscopy, differential scanning calorimetry and *in-vitro* drug release study. The obtained microspheres were spherical in shape & free flowing. The surface morphology revealed that the prepared microspheres are spherical, having smooth and dense surface. With an increase in cross linking agent, the microsphere size was decreased, whereas with increase in polymer and drug concentrations microsphere size increased. The drug entrapment efficiency of the microspheres was increased with increased in glutaraldehyde concentration. The DSC analysis indicates that there was no drug polymer interaction. The F2 formulation which shows satisfactory release *in vitro* was tested for its integrity. The coefficient of regression (R^2) value shows drug release mechanism followed anamolous non Fickian diffusion.

KEYWORDS

Meloxicam, Eudragit S-100, Colon Targeting, Microspheres, Cross Linking Agents

INTRODUCTION

Oral drug delivery is the most preferred and convenient option as the oral route provides maximum active surface area among all drug delivery system for administration of various drugs. The attractiveness of these dosage forms is due to awareness to toxicity and ineffectiveness of drugs when administered by oral conventional method in the form of tablets & capsules. Usually conventional dosage form produces wide range of fluctuation in drug concentration in the bloodstream and tissues with consequent undesirable toxicity and poor efficiency.

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The maintenance of concentration of drug in plasma within therapeutic index is very critical for effective treatment. These factors as well as factors such as repetitive dosing and unpredictable absorption lead to the concept of oral Sustained release (SR) drug delivery systems. Sustained release drug delivery system works on many different mechanisms to control the release rate of drugs[1]. But there are certain conditions which demand release of a drug after a lag time i.e. chronopharmacotherapy of diseases which shows circadian rhythms in their pathophysiology. Recent studies have revealed that diseases have predictable cyclic rhythms and that the timing of medication regimens can improve outcome in selected chronic conditions[2].

MATERIALS AND METHOD

Materials

Eudragit S-100 was obtained as gift sample from Evonik industries and Meloxicam is obtained as gift sample from Arbro Pvt. Ltd. All chemicals and reagents used were of analytical grade, and solvents used for HPLC were of high performance liquid chromatography (HPLC) grade.

Differential Scanning Calorimetry (DSC)

DSC of the pure drug was taken by using differential scanning calorimeter[3] (Outsourced Jamia Hamdard New Delhi)

The instrument was adjusted to the following parameters:

a) Atmosphere : Nitrogen inert.

b) Heating rate : 10ºC/min

c) Gas flow rate : 20ml/min

d) Temperature range : 0-300ºC

e) Sample size : 0.5 mg

HPLC Method for Meloxicam

The HPLC method reported by (Shukla et al., 2007) was modified and validated for *in vitro* sample analysis in phosphate buffer pH 7.4 HPLC condition. The samples were analysed on a reverse phase column C_{18} (25 cm × 4.6 mm, 5 µm) with the mobile phase of a mixture of 65% water: acetic acid (99:1, v/v) and 35% acetonitrile. Flow rate of the mobile phase was adjusted to 1 ml/min. Elution of drug was measured at 363 nm[4].

a. Equipment: Shimadzu HPLC with an attached UV detector.

b. Column: C_{18} reversed- phase column (25 cm ×4.6 mm, particle size 5µm)

c. Mobile phase: Water: acetic acid (99:1): Acetonitrile (65:35)

d. Flow rate: 1.0 ml/min.

e. Detection: UV, 363 nm

f. Injection: 20 microlitre (µl)

Procedure: A stock solution of meloxicam of 0.1mg/ml was prepared by taking 10 mg of drug and diluting to 10 ml acetic acid: acetonitrile (1.1). From this stock solution (1000µg/ml), the serial dilutions were prepared for concentrations ranging from 2 -20 µg/ml. A graph was plotted between concentration (x- axis) and area under curve (y-axis). The calibration curve of meloxicam was prepared by HPLC method for the determination of drug content and *in vitro* analysis[4].

Preparation of Meloxicam Microspheres

Meloxicam microspheres were prepared by emulsification solvent evaporation method. Accurately weighed polymer (200 mg) was dissolved in 10 ml of ethanol. In this polymeric solution dispersed the drug (100 mg) and mixed thoroughly. The above organic phase was slowly poured into liquid paraffin (10 ml) containing span 80 of (0.5%) concentration. Thereafter, it was allowed to attain room temperature and stirring was continued until residual ethanol evaporated and smooth walled, rigid and discrete microspheres were formed. When the microsphere separates out then the glutaraldehyde 25 % (2%) was added drop wise into the emulsion for cross linking of the microspheres. The microspheres were collected by decantation and the product was washed with petroleum ether, three times or till complete removal of glutaraldehyde and dried at room temperature for 3 h. The microspheres were then stored in a desiccator over fused calcium chloride for further use[5,6,7,8]. To optimize the formulation various batches were made which is given in Table 2.1 and Table 2.2.

Characterization of Chitosan Microspheres

Percentage Yield

The prepared microspheres were collected and weighted. The actual weight of obtained microspheres divided by the total amount of all material that was used for the preparation of the microspheres.[9]

$$\%\text{Yield} = \frac{\text{Actual weight of product}}{\text{Total weight of excipients and drugs}} X100$$

Table 1: Batch design of microspheres by varying span 80 and glutaraldehyde concentration

Batch No.	Drug (mg)	Polymer (mg)	Span 80 (%)	Glutaraldehyde (%)
SA1	100	200	0.5	1
SA2 (F)	100	200	0.5	2
SA3	100	200	0.5	3
SB1	100	200	1	1
SB2	100	200	1	2
SB3	100	200	1	3
SC1	100	200	1.5	1
SC2	100	200	1.5	2
SC3	100	200	1.5	3

Table 2: Batch design of microspheres by varying polymer concentration

Formulation Code	Drug (mg)	Polymer (mg)	Surfactant concentration Span (80%)	Glutaraldehyde (25%)
F1	100	100	0.5	2
F2	100	200	0.5	2
F3	100	300	0.5	2

Entrapment Efficiency

Microspheres prepared were dried at room temperature after that they were allowed for shaking on orbital shaker for 3 hours in 0.1 N HCl. The solution was filtered using whatmann filter paper and filtrates was analysed using a UV spectrophotometric method at 343.2 nm in the presence of a blank prepared from microspheres containing all material except the drug[9,10].

$$\text{Entrapment Efficiency} = \frac{\text{Weight of drug} - \text{Weight of free drug}}{\text{Weight of drug}} X100$$

Loading Capacity

Microspheres obtained after treatment with HCl are filtered and dried at room temperature and weighed.

$$\text{Loading Capacity} = \frac{\text{Weight of drug} - \text{weight of free drug}}{\text{Weight of Microspheres}} X100$$

Particle Size Analysis

The particle size of microspheres was measured by using Malvern, ZS 90 particle size analyzer[11].

Scanning Electron Microscopy

Scanning electron microscopy has been used to determine the surface morphology and texture. SEM studies were carried out by using zeiss scanning microscope[12].

Transmission Electron Microscopy

Scanning electron microscopy has been used to determine the surface morphology and texture. SEM studies were carried out by using zeiss scanning microscope[12].

Zeta Potential

Zeta potential is measured by zeta potential analyzer outsourced from Jamia Hamdard[13].

Particle Size Distribution of Meloxicam Microspheres

It is measured by zeta sizer instrument Malvern Instrument outsourced from Jamia Hamdard[13].

In Vitro Drug Release

The dissolution studies were performed in six station dissolution test apparatus (37±0.5^{0}C, 50 rpm) using the USP rotating basket method in simulated gastric media (0.1 N HCl, 900ml) for first 2 hrs and phosphate buffer media (pH 7.4, 900 ml) for next 6 hrs. A quantity meloxicam microspheres equivalent to 7.5 mg for each formulation was employed in all dissolution studies. The sample of 5 ml each was withdrawn at predetermined time interval and were replenished immediately with the same volume of gastric/phosphate buffer maintaining sink condition throughout the experiment. The aliquots, following suitable dilution with gastric/ phosphate buffer were analysed spectrophotometrically at 343.2 nm and 362.6 nm respectively[14,15]. The concentrations of meloxicam in the test samples were calculated using a regression equation (Absorbance = 0.05363x + 0.01448; r^2 = 0.99686) of the calibration curve in phosphate buffer.

Release Kinetics

The cumulative drug release obtained from the optimized formulation was used for the calculation of release kinetics i.e. zero order, first order, Higuchi's square root of time equation plot, and Korsmeyer-peppas' power law equation model. All the models are compared for the best fitting of the model[16].

RESULTS

Drug-Excipient Incompatibility Study

Differential Scanning Calorimetry (DSC)

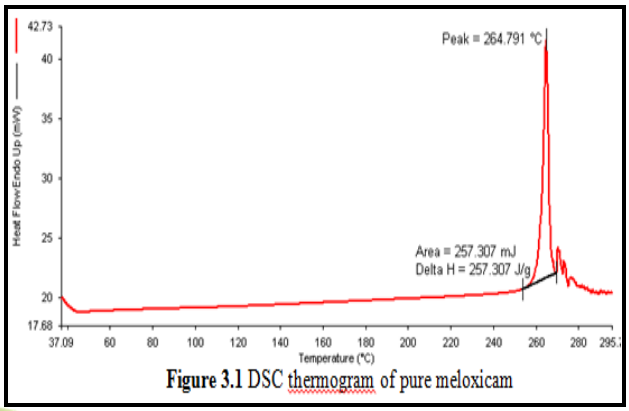

Figure 1: DSC thermogram of pure meloxicam

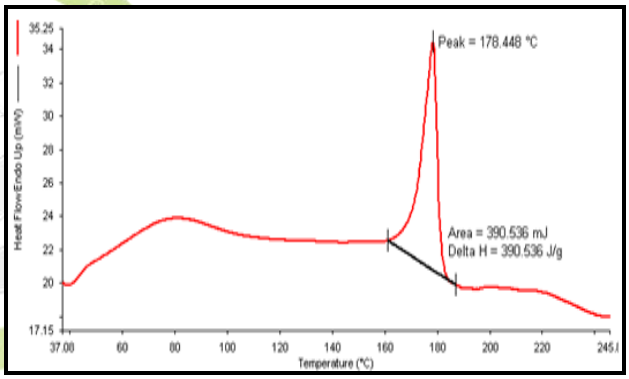

Figure 2: DSC thermogram of Eudragit S-100

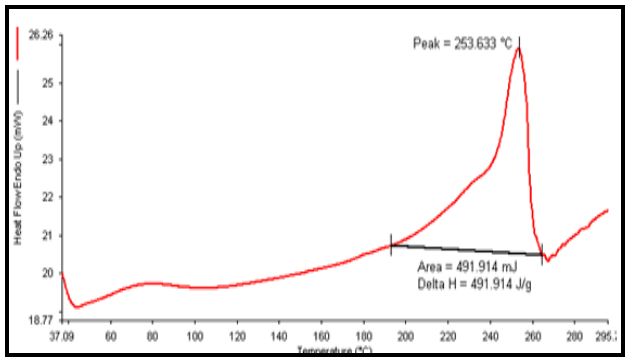

Figure 3: DSC thermogram of Meloxicam and Eudragit S-100

The purpose of interaction studies was to determine any interference & interaction of the drug with other ingredients of the formulation. However, in the interaction studies the drug was

shown to be fairly stable and inert with the excipients used in the microsphere formulation.

High Performance Liquid Chromatography

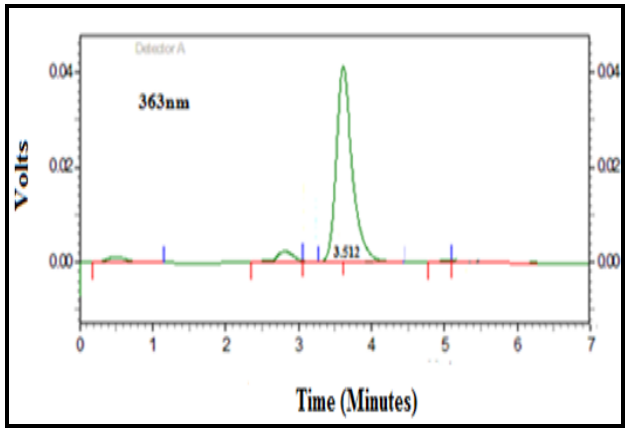

Figure 4: HPLC chromatogram of meloxicam

HPLC chromatogram of meloxicam in mobile phase shows that the retention time is 3.512 minutes.

Evalution of Microspheres on the basis of Entrapment Efficiency, loading Capacity and Percentage Yield

Table 3: Entrapment efficiency, loading capacity and Percentage yield of meloxicam microspheres by varying span 80 and glutaraldehyde concentration

Batch No.	Entrapment Efficiency	Loading Capacity	% Yield
SA1	65.03038194	42.50351761	51
SB1	63.03385417	46.34842218	45.33333
SC1	59.04079861	57.3211637	34.33333
SA2	75.88107639	37.5648893	67.33333
SB2	71.19357639	33.58187566	70.66667
SC2	68.06857639	30.94026199	73.33333
SA3	63.98871528	56.130452	38
SB3	56.43663194	54.79284655	34.33333
SC3	58.17274306	59.35994189	32.66667

With increase in span-80 concentration entrapment efficiency and percentage yield increases and with further increase in span 80 concentration entrapment efficiency and percentage yield decreases. With increase in glutaraldehyde concentration entrapment efficiency and percentage yield increases.

Table 4: Entrapment efficiency, loading capacity percentage yield of microspheres by varying polymer ratio

Batch No. (Drug: Polymer)	Entrapment Efficiency	Loading Capacity	% Yield
F1 (1:1)	76.40190972	57.4450449	66.5
F2 (1:2)	78.48524306	34.88233025	75
F3(1:3)	79.52690972	22.46522874	78.5

With increases in polymer concentration from 100mg – 300 mg the entrapment efficiency increases from 76- 79%, loading capacity decreases from 57 – 22 % and percentage yield increases from 66.5 – 78.5 %. Whereas the optimum value is seen using 200 mg polymer where entrapment efficiency is 78.48%, loading capacity is 34.88% and yield is 75%.

Particle Size and Surface Morphology

Scanning Electron Microscopy

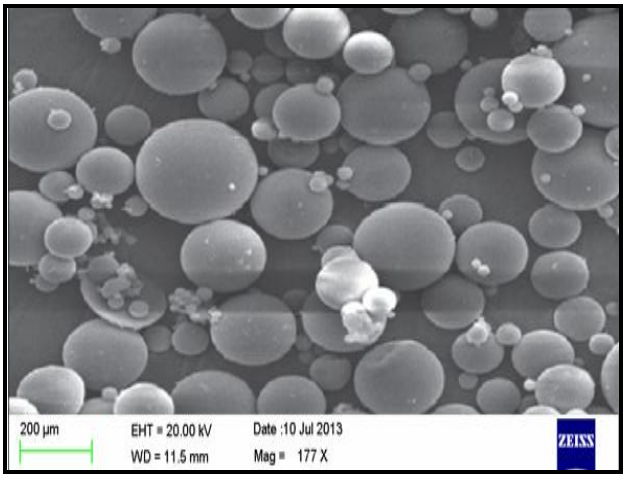

Figure 5: SEM photograph of Meloxicam

Transmission Electron Microscopy

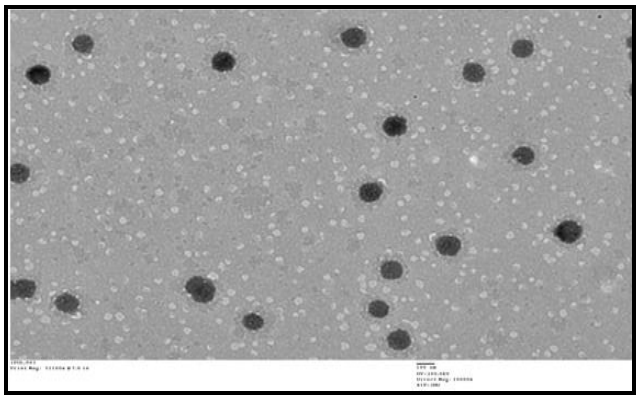

Figure 6: TEM photograph of Meloxicam microspheres

Particle Size Distribution of Meloxicam Microspheres

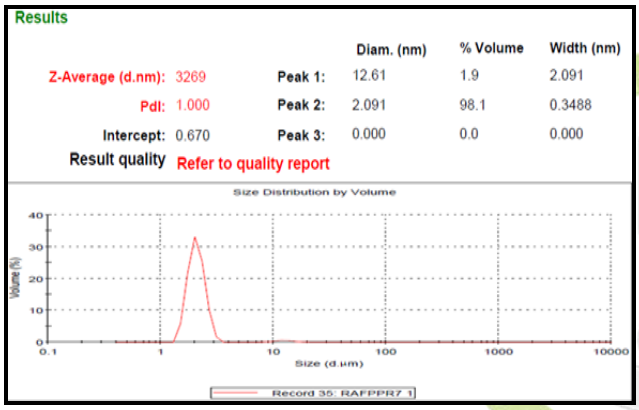

Figure 7: Particle size distributions of Meloxicam microspheres

SEM shows that the particle is smooth and spherical and drug is entrapped within the microspheres and TEM shows that microsphere size is 5-7 µm.

Particle size distribution of meloxicam microspheres shows that the average particle size range of meloxicam microsphere is 3.269 µm. 98% of the microspheres are in the size range of 2.091 µm.

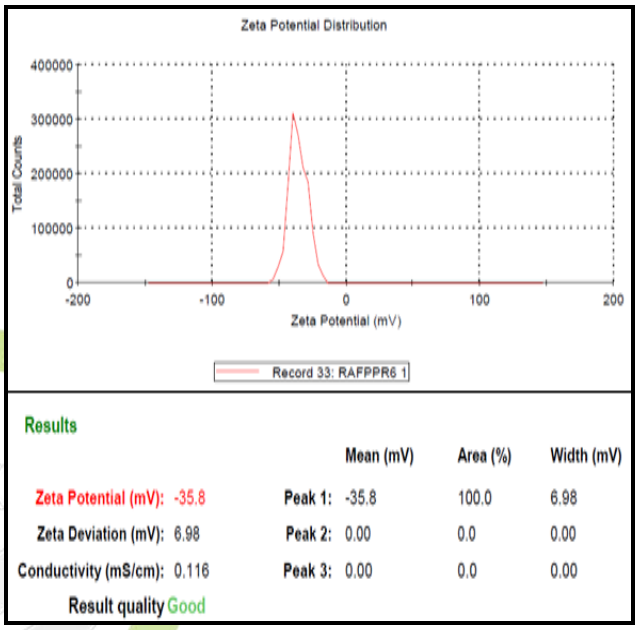

Figure 8: Zeta Potential of Meloxicam

The zeta potential value (-35.8) shows that the polymer is anionic and the formulation is stable.

Drug Release

Table 5: Drug release of Meloxicam microspheres containing drug polymer ratio (1:1)

Time	Absorbance	Concentration	Cumulative Drug Release	CPDR
0	0	0	0	0
2	0	0	0	0
3	0.125	2.060786873	1.854708186	24.72944248
4	0.176	3.011747156	2.710572441	36.14096588
5	0.196	3.384672758	3.046205482	40.61607309
6	0.251	4.410218161	3.969196345	52.92261794
7	0.267	4.708558643	4.237702778	56.50270371

CPDR= Cumulative percentage drug release

Table 6: Drug release of Meloxicam microspheres containing drug polymer ratio (1:2)

Time	Absorbance	Concentration	Cumulative Drug Release	% CPDR
0	0	0	0	0
2	0	0	0	0
3	0.25	4.391571881	3.952414693	52.69886258
4	0.299	5.305239605	4.774715644	63.66287526
5	0.352	6.293492448	5.664143203	75.52190938
6	0.406	7.300391572	6.570352415	87.60469886
7	0.414	7.449561812	6.704605631	89.39474175

Table 7: Drug release of Meloxicam microspheres containing drug polymer ratio (1:3)

Time	Absorbance	Concentration	Cumulative Drug Release	% CPDR
0	0	0	0	0
2	0	0	0	0
3	0.119	1.948909193	1.754018273	23.38691031
4	0.136	2.265895954	2.039306358	27.19075145
5	0.187	3.216856237	2.895170613	38.60227485
6	0.231	4.03729256	3.633563304	48.44751072
7	0.262	4.615327242	4.153794518	55.38392691

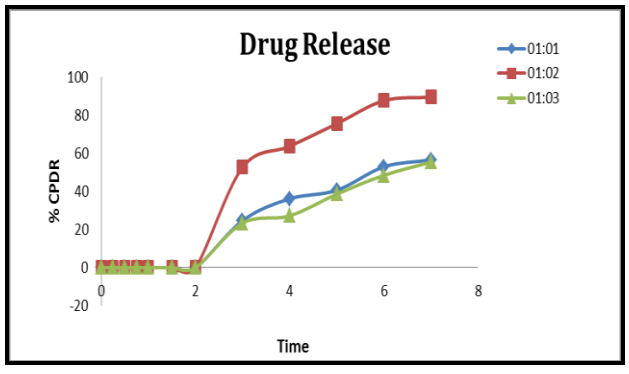

Figure 9: Drug release of formulation containing drug polymer 1:1, 1:2, 1:3

Drug release from the F1, F2, and F3 shows that F2 formulation has maximum drug release i.e. 89% at 7th hour in comparison to the F1 and F3 in which drug release is 55% and 56% respectively, since in F1 formulation less drug is entrapped in the polymer since drug and polymer ratio are equal and in F3 formulation since drug polymer ratio is 1:3 hence more drug is entrapped in the polymer but due to higher amount of polymer drug is released in longer period of time.

Drug Release Kinetics

Table 8: Drug release kinetics of optimized F2 formulation

Dose(mg)	Time	CDR	Qt	√t	ln T	ln CDR
7.5	0	0	7.5	0	0	0
7.5	3	3.952415	3.547585	1.732051	1.098612	1.374326707
7.5	4	4.774716	2.725284	2	1.386294	1.563334421
7.5	5	5.664143	1.835857	2.236068	1.609438	1.734155639
7.5	6	6.570352	0.929648	2.44949	1.791759	1.882567471
7.5	7	6.704606	0.795394	2.645751	1.94591	1.902794698

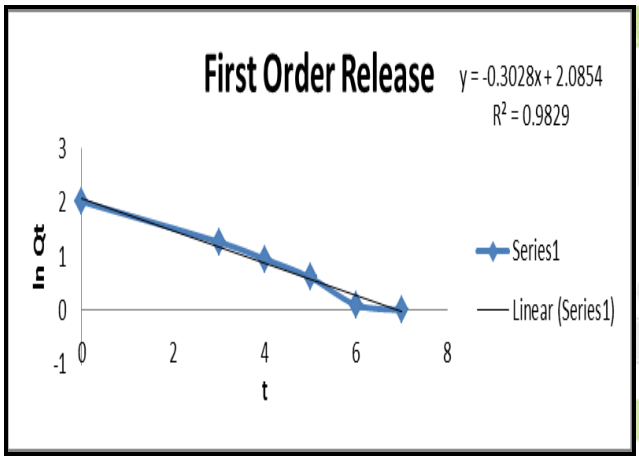

Figure 10: First order release of F2 formulation

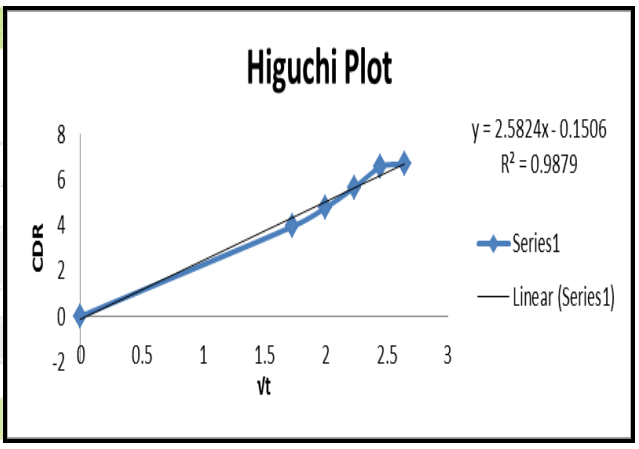

Figure 12: Higuchi plot of F2 formulation

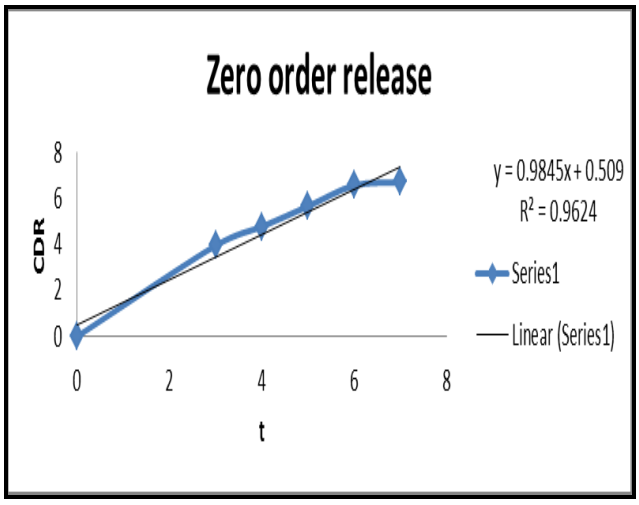

Figure 11: Zero order release of F2 formulation

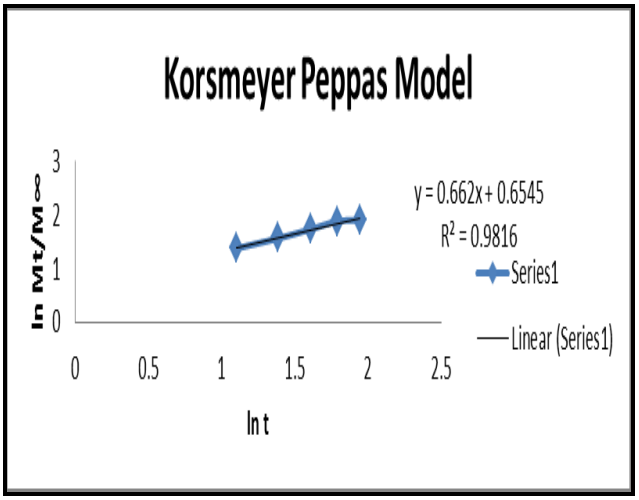

Figure 13: Korsmeyer peppas model of F2 formulation

Table 9: Model fitting of the drug release profile of formulation F2

Release Model	R^2	Slope = K
Zero order model	0.962	0.984
First order model	0.982	0.302
Higuchi's square root of time plot	0.987	2.582
Korsmeyer Peppas plot	0.981	0.662

Interpretation of Release Models (*In Vitro*)

The formulation so proposed is following first order release kinetics (first order release model, r^2 = 0.9624), where drug is being released through diffusion process from the polymer matrix (Higuchi model r^2 = 0.9879). The meloxicam microspheres follows anomalous non-Fickian diffusion (n = 0.662 from Korsmeyer- Peppas law equation) i.e. the rate of solvent penetration & drug release are in the same range. This deviation is due to increase drug diffusivity from the matrix by the solvent induced relaxation of the polymers.

CONCLUSION

From the result of present study, it can be concluded that Eudragit S-100 based meloxicam microspheres offer high degree of protection from premature drug release in simulated upper GIT conditions and deliver most of the drug load in the distal part of the small intestine. Based on the results of the physiochemical characterization and in vitro drug release studies, it possesses all the required physiochemical characters with drug releases up to 89% at 7 hours.

DSC study revealed that there is no drug polymer interaction. SEM shows that the microspheres prepared are smooth and spherical. TEM study shows that the microspheres prepared are in the range of 3-7µm. Microspheres were prepared by solvent evaporation method and F2 formulation was optimized from all the batches having span concentration 0.5%, glutaraldehyde 2% and drug polymer ratio was taken (1:2). In the optimized formulation the drug entrapment and drug loading was found to be 78.48% and 75% respectively. Release mechanism follows first order kinetics where drug is being released through diffusion process from the polymer matrix (Higuchi model r^2=0.9879). The meloxicam microspheres follows anomalous non-Fickian diffusion (n= 0.662 from Korsmeyer- Peppas law equation) i.e. the rate of solvent penetration & drug release are in the same range. This deviation is due to increase drug diffusivity from the matrix by the solvent induced relaxation of the polymers. Thus, Eudragit S-100 based meloxicam microspheres are a potential system for the management of rheumatoid arthritis.

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